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SEED DESICCATION TOLERANCE AND GERMINATION OF SEVEN IMPORTANT FOREST TREE SPECIES IN GHANA

By

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To the Almighty God, I express my innermost appreciation for making me, who I am today.

DECLARATION

I hereby declare that this submission is my own work towards the PhD and that, to the best of my knowledge it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the university, except where due acknowledgement has been made in the text.

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DEDICATION

This thesis is dedicated to my four lovely children namely: Nana Sakyiwa, Maame Pokuaa, Papa Mireku and Kwadwo Obeng for their patience and understanding when I had to scale down my time and attention for them due to my desire to complete this PhD programme.

ABSTRACT

Seed desiccation and germination studies were conducted on seven important tree species in Ghana namely: *Garcinia kola, Garcinia afzelii, Terminalia superba, Terminalia ivorensis, Khaya anthotheca, Entandrophragma angolense* and *Mansonia altissima.* Seed desiccation was carried out using silica gel as well as drying under shade and seed germination trials conducted at various moisture contents to assess the effect of drying on seed viability. Drying was carried out on whole seeds of *G. afzelii, T superba, T. ivorensis, K. anthotheca, E. angolense* and *M. altissima.* In the case of *G. kola* whole seeds, and seed sections/fragments namely: the proximal section (PS) and the distal sections (DS) were desiccated. Seeds of *T. superba, T. ivorensis, K. anthotheca, E. angolense* and *M. altissima* survived desiccation to moisture contents of approximately 4-5% with very little or no loss in viability. Seeds of *G. afzelii* declined in viability as they were desiccated and viability drastically reduced below its "critical moisture content" of 25%. Seeds and seed fragments of desiccated *G. kola* also decreased in viability as they were desiccated and viability drastically reduced below their 'critical moisture contents' of 30%. Germination of *T. superba,* T. *ivorensis and K. anthotheca* were enhanced by alternating temperatures using the Grant 2-way thermogradient plate. Seeds of *K. anthotheca* placed at alternating temperatures of 20/25ºC; 20/35ºC; 25/15ºC; 25/30ºC and 30/20ºC gave 100% germination. *T. ivorensis* seeds set at 15/35ºC; 20/35ºC and 30/35ºC resulted in germination percentages above 73%. On the other hand seeds of *T. ivorensis* placed at a constant temperature of 30/30ºC gave 87% germination.

T. superba seeds incubated at alternating temperatures of 35/15ºC; 35/40ºC; 40/25ºC and 40/30ºC gave germination percentages of 93% and beyond. Normal seedlings of *Garcinia kola* could be raised from the proximal; half proximal; distal end chipped off and proximal end chipped off sections of *G. kola* seed. Water sorption isotherm curves of all the species exhibited the reversed sigmoidal shape. Electrical conductivity measurements of leachates from *Garcinia kola* seeds increased as seeds were dried to lower moisture content.

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5.0. DISCUSSIONS

5.1 Equilibrium Relative Humidity (eRH) of Seed Samples

5. 1.1 *Garcinia kola and Garcinia afzelii*

Equilibrium relative humidity (eRH) is the value of relative humidity into which a hygroscopic product can be placed where no net exchange of moisture between the product and the surrounding environment can take place**.** In the seed industry, knowing the seed eRH at harvest can inform post-harvest handling (MSBP, 2002). The equilibrium relative humidity (eRH) and moisture content (MC) values of 97.0% and 58.0% respectively recorded for the matured fresh seeds of *Garcinia kola* and an eRH of 93.3% and moisture content of 38.2% recorded for matured fresh seeds of *Garcinia afzelii* when they were received at the laboratory were very high.

According to MSBP (2002), very high eRH close to 100% indicates that seeds are potentially immature or probably seeds are in post-abscission phase. This may be applicable to orthodox seeds which go through the process of maturation drying. Desiccation sensitive (recalcitrant) seeds unlike orthodox seeds do not undergo maturation drying, and even after shedding from the mother plant they have high moisture content, ranging from 30 to 70% (Chin *et al*., 1984). *Garcinia kola* and *Garcinia afzelii* most probably had not gone through maturation drying during their development and therefore had high moisture content at shedding (Robert,1973), leading to their "wetness " and high eRH values (MSBP, 2005).

This is an important characteristic of the Genus *Garcinia* (family Clusiaceae) as reported by Morton (1987). *Garcinia kola* desiccated in silica gel for 30 days still had relatively high eRH and

MC of 57.7% and 20.0% respectively. This observation may be attributed to the large seed sizes of the species (8.9 g ± 2.3) which resulted in the slow drying rate. Troftgruben (1977) observed that small fruits dry faster than thick ones.

5. 1.2 *Terminalia superba and Terminalia ivorensis*

When fresh seed samples of *Terminalia superba* arrived at the laboratory, eRH and MC values of 61.5% and 13.8%, respectively, were recorded while *Terminalia ivorensis* seed samples on arrival recorded an eRH and MC values of 70.5% and 18.5%, respectively. These initial eRH and MC values indicate that seeds of the two species could be placed in the "damp" moisture status (MSBP, 2005) at the time they were harvested. Such seeds need to be dried as soon as possible (MSBP, 2005). The two species, unlike *Garcinia kola* and *Garcinia afzelii* could be said to have gone through maturation drying during their development (Pammenter and Berjak, 2000) and therefore their relatively low eRH and seed moisture content compared to the two *Garcinia* species. After 14 days of desiccation in silica gel, the eRH and MC of 7.2% and 3.5%, respectively for *T. superba*; 13.0% and 3.6%, respectively for *T. ivorensis* gave an indication of how fast these species dried which was attributed to their relatively small seed sizes.

5.1.3 Khaya *anthotheca* and *Mansonia altissima*

Khaya anthotheca and *Mansonia altissima* seeds at the time of receipt at the laboratory registered eRH and MC values of 54.7% and 14.2%, respectively (*K. anthotheca*) and eRH and MC values of 68 0% and 13.4%, respectively (*M. altissima*). According to MSBP (2005), the two species could be placed in the ''damp'' moisture status at the time they were received at the laboratory**.** These two species could be said to have gone

through maturation drying like all orthodox seeds (Pammenter and Berjak, 2000). These seeds registered eRHs less than 85% and thus could be said to be fully matured, but could be dried further (MSBP, 2002) before being packed in airtight containers for storage (MSBP, 2005).

5.1.4 *Entandrophragma angolense*

The eRH and MC values of 34.9% and 6.4% respectively of seed samples of *E. angolense* suggested that the species passed through maturation drying resulting in the very low moisture content and eRH. *E. angolense* could be placed in "dry" moisture status (MSBP, 2005) at the time they were received at the laboratory. Seeds with such low eRH and moisture content would not need any further drying and they could be stored immediately (MSBP, 2005).

5.2 Initial moisture content of whole seed and seed components

In carrying out a seed desiccation study, it is important to know the whole seed moisture content as well as moisture content of individual seed components. This helps in determining the variation in moisture content within the seed (IPGRI- DFSC, 1999).

5.2.1 *Garcinia kola* and *Garcinia afzelii*

The initial test indicated that the mesocarps of *Garcinia kola* and *Garcinia afzelii* had a significantly higher moisture content compared to the whole fruit, whole seed and seed coat. Since the fruits of the two species were fleshy (Thomsen, 2000; Geeta *et al*., 2006) their mesocarps were expected to contain a lot of water. The whole fruit of *G. kola* and *G. afzelii* had a higher moisture contents compared to the whole seed and seed coat.

These observations might be due to the overwhelmingly high moisture content of the mesocarps which greatly enhanced the fruit moisture content. The seed coat (testa) of the two *Garcinia* species although thin and leather-like as reported by Gyimah (2000), had significantly higher moisture contents than the whole seed.

5.2.2 *Terminalia superba* and *Terminalia ivorensis*

The moisture content of decoated *Terminalia superba* and decoated *T. ivorensis* seeds was significantly higher than whole seed moisture content and seed coat moisture content of the two species. These observations might be due to the presense of the thick and lignified seed coat, especially, of *Terminalia ivorensis* seeds (Corbineau and Come, 1993) which contain less water.

5.2.3 *Khaya anthotheca*, *Entandrophragma angolense* and *Mansonia altissima*

The moisture content of the seed and seed components of *Khaya anthotheca*, *Entandrophragma angolense* and *Mansonia altissima* showed that the seed coat moisture content was higher than whole seed as well as decoated seed moisture content for the three species. Similar observations were reported on the seeds of *Sclerocarya birrea* from Burkina Faso by Gaméné *et al*. (2004). These observations made from the present study and by Gaméné *et al*. (2004), however, say the opposite to the situation in *Terminalia superba* and *T. ivorensis* in the present study. There are a lot of variations in moisture content within the individual component parts of the various seed species. Variation in moisture content of components within a seed may be due to the type of seed as well as the stage of maturity at which the seed was harvested, among other factors. It is important to know these variations, because water content is crucial to the long-term survival of

stored seeds, as it affects the rate of metabolic and deterioration reactions (Vertucci and Roos, 1990).

5.3 Desiccation and germination trials on species

5.3.1 *Garcinia kola* and *Garcinia afzelii*

The relationship between seed moisture content and germination capacity revealed that fresh *Garcinia kola* seeds and seed parts with moisture content 58.0% will germinate to 80% (whole seed), and 85-95% (proximal and distal parts). Seed and seed parts gradually but significantly lost their viability as seed moisture reduced. Desiccation to relatively high moisture content (approximately 25-27%) reduced seed viability to about 10% or less. *Garcinia kola* can therefore be categorized as a recalcitrant seed. Geeta *et al*. (2006), reported that the Genus *Garcinia* (family Clusiaceae) are recalcitrant in storage behavior. Besides, the seed possesses most of the characteristics of recalcitrance such as having large seeds, the trees growing in the humid forest environment and fruits/ seeds being fleshy (Thomsen, 2000). Drying of seed or seed parts of *G. kola* in silica gel (SGD) or under shade (SD) at ambient temperature (27-30 ºC) did not significantly affect the critical moisture content (CMC) which is the lowest-safe moisture content of the species which was recorded to be between 30 and 32% in the present study. In all cases whether seed or seed parts were dried in silica gel (fast drying or under shade (slow drying), the critical moisture content (30-32%) was not affected. This might probably be due to the fact that the seeds as well as the seed parts were still too large in size to ensure a very fast drying rate using silica gel, even with the regular renewal of the drying agent.

For *Garcinia afzelii*, the relationship between seed moisture content and germination capacity revealed that fresh seeds with 31.5% seed moisture germinated 100%.

Desiccation of the seed either by silica gel or under shade at ambient temperature ((2730 ºC) resulted in the reduction of seed viability gradually with decreasing seed moisture content. At seed moisture contents of 23.3% (silica gel drying) and 23.6 % (shade drying), seed germination drastically reduced to 43% in both situations. *Garcinia afzelii* can therefore be categorized as a recalcitrant seed just like *Garcinia kola*. The critical moisture content (CMC) of *Garcinia afzelii* seed was found to be 25.3%.

Similar to *Garcinia kola*, the drying rate of seeds of *Garcinia afzelii* in silica gel (relatively fast) and under shade (slower rate), did not significantly affect the critical moisture content of *Garcinia afzelii*. This had been achieved with the excised embryos of some recalcitrant seeds since it is difficult to have a fast drying of large seeds (Thomsen, 2000).

King and Roberts (1980) suggested that seed death resulting from desiccation occurs at or below a critical moisture and is caused by membrane related physiological damages or an accumulation of by-products of biochemical enzymatic breakdown. Hanson (1984), postulated that in desiccation tolerant seeds, membrane permeability and structure remain intact during desiccation, while in the desiccation sensitive seeds some membrane dysfunction occur during desiccation. When fresh recalcitrant seeds begin to dry, viability is first slightly reduced as moisture is lost, but starts to decline considerably at a certain moisture content termed the "critical moisture content" (King and Roberts, 1980) or "lowest safe moisture content" (Tompsett, 1984). If drying continues further, viability is

eventually reduced to zero (Hong *et al*., 1996). Critical levels of moisture content vary greatly among species (Chin, 1988) and even among cultivars and seed lots (King and Roberts, 1979).

5.3.2 Seed desiccation and viability of *Terminalia superba:*

Terminalia superba seeds survived desiccation to moisture content of 5%. Drying the seed had little effect on germination levels and thus the species can be said to have an orthodox storage behavoiur as suggested by Hong *et al*. (1998). This findings however contrast with the views of Schaeffer (1990), who reported that seeds of tropical genus *Terminalia* are recognized as short-lived and recalcitrant and that both chemical and physical seed coat dormancy has been observed.

5.3.3 Seed desiccation and viability of *Entandrophragma angolense*

Entandrophragma angolense seeds were dehydrated from 13% to 4% moisture content using silica gel. Germinated seeds remained in the range from 75.3 ± 2.5 to 77.5 ± 2.9 percent. This indicated that there was no adverse effect of drying on germination of the species. Seeds of *E. angolense* are desiccation tolerant maintaining high viability after drying to 4% moisture content. Tompsett (1994), observed that the seeds of *E. angolense* tolerated desiccation to 5% moisture content and resulted in 76% germination following 150 days subsequent hermetic storage at 2°C.

5.3.4 Seed desiccation and viability of *Khaya anthotheca*

Khaya anthotheca seeds were dehydrated from 14% to 4% moisture content using silica gel. Germination percent was between 76.3 ± 2.5 to 77.5 ± 2.9, indicating that there was no adverse effect of drying on germination of the species. *Khaya anthotheca* seed can therefore be classified as desiccation tolerant. Joker (2003), reported that seeds of the species are tolerant to desiccation and should be dried down to a low moisture content (5-7%) and stored in an airtight container.

5.3.5 Seed desiccation and viability of *Mansonia altissima*

Seeds of *Mansonia altissima* were dehydrated from 13% to 4% moisture content using silica gel. Viability of the seeds were recorded to be between 68.8 ± 2.5 to 71.3 ± 2.8 percent, indicating that there was no adverse effect drying on germination of the species. It can be said that this species is not recalcitrant but orthodox in response to drying as suggested by Hong *et al*. (1998).

Hanson (1984), postulated that in desiccation tolerant seeds, membrane permeability and structure remains intact during desiccation, while in the desiccation sensitive seeds some membrane dysfunction occur during desiccation.

5.4 Prediction of drying periods from mean drying curves of species

Seeds of *Terminalia superba***,** *Terminalia ivorensis*, *Entandrophragma angolense* and *Khaya anthotheca* dried at an exponential rate until equilibrium moisture contents were reached. This is in line with the findings of (Rao *et al*., 2006).

Seed moisture content declined steeply during the initial stages of drying in silica gel and as seed moisture content reduced the rate of seed moisture losses also reduced. In other words, the drying rates of the seeds decreased with moisture content as observed by FAO (1994). This observation could be explained by what Vertucci and Farrant (1995), described as levels of hydration in seed tissues or the types of seed water. The moisture contents of all the species under consideration before they were dried were all above 20% corresponding to type 3 water in seeds (20-33%). This type of water is freezable and is easily lost by drying (Vertucci and Farrant, 1995). Hence the relatively faster rates of moisture loss from seeds at the beginning of drying. As drying progressed, the water present in the seeds entered the type 2 corresponding to water content between 7.5 and 20%. This water is tightly associated with macromolecular surfaces of the cells, its mobility is reduced and it constitutes the "bound "or unfreezable water which is difficult to remove by drying (Verticci and Farrant, 1995). When seeds were dried further, the water status entered the type 1 (corresponding to water content < 7.5%). This water strongly binds to macromolecules as structural component and maintains its integrity. This type of water is present in very dry seeds and it is very difficult to remove by further drying and that could be the reason for the slow rates of drying when the seed moisture content were approaching very low levels (Vertucci and Roos, 1993).

5.4.1 Germination pattern observed in *Garcinia kola* seed and seed sections

The pattern of germination described as 'garcinia-type' of seed germination by de Vogel (1980) in other *Garcinia* species including *Garcinia gummi-gutta* (Geeta *et al*., 2006) and in *Garcinia indica* (Malik *et al*., 2005) was observed in the present germination trials on *Garcinia kola*. The primary root (PR) emerged from the distal end of the seed whilst the shoot emerged from the opposite end called the proximal end (end towards the peduncle). The proximal extremity or end of the seed is slightly broader than the distal end (Agyili *et al*., 2007). Subsequently, prior to leaf differentiation, an adventitious root (AR) originated from the base of the shoot. The primary root (PR) disintegrated later and eventually the adventitious root took over as the main root system of the plant. In the present germination studies, it was observed that whole *Garcinia kola* seeds (WS), proximal sections (PS) , half of proximal sections (HPS) , proximal end cut- off sections (PEC), distal end cut -off sections (DEC) of the seed germinated and developed to complete seedlings.

On the other hand, distal sections (DS) and half of distal sections (HDS) of the seed developed only primary roots which soon degenerated to end the life of the potential seedlings. Middle section (MS) and half of middle section (HMS) of the seed produced neither roots nor shoots.

Malik *et al*. (2005), also reported that the middle segments of *Garcinia indica* failed to produce either shoots or roots but the proximal segments showed development of shoots and roots while the distal segments developed only roots. These observations from the germination patterns in *Garcinia kola* points to the fact that root- shoot polarity is exhibited by the embryo of the seed.

Geeta *et al*. (2006), reported of root- shoot polarity in *Garcinia gummi-gutta* seeds. Kumar and Rangaswamy (1977), also observed monopolar and bipolar pattern of seedling development in *Orabanche aegyptiaca* treated with certain growth substances.

The embryo in *Garcinia kola* seed is rudimentary at the time the seed is matured (Watson and Dallwitz, 2009. It is not well differentiated into cotyledons and embryonic axis. Rather, most of the seed is a mass of undifferentiated tissue (Agyili *et al*., 2006). An attempt to locate the embryo of the seed during the current experiment through tetrazolium testing (TZ) was not possible because no staining of the seed parts was achieved by this method (results of TZ test not presented).

It is clear *Garcinia kola* seed exhibits bipolar as well as monopolar pattern of seedling development. The bipolar mode of seedling formation, is that a shoot originates from the plumular pole and roots from the radicular pole when the complete/whole seed is used. The monopolar mode of germination is when both roots (adventitious) and shoots originate from the morphological plumular pole of the embryo (the proximal end) when the proximal fragment/section of the seed is set for germination.

One marked difference between the germination pattern observed in *Garcinia kola* in the present study and in *Garcinia gummi-gutta* as carried out by Geeta et *al*. (2006) is that in *Garcinia kola*, the Middle section (MS) and half of middle section (HMS) of the seed produced neither roots nor shoots but in *Garcinia gummi-gutta* seedlings were produced from the middle sections of the seed. Malik *et al*. (2005) reported that the middle fragments of *Garcinia indica* would not develop roots nor shoot, an observation similar to the situation observed in *Garcinia kola*.

5.4.2 Effect of chemical pretreatment and temperature on root emergence from distal sections of *Garcinia kola* seeds

The combined effect of GA_3 (500mg/l) as chemical pre-treatment and the incubation temperature of 30°C to give a significantly higher rooting percentage than the other treatments including GA_3 (1000mg/l) at 30°C indicate that GA_3 is a strong rooting stimulating chemical compared with the others and that it is required in smaller concentrations. Abu-Shakra *et al*. (1970), observed that presoaking of seeds of *Orabanche ramosa* in GA_3 solution (100mg/l) resulted in the highest germination compared with other chemicals. Better stimulation of seed germination and root growth by GA_3 have also been reported for citrus, lettuce and douglas fir (Johri and Varner, 1967; Van Overbeek, 1966; Rangaswamy, 1961).

The highest cummulative root emergence was recorded at 30°C followed by cummulative root emergence at 25 and 35°C. Cummulative root emergence at 20°C was the least among temperatures where rooting was recorded. Distal sections of *G. kola* incubated at temperatures between 5 to 15°C showed no response to root development. The observation that no root emergence occurred at temperatures between
5 to 15°C but rather at 20°C to 35°C was not unexpected as *G. kola* is a tropical species. For most tropical tree seeds, temperatures of 25 to 30°C as exist in the tropics will be quite suitable for maximum germination (Smith *et al*., 2002).

5.4.3 Responses of Proximal Sections, Half Proximal Sections, Proximal End Cut Sections and Distal End Cut Sections of *Garcinia kola* seed to germination temperatures

Performances of the various seed sections namely: proximal section (PS), half proximal section (HPS),proximal end cut (PEC), and distal end cut (DEC) at 25, 30 and 35°C in terms of germination percentages were superior to their performance at 20°C. None of the seed parts germinated at 5, 10 and 15°C. This observation was also made in the experiment on distal section of the seed and rooting temperatures. Germination at these temperatures (25-35°C) or even higher is typical for many tropical species (Tompsett and Kemp, 1996; Smith *et al*., 2002). This is probably explained by the fact that in the natural environment, dispersed seeds are likely to experience soil temperatures of 36°C or more (Daws *et al*., 2002). It can, therefore, be concluded that whether seed fragments of *G. kola* will only produce root or produce root as well as shoot they will require similar temperature conditions for optimum root development as well as shoot development.

5.4.4 Germination temperature and period of germination on cumulative germination percentage of *Garcinia kola* seed sections

Cummulative germination percentages recorded weekly for *G. kola* seed parts over a period of 8 weeks revealed that from the first week to the eighth week cummulative germination at the temperatures of 25 and 30°C were consistently significantly higher than what was recorded at 35°C and lowest at 20°C. This has established the fact that G. *kola* seed parts gave higher weekly germination at 25 and 30°C and that in a venture to raise large

quantities of *G. kola* seedlings, seed parts should be incubated at these two temperatures (25 and 30°C and to a lesser extent at 35°C but not at 20°C which consistently gave the lowest cummulative germination percentage over the period.

5.4.5 Responses **of seed sections (seedling producing sections) of *Garcinia kola* to germination temperature and chemical pre-treatment**

All the chemical pre-treatments applied significantly enhanced the germination of *G. kola* seed part at 25 and 30°C and to a lesser extent at 35 and 20°C compared to the control. This is an indication that *G. kola* seed parts responded to the chemical treatments during the period of the experiment. Pre-treatment of eastern red cedar seed with citric acid increased both speed of germination and total germination (Cosimo, 1963). Hare (1981), found out that germination of a number of recalcitrant seed lots of loblolly, slash and longleaf pine was improved markedly following treatment with 0.1 percent nitric acid. Batchelard (1978), reported that the germination of dormant seeds of *Eucalyptus delegatensis*, *E. fastigata* and *E. regans* could be improved by 24 hours' immersion in gibberellic acid (GA_3) at the concentrations of 50 and 100 milligrams per litre. Agyili *et al*.(2007), reported that soaking *G. kola* seeds in 70% ethanol solution boosted germination. .

5.4.6 Relationship between fruit, seed moisture contents and other vigour parameters of *Garcinia afzelii* dried under shade at room temperature (27-30°C)

The observation that seed moisture content reduced with reduction in fruit moisture content during drying is a normal occurrence as water is lost from the fruit and seed simultaneously during drying, though, the rate of moisture lost from the two may differ. There is a strong correlation between seed moisture content, germination and vigour in recalcitrant seeds under which *G. afzelii* falls (Raja *et al.,* 2001; Pritchard *et al*., 1995). In the present investigation, it was observed that reduction in speed of germination, seedling vigour and vigour index was associated with seed desiccation in *G. afzelii*. Raja *et al*. (2001); Bhattacharyya and Basu (1992) also observed decreased viability and seedling vigour during the desiccation of recalcitrant jackfruit and avocado seeds.

Conversely, seed vigour may increase during the early stages of drying in recalcitrant seeds, for example in lychee (*Litchi chinenses*) and longan (*Dimocarpus longan*) (Xia *et al*. (1992) and in horse chestnut (*Aesculus hippocastanum*) (Pritchard and Tompsett, 1995). The present study also revealed a similar trend in which the seedling dry weight and vigour of seedlings of *G. afzelii* seeds increased with desiccation up to a certain level and then decreased.

5.5 Media, temperature and chemicals on seed germination of seeds

5.5.1 Germination media and chemical pre-treament on the germination of *T. superba*

Normally seeds of *Terminalia superba* do not pose any problems in seed germination (Roederer, 1988). However, the present investigation has revealed that chemical pretreament of the seed with K_2SO4 (1g/l) + GA_3 (200mg/l) interacted with the germination media to significantly enhance germination on agar and seed testing paper compared to its performance in soil and the control experiment. The significantly higher germination recorded on agar compared to germination on seed testing paper and in soil in the control, coupled with their better performance on agar when seeds were chemically pre-treated is an indication that agar is a better medium for germinating *T. superba* seeds compared with seed testing paper and soil.

5.5.2 Temperature and chemical pre-treatment on the germination of *T. superba*

T. superba seed pre-treated with K_2SO4 (1g/l) + GA_3 (200mg/l) and incubated at 30 and 35°C germinated significantly higher than pre-treated samples incubated at 25°C This has reiterated the fact that the species is a typical tropical plant which requires a relatively higher temperature for optimum germination. Germination at these temperatures (25-35°C) or even higher is typical for many tropical species (Smith *et al*., 2002; Tompsett and Kemp, 1996). This is probably explained by the fact that in the natural environment, dispersed seeds are likely to experience soil temperatures of 36°C or more (Daws *et al*. 2002).

Another important observation made is that at 25°C, *T. superba* seeds will not germinate unless it was pre-treated with chemicals. The chemical pre-treatment probably enhanced germination at 25°C by lowering the threshold temperature of the species below which germination will not occur. Germinating the species at 35°C may not require the use of any chemical pre-treatment since both the pre-treated and the control samples gave significantly high germination percentages at this temperature.

5.5.3 Chemical pre-treatment and germination media on mean germination time (MGT) of *Terminalia superba.*

Mean germination time is an indication of the spread of germination of a seed lot. On agar it took significantly less days (19.1 days) to complete germination at 25°C compared to 23.4 and 21.9 days at 30 and 35°C in the chemical pre-treament experiment. The 84% germination recorded with the mean germination time of 21.9 days at 35°C was, however, significantly higher than the 70% germination recorded with the mean germination time of 19.1 days at 25°C. Therefore, it must be noted that certain germination conditions may give minimum mean germination time but not necessarily the highest germination percentages. On the other hand when soil was used as medium, there were no significant differences between mean germination times recorded at temperatures 25, 30 and 35°C. Significant differences in the germination percentages were however, recorded at these mean germination times.

The highest of 86% was recorded at 35°C. It must be noted that at 35 °C with soil as media under the control experiment a significantly lower mean germination time (18 days) was used to achieve the same level of germination as when seeds were chemically pre-treated and germinated at 35°C on soil. It can be concluded that germinating *T.*

superba at 35°C may not require the use of any chemical pre-treatment since both the pre-treated and the control samples gave significantly high germination percentages.

5.5.4 Germination of *Terminalia ivorensis* seeds pre-treated with chemicals

The chemical pre-treaments applied to *Terminalia ivorensis* seeds significantly enhanced germination especially at 30°C and to some extend at 25 and 35°C compared to the control. This is against the background that *T. ivorensis* seeds are very difficult to germinate due to the presence of thick seed coat which is lignified (Corbineau and Côme, 1993). The significant enhancement in this "difficult to germinate" seed by the various chemical pre-treatments especially the GA_3 could partly be due to the fact that the seed samples were sufficiently matured and therefore the embryos had reached their maximal development. Corbineau and Côme (1993) reported that GA_3 at high concentrations significantly facilitated the germination of complete seeds, if the seeds were sufficiently matured. Comparing *Terminalia ivorensis* and *T. superba* it shows the two species achieved their maximum germination at 30°C and 35°C respectively.

5.5.5 Temperature and germination of whole and decoated seeds of *T. ivorrensis*

Results indicated that decoated seeds of *Terminalia ivorensis* germinated to a significatly higher percentage and much more easily than whole/complete seed. This was probably because the hard and lignified seed coat which possibly served as a barrier to germination was absent. Corbineau and Côme (1993), had also reported that

seeds of the species with removed testa performed much better than un-cut seeds. The performance of the decoated seeds at the various temperatures was similar to the performance of whole seed pre-treated with chemicals with the maximum germination

occuring at 30°C followed by germination at 25 and 35°C. At 20°C, significantly lower germination percentages of decoated seeds extracted from fully matured brown seed (FMB) and matured green seed (MSG) were recorded. No germination of whole seed (WSB) and decoated seeds extracted from immature green seed (IMG) occurred. This affirms the fact that the optimal germination temperature of tropical species is generally relatively high (Daws *et al*. 2002). Tompsett and Kemp (1996), found the optimum germination temperatures of two other *Terminalia* species namely: *T. brassii* and *T. calamansanai* to be 31°C.

5.5.6 Germination media and temperature on germination percentage and mean germination time of *Khaya anthotheca*

Khaya anthotheca germinated significantly better on agar and in soil than on seed testing paper. The seeds germinated equally well over the temperature range of 2035°C. Germination percentages recorded at 20, 25, 30 and 35°C for *K. anthotheca* were not significantly different from each other. Germination above 85% was recorded at each of these temperatures without any chemical pre-treatment. Jøker (2003), reported that freshly harvested seeds of *K. anthotheca* will normally germinate 60-90%. It is obvious that *K. anthotheca* seeds germinate over a wider range of temperature (20 to 40°C) compared to *T. superba*, *T. ivorensis*, *E. angolense* and *M. altissima.*

Despite the observation that there were no significant differences in the germination percentages at 20, 25, 30 and 35°C, these germination temperatures and germination media interacted to give significant differences in mean germination times. The minimum

mean germination times on all the germination media were recorded at 30°C followed by 35°C and 25°C. Even though percentage germination of the seed at 20°C was similar to the levels at 25, 30 and 35°C, mean germination times at 20°C on all the germination media were longer. Thus it will be prudent to incubate the seeds at 25, 30 and 35°C to achieve higher germination rates within fewer days and not at 20°C.

5.5.7 Germination media and temperature on percentage germination and mean germination time of *Entandrophragma angolense*

At the germination temperature of 30°C, all the seed samples of *E. angolense* germinated to the same percentage on all the three germination media with no significant differences among them. Germination at this temperature (30°C) on the three germination media as well as at 35°C on agar was significantly higher than at the other temperatures. Thus germination on any of the three media at 30°C and on agar at 35°C could conveniently be adopted for the species. In addition germination on any of the media at 25°C could also be adopted since the seeds germinated very well on all the media. At 35°C, *E. angolens**e*** could also be germinated in soil and on seed testing paper with good levels of germination. At 40°C, germinating the species on any of the three media will be a complete failure since no seed germinated under these conditions. Similar to the other tropical species, *E. angolense* germinated well at relatively higher temperatures of between 25-35°C (Smith *et al*., 2002). Tompsett and Kemp (1996), also reported that germination recommendations for the species are within the range of 26- 31°C.

The minimum mean germination time for *E. angolense* was achieved at 35°C on all the three media and in soil at 30°C. Germination at 25°C on all the media and at 30°C on

agar as well as on seed testing paper also resulted in appreciable minimum mean germination times. Even at 20°C where mean germination time was maximum, the longest mean germination time which was recorded on agar was less than 14 days. This implied that *E. angolense* germinates very well and at a fast rate on agar, soil and seed testing paper at temperatures between 20-35°C.

5.5.8 Germination media and temperature on seed germination and mean germination time of *Mansonia altissima*

The best germination condition for *Mansonia altissima* was at 30°C using agar and soil as media. The species also germinated relatively well at 20°C and 25°C on agar and in soil. It appeared seed testing paper was not a suitable germination media for *M. altissima* as the seed performance on this media at temperatures 20-30°C was significantly much lower compared to the use of agar or soil as media. However, at 40°C, where zero germination was recorded on agar and in soil, some level of germination was achieved on seed testing paper similar to what was recorded at 20 and 25°C on seed testing paper and at 35 °C. The argument can therefore be developed for germinating the seed at 40°C using seed testing paper rather than the use of agar or soil.

The minimum mean germination times for the germination of the seed were achieved at 30°C on agar and in soil. At 20°C in soil and at 20°C and 25°C on agar, mean germination times were relatively low, compared to the levels of germination. recorded. The present study has revealed that the optimal germination temperature of *Mansonia altissima* is between 20-35°C. This is in line in with the reports of Daws *et al*.

(2002); Tompsett and Kemp (1996) on the germination temperatures of tropical species.

5.6.1 Germination of *Terminalia superba, T. ivorensis* and *Khaya anthotheca* on the Thermogradient plate.

The thermogradient plate experiment has revealed that *Terminalia superba* and *Terminalia ivorensis* seeds will not germinate at low constant temperatures and low alternating temperature. Seed of the two species will also germinate poorly at alternating temperatures, one very low and the other being less than 35°C (< 30°C in the case of *T. ivorensis*). It was expected that seeds of *T. superba* and *T. ivorensis* will not germinate under very low temperature conditions whether constant or alternating, considering the fact that they are typical tropical species. According to Bonner *et al*. (1994), it is seeds of temperate species that can germinate at very low temperatures from 2 or 3 °C. Northern red oak (*Quercus rubra* L.) germinated at 1°C in a trial reported by Godman and Mattson (1980). The Australian Tree Seed Centre and Mortlock (1999), gave the lower temperature limit at which most tropical species tend to germinate better as 25°C.

It is evidently clear that germinating at alternating temperatures greatly boosted germination of *T. superba*. The constant temperatures of 25/25°C; 30/30°C and 40/40°C gave 0%, 30% and 45% germination respectively. The highest germination percentage recorded at a constant temperature was 88% at 35/35°C. Very high germination percentages were recorded at several alternating temperatures like 15/35°C (90%); 20/35°C (90%); 30/35°C (88%); 35/15°C (95%); 35/25°C (85%); 35/40°C (100%) and 40/25 (95%).

According to Schaeffer (1990), seeds of tropical genus *Terminalia* are recognized as short-lived and that both chemical and physical seed coat dormancy have been observed.

Even though Roederer, (1988), had reported that *T. superba* has no problems in seed germination, Corbineau and Côme (1993), reported that *T. ivorensis* seeds germinate with great difficulty due to the presence of a lignified seed coat. BrookmanAmissah (1973), had also reported that chemical dormancy apparently occurs in *Terminalia ivorensis*.

Germinating at alternating temperatures greatly enhanced the germination of *Terminalia ivorensis* similar to that of *T. superba*. For instance, alternating temperatures: 15/35°C, 20/35°C, 35/40°C and 40/30°C gave very good germination percentages above 73 % in *T. ivorensis* (considering the great difficulty which is usually encountered when the seed is being germinated). Alternating temperatures are preferred to constant temperatures because they can overcome shallow seed dormancy and enhance uniform germination (Willan, 1985). In Nigeria, alternating temperatures of 34°C and 24°C gave 93% germination in 41 days compared with 27% at a constant temperature at 30°C both with continuous light (Okoro, 1976). Willan (1985), reported that alternating temperatures of 20°C and 30°C are commonly prescribed for tree species. Bonney (1994), had reported that alternating day/night temperatures (when germinating seeds from semi-arid and arid areas of South Australia) play a large role in softening hard

seed. Grose (1962), however found little differences in germination using constant or alternating temperatures; but the rate of germination was slower under alternating temperatures.

In the present study, germinating *Terminalia ivorensis* at a constant temperature of 30/30°C gave 87% germination which is equal in magnitude to the highest germination percentage recorded at an alternating temperature (40/30°C).

The thermogradient plate experiment also revealed that like *Terminalia superba* and *Terminalia* ivorensis, seeds of *Khaya anthotheca* would also not germinate at low constant temperatures and low alternating temperatures. *K. anthotheca* germinated very well at alternating temperatures, one very low (5 and 10°C) and the other at 30°C compared to the very low germination when the higher alternating temperature went up to 35°C and beyond . It is evident that amplitude of change between day and night of 20 to 25°C and not more was good for germination of the seed. Germination of many temperate species will occur at a wide range of temperature regimes, and that amplitude of change between day and night of 10 to 12°C may be more important than the cardinal points (Bonner, 1983).

Germination of *K. anthotheca* was greatly enhanced by alternating temperatures compared to germination percentages recorded at constant temperatures even though the species is not known to have any dormancy problem. Germination at alternating temperatures such as 5/30°C; 10/30°C; 15/30°C; 20/25°C; 20/35°C; 25/15°C; 25/20°C; 25/30°C; 30/5°C; 30/10°C; 30/15°C; 35/10°C; and 35/15°C among others gave between 90 and 100% germination. Between each of these alternating temperatures, amplitude of change between day and night was not more than 25°C.

5.6.2 Water Sorption Isotherms (Moisture Release) Curves of the Species

Conservation of genetic resources of forest trees in Africa is a challenging task because of limited information available. In seed gene banks, temperature and relative humidity are the most prominent factors determining seed water content and therefore directly influence seed longevity during storage (Walters 1998; Vertucci and Roos, 1993). The

importance of these factors made it essential to understand the interaction among storage parameters in order to develop optimal seed storage protocols. Seeds are generally hygroscopic and will equilibrate according to the water potential gradient between the seed and the surrounding air (Roberts and Ellis, 1989). Lowering the relative humidity or increasing the temperature decreases the water potential of the air, thus there is a net water movement out of the seeds and lower water content is reached (Walters 1998; Roberts and Ellis, 1989).

In the present study, moisture sorption isotherm curves were developed for the species under consideration in order to study the behaviour of water in their tissues (Baldet *et al*., 2008). *Garcinia kola* seed samples placed at all relative humidity chambers from 3% to 93% lost fresh weight). This implied that there was a net loss of seed moisture (desorption) to the environment (Relative humidity chambers). This observation could be explained by the fact that all seeds are hygroscopic and automatically absorb or desorb moisture by diffusion along a water potential gradient between the seed and the surrounding air. If the water potential of the seed is greater than the surrounding air, the seed will lose water and become drier (Probert, 2003). In this sense, *Garcinia kola* seeds placed at all RHs lost moisture to their environments due to their initial high moisture content (approximately 58%), consequently, their greater water potential than the water potential in the environment (relative humidity chambers).

Seeds of *Terminalia superba, T. ivorensis*, *Khaya anthotheca*, E*ntandrophragma angolense* and *Mansonia altissima* placed in the various relative humidity chambers either lost weight (desorption) or gained weight (adsorption) depending on the relative chambers they were placed in and the seed water potential.

The behavior of moisture sorption isotherms can be illustrated by the relationship between equilibrium moisture content storage temperature and relative humidity. Sorption isotherms can be used to estimate which moisture content seeds can be dried in a given environment (Thomsen and Stubsgaard, 1998), and is essential for developing optimal seed storage protocols (Merritt *et al*., 2003).

The moisture sorption isotherms curves generated for the species showed that seed moisture content increases with increasing relative humidity. Moisture sorption isotherm curves of *T. superba*, *T. ivorensis*, *E. angolense*, *K. anthotheca* and *M. altissima* have been divided into three regions and exhibited a reverse sigmoidal shape characteristic of orthodox species (McDonald and Copeland, 1996), but they vary from species to species due to differences in composition (Vertucci and Roos 1993; Vertucci and Leopold 1987). The three regions of an isotherm curve have been classified by the strength with which water is bound and the nature of the binding site: water which is tightly bound to ionic groups (Region 1), weakly bound to polar nonionic sites (Region 2), and very weakly arrayed as bridges over hydrophobic moities (Region) (Rupley *et al*., 1983).

In the present study, the isotherms for *T. superba*, *T. ivorensis*, *E. angolense*, *K. anthotheca* and *M. altissima* are consistent with those of native species of Australia generated by Merritt *et. al.* (2003), having inflection points at approximately 13-25% RH for the boundary of sorption zones 1 and 2 and another at 60-80% RH for the boundary of sorption zones 2 and 3 at 20°C, the temperature at which the isotherms were generated. Water in sorption zone 1 is thought to be important in maintaining the structural integrity

of membranes (Walters, 1998); thus removal of this water may have detrimental effects on membrane structure and function (Walters, 1998).

5.6.3 The shape of moisture sorption isotherm curve of *Garcinia kola*

The fact that *Garcinia kola* trees grow in humid ecosystems; the fact that the fruit/seeds are shed at the time when they have very high moisture content (Agyili *et al*., 2007); the fact that the fruits and seed are large and fleshy (Thomsen, 2000); the fact that seed viability of the species reduces drastically when seed moisture content falls below 30% (from the present study) among others, are points which place *Garcinia kola* into the desiccation intolerant category so far as seed storage physiology is concerned.

According to Vertucci and Leopold (1987), in the desiccation intolerant condition isotherms were more hyperbolic indicating that the water binding differs between plant tissues that are intolerant versus those that are tolerant to desiccation. Isotherms of hyperbolic form (monotonic isotherms) have also been reported for some recalcitrant seeds including cacao and acorn (Leopold and Vertucci, 1986). According to the Langmuir sorption theory, these monotonic isotherms indicate that there is little or no contribution to the water absorption characteristics by the strong binding sites (Atkins, 1982; D' Arcy and Watts, 1970).

In the present study, however, the shape of the sorption isotherm developed for *G. kola* described as recalcitrant (Agyili *et al*. 2007) and in the present study appear to possess the sigmoidal character which is typical of desiccation tolerant species (Vertucci and Leopold, 1987). It is not certain the maturity stages of the cacao and acorn seeds used by Vertucci and Leopold (1987) to develop the isotherms for the two species which the

authors described as hyperbolic in shape. The stage of maturity of seeds has a marked effect on the level of desiccation sensitivity (Finch-Savage and Blake, 1994). Immature seed tissues tend to be more desiccation intolerant than mature tissues of the same species (Aldridge and Probert, 1993). In the present study, *G. kola* seeds which were used in developing the isotherm curve were fully matured. This probably may have influenced the shape of the curve. Another possible reason for the contradiction between the shapes of the isotherm developed for *Garcinia kola* on one hand in the present study and that for cacao and acorn as observed by Leopold and Vertucci (1986), is that, probably not all desiccation intolerant species (recalcitrant species) possess strong binding sites that do not contribute to water absorption as proposed by the Langmuir sorption theory. Probably in the tissues of *G. kola* seeds, the strong binding sites contribute strongly in water absorption just like it happens in the tissues of desiccation tolerant species. Kapseu *et al*. (2006), observed that sheanut kernels (*Vitellaria paradoxa*) which also have recalcitrant storage behavior, losing viability below 20% moisture content, as observed by Gaméné *et al*. (2004), showed the typical sigmoid shape curve similar to that of orthodox species and the curve developed for *G. kola* in the present study.

5.7 Electrical conductivity measurement of leachate from desiccated *Garcinia kola* seed

Measurement of electrical conductivity of leak water from imbibing tree seeds can be used as a vigour test (Sørensen *et al*., 1996). Seeds of many tropical and temperate plant species do not survive dehydration (Chin and Roberts, 1980). It is known that the germination of desiccation-sensitive seeds declines rapidly as seed moisture content is decreased (Becwar *et al*., 1982). The seed of *G. kola* is an example (Agyili *et al*., 2007).

It has been suggested that water uptake by desiccation-tolerant seeds reinstates the original structure of the cellular membranes, whereas membranes of desiccationsensitive seeds that have been dehydrated are unable to reform completely (McKersie and Stinson, 1980). If dehydration stress disrupts membrane integrity in desiccation sensitive seeds, then changes in the leakage rates and increases in the amount of solutes leaked may be detectable in response to dehydration, and these changes should be associated with loss of viability (Becwar *et al.,* 1982).

The dehydration and electrical conductivity measurements on *G. kola* has indicated that seeds of *G. kola* as they were dehydrated from the moisture content of 50% to lower moisture content, and were soaked in water released more electrolytes into solutions. This reflected in the higher electrical conductivity measurements recorded with reduction in seed moisture content. The rate of increase in leakage from hour to hour as seeds were soaked increased gradually from the initial moisture content of 50% till after 35% seed moisture content. At the moisture content of 30% (which has been established as the 'critical moisture content' for the species from the present experiment) and below, hour to hour increases in solutes leakages as indicated by the electrical conductivity values were drastic. The results from this study have shown that cellular membranes of desiccation sensitive *G. kola* seeds were damaged as seeds were dried further and for that matter increases in the levels of solute leakages observed. Excessive dehydration of the seeds beyond the ''critical content moisture'' severely disrupted the integrity of the cellular membranes of seed tissues resulting in the uncontrollable rate of solute losses from the seed.

The results from the present study are complementary to findings made by Becwar *et al*. (1982) on desiccation sensitive silver maple (*Acer saccharinum* L.) and areca palm (*Chrysalidocarpus lutescens* [Bory] Wendl.). The authors reported from their studies that desiccating silver maple and areca palm seeds below their critical moisture contents of 40% and 55% respectively resulted in massive solute leakage as membranes were no more effective barriers to solute leakage during imbibitions.

The results from the study on dehydration and solute leakage in *Garcinia kola* seeds could also be used to draw a conclusion that there is a close correlation between increased leakage and loss of seed viability and therefore seed quality (Sørensen *et al*., 1996 ; Becwar *et al*., 1982).

Results clearly indicated that seed weight significantly affected electrolyte leakage from desiccated *G. kola* seeds as reported by Hepburn *et al*. (1984). Electrolyte leakage and for that matter electrical conductivity measurement increased as seed weight increased. This observation could only be possible if there is enough de-ionized water to ensure the full submersion of the electrodes as well as to receive all the leachate from imbibing and leaking seeds. The larger the seed, the more potential electrolytes can be leaked into the water from imbibing seeds. Another factor which is possible to disprove this statement is when there are variations in the volume of de-ionized water in the various trays receiving the leachates from imbibing seeds. Trays that contain less amount of deionized water could reduce the potential of seeds to release electrolytes into solution which could result in inaccurate reading as the electrodes will not be fully submerged.

LIST OF ABBREVIATIONS

Abscisic acid	ABA
Adventitious root	AR
Association of Official Seed Analysts	AOSA
Centimetre	cm
Coefficient of variation	cv
Danida Forest Seed Centre	DFSC
Degree Celcius	°C
Degrees of freedom	df
Deoxyribonucleic acid	DNA
Distal end cut off	DEC
Distal section	DS
Endoplasmic reticulum	ER
Equilibrium relative humidity	eRH
Et alii (and so on)	*et al.*
Food and Agriculture Organization	FAO
Forestry Research Institute of Ghana	FORIG
Fresh weight	f. wt.
Fully matured brown seed	FMB
Gibberellic acid	GA_3
Gram	g
Gram per litre	g/l
Gram per gram	g/g
Half of distal section	HDS
Half of middle section	HMS
Half of proximal section	HPS
Immature green seed	IMG
International Board for Plant Genetic Resources	IBPGR
International Institute of Tropical Agriculture	IITA
International Plant Genetic Resource Institute	IPGRI
International Seed Testing Association	ISTA
Late embryogenic accumulation/abundant proteins	LEA's
Matured green seed	MSG
Mean germination time	MGT
Mega Pascal	MPa
Microsiemens	µS
Middle section	MS
Millennium Seed Bank Project	MSBP
Milligram per litre	mg/l
Millilitre	ml

Millimetre	mm
Moisture content	MC
Nitrogen	N
Part per million	ppm
Percentage	%
Primary root	PR
Distal and proximal ends cut off	DPEC
Proximal end cut off	PEC
Proximal section	PS
Regional Soil Conservation Unit	RSCU
Relative humidity	RH
Ribosomal ribonucleic acid	rRNA
Standard error of the difference	s.e.d
The World Conservation Union	IUCN
United Nations Environment Programme	UNEP
Vigour index	VI
Voltage	V
Wet mass basis	wmm
Whole seed	WS

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3.0 MATERIALS AND METHODS

3.1 Collection and Processing of Seed Samples

Seven important indigenous species in Ghana namely; *Garcinia kola* (Tweapea), *Garcinia afzelii* (Nsokodua), *Terminalia superba* (Ofram) *Terminalia ivorensis (*Emire*), Khaya anthotheca* (Dubini), *Entandrophragma angolense* (Edinam) and *Mansonia altissima* (Aprono) were selected for the desiccation and germination studies in the United Kingdom and in Ghana. Seeds were harvested at full maturity for two seasons from October to December 2005 and 2006.

3.1.1 Collection and processing of *Garcinia kola* seeds.

Garcinia kola seeds were obtained from trees at Amantia in the Ashanti Region of Ghana in October 2005 and 2006. Matured fleshy fruits of the species were processed by depulping soon after collection to avoid fermentation and heating as recommended by Willan (1985). Fruits were placed in a can with water for 3 days for the pulp to soften. The fruits were then mashed carefully with a wooden block without crushing the seeds. Plenty of water was then added for the pulp to float while the seeds sunk to the bottom of the can (Seeber and Agpaoa, 1976). Seeds were picked and were rubbed with a clean cloth to remove any pieces of remaining pulp (Figure 3.1a, Panel A). Seeds were then packed in cotton bags filled with moistened sawdust to prevent loss of seed moisture. In order to establish the exact shedding moisture contents of seed and seed parts of *G. kola*, samples to be used for the initial moisture content tests were depulped without seeds being soaked in water.

3.1.2 Collection and processing of *Garcinia afzelii* seeds

Garcinia afzelii seed were harvested from trees at Ho in the Volta Region in January 2005. Matured fleshy fruits of the species were processed by depulping soon after collection to avoid fermentation and heating as recommended by Willan (1985). Fruits were placed in a can with water for 2 days for the pulp to soften. The fruits were then mashed carefully with the hands without crushing the seeds. Plenty of water was then added for the pulp to float while the seeds sunk to the bottom of the can (Seeber and Agpaoa, 1976). Seeds were picked and rubbed with a clean cloth to remove any pieces of remaining pulp. Seeds were then packed in cotton bags filled with moistened sawdust to prevent loss of seed moisture. In order to establish the exact shedding moisture contents of seed and seed parts of *G. afzelii*, samples to be used for the initial moisture content tests were depulped without seeds being soaked in water (Fig. 3.1b, Panel C)

3.1.3 Collection and processing of *Terminalia superba* and *Terminalia ivorensis* seeds

These species were harvested from trees at Gambia No. 1 in the Brong Ahafo Region in November 2005 and 2006. Seeds were spread on wheat sacks and cleaned of twigs, bark, foliage and other impurities (Turnbull, 1975). They were then shade-dried for 3 days and packed into cotton bags (Figure 3.1a, Panels C and D).

3.1.4 Collection and processing of *Mansonia altissima* seeds

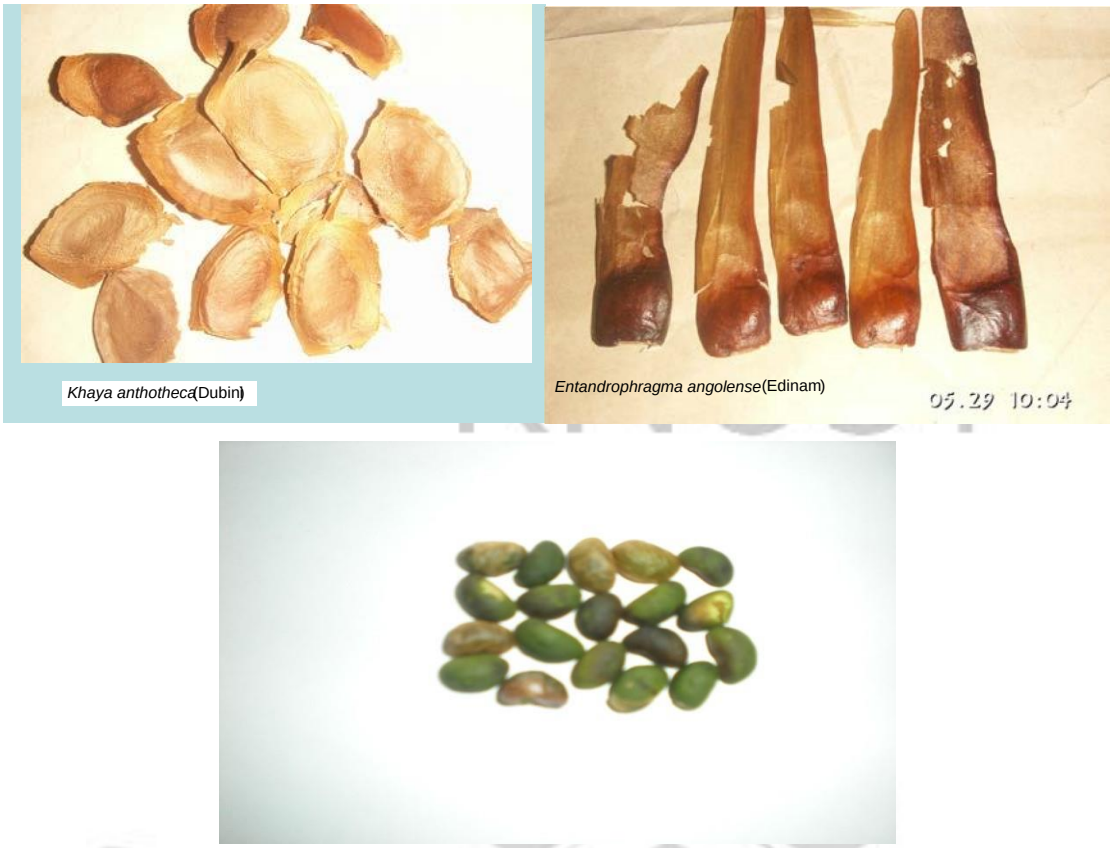
Mansonia altissima seeds were harvested from Dormaa Ahenkro in the Brong Ahafo Region in January. Harvested fruits were sun dried for 2 days on wheat sacks after which shells covering the seeds were gently cracked with the hand to take out the seeds. They were then packed into cotton bags (Fig. 3.1a, Panel B)

3.1.5 Collection and processing of *Khaya anthotheca* and *Entandrophragma angolense* seeds

Khaya anthotheca and *Entandrophragma angolense* seeds were collected from trees at the Kwahu Mountains in the Eastern Region of Ghana in December 2005 and 2006. Harvested fruits (capsules) were spread on wheat sacks in the sun for capsules to split open (Turnbull, 1975). Seeds were removed from open capsules, cleaned of all debris (Willan, 1985) and shade dried for 2 days after which they were packed in cotton bags (Figure 3.1b, Panel A and B).

The first season's samples of all the species were immediately sent by air to the Seed Conservation Department of the Royal Botanic Gardens, Kew, in the United Kingdom where part of the experiment was carried out. The second season's collection was used for the second part of the work at the Forestry Research Institute of Ghana (FORIG), in Kumasi, Ghana.

FIGURE 3.1a: Sample pictures of four of the seed species on which the desiccation and germination studies were conducted. **Panel A**, seeds of *Garcinia kola* (Tweapea). **Panel B**, seeds of *Mansonia altissima* (Aprono). **Panel C**, seeds of *Terminalia superba* (Ofram). **Panel D**, seeds of *Terminalia ivorensis* (Emire).



***Garcinia afzelii* (Nsokodua)**

FIGURE 3.1b: Sample pictures of three of the seed species on which the desiccation and germination studies were conducted. **Panel A**, seeds of *Khaya anthotheca* (Dubini). **Panel B**, seeds of *Entandrophragma angolense* (Edinam). **Panel C**, seeds of *Garcinia afzelii* (Nsokodua).

3.2 Moisture content determination

Moisture contents of seeds were determined gravimetrically by weighing the following samples:

(i) 5 gram sample each in four replications of whole seeds; 5 gram sample of seeds with testa removed in four replications and testa extracted from 5 seeds in four replications of *Terminalia superba*, *Terminalia ivorensis*, *Entandrophragma angolense* , *Khaya anthotheca* and *Mansonia altissima*,.

(ii) For *Garcinia afzelii*, moisture content was determined using 5 whole fruits in four replications; mesocarp extracted from 5 fruits in two replications; 5 whole seeds in four replications as well as the testa extracted from 5 seeds in four replications. (iii). in the case of *Garcinia kola* moisture content was determined using 5 whole fruits in four replications; mesocarp extracted from 5 fruits in two replications; 5 whole seeds in four replications, and testa extracted from 5 seeds in two replications. Samples were dried in an oven at 103ºC for 17 h (ISTA, 1999) and cooled after drying for 45 mins to 1hr in a dessicator (over silica gel). Dried samples were weighed again and moisture contents expressed as a percentage of fresh weight (f.wt.)

3.3 Equilibrium Relative Humidity (eRH) of seed samples

The equilibrium relative humidity (eRH) or water activity (aw) of seed samples were monitored soon after their arrival in the United Kingdom and also after some days of desiccation (11 to 30 days) in silica gel using a Rotronic AWVC-DIO sensor manufactured by Rotronic Ltd. of the United Kingdom shown in Figure 3.2. The essence of this experiment was to measure the relative humidity of air above the seed samples. Samples drawn from the species were placed in the WP40 sample chambers of the instrument to cover a minimum of 20% of the volume as recommended by the Millennium Seed Bank Project (MSBP) (2002). The water activity probes 1 and 2 of the application were then inserted into the sample chambers and the clamp sealing

mechanism of the AW DIO probes were put in place to prevent external conditions from influencing the samples. Measurements were logged on a laptop computer using the HW3 software. The set up was allowed to run for at least 30 minutes whenever measurement was being taken for seeds to reach equilibrium (when a stable result had been obtained) and the readings recorded.

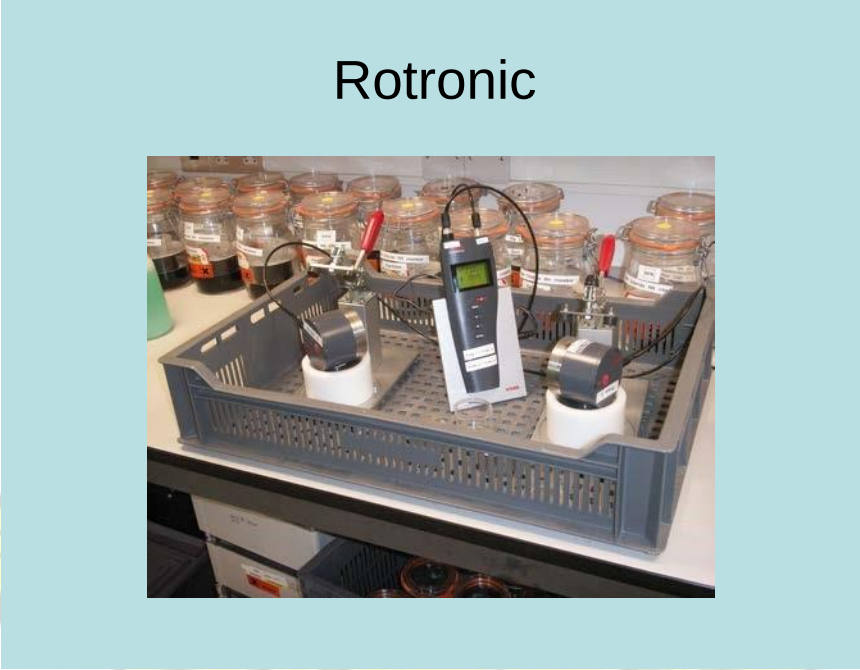


FIGURE 3.2: A Rotronic set up for measuring the equilibrium relative humidity (eRH) of seed samples showing a HygroPalm AW1 water activity instrument in the middle and two water activity probes resting on sample chambers.

3.4 Seed **dimensions and seed weight measurement**:

Seed dimensions such as seed length, seed breath and seed width were taken by measuring 50 individual seeds sampled from each species using Vernier calipers. Seed weight was also determined by measuring the 50 individuals using a laboratory balance (IPGRI-DFSC, 2000) in the seed desiccation and storage protocols.

3.5 Desiccation (drying) trials and germination (viability) trials:

Desiccation trials were undertaken in the United Kingdom and in Ghana. Seed samples with initial seed moisture contents as presented in Table 4.1 were placed in plain polythene bags and desiccated by mixing them with silica gel (1kg of seeds for 1kg of silica gel). In the case of *E. angolense* seeds were rehydrated over water to raise the moisture content to 13% due to their low initial moisture content (6.4%) prior to the trials. During the desiccation periods, samples were drawn every two days and the moisture contents and germination percentages assessed

In view of the well-known differential effects of drying rate on the viability of recalcitrant seeds (Liang and Sun, 2000; Pritchard 1991), desiccation experiments were also carried out by spreading whole seeds of *Garcinia afzelii* and seed portions (i.e. the distal and proximal portions of *Garcinia kola* on plastic sheets as a monolayer and kept under ambient temperature (28-30ºC) condition to facilitate shade drying apart from silica gel drying as suggested by Erdey *et al*. (2004). Samples of *G. afzelii* and *G. kola* were drawn at 2 and 3 days intervals respectively for up to 18 and 24 days for the determination of seed moisture content, seed viability and seedling vigour index for *Garcinia afzelii* and in the case of *G. kola* for moisture content and viability of desiccated samples. The vigour index values were calculated by multiplying the germination percentage with seedling length (Abdul-Baki and Anderson, 1973).

3.6 Drying curve experiment

In another desiccation experiment, 25 seeds of each of the species were weighed and mixed with equal weight of silica gel (1kg of seed for 1kg of silica gel) and put in plain polythene bags. Desiccating seeds were placed at a temperature and relative humidity of 20 ºC and 67%, respectively, and weighed every 2-3 days to construct drying curves for the species (Pritchard *et al*., 2004; Hanson, 1985). Seeds were aerated by mixing

once daily to avoid anoxia (IPGRI-DFSC, 2000) and silica gel changed frequently. As a control, samples of the species were mixed with moistened vermiculite in place of silica gel in polythene bags and held at 20ºC for equivalent periods (Gamene *et al*., 2004). Changes in seed weight were recorded at 2-3 days interval. Seed weight in grams was plotted against period of drying in days.

3.7 Germination trials

Germinations at each moisture under activity 3.5 were assessed by sowing 100 seeds in four replicates of 25 seeds each for *Terminalia superba*, *T. ivorensis*, *Khaya anthotheca*, *Entandrophragma angolense* and *Mansonia altissima*. In the case of *Garcina kola* and *G. afzelii*, germination at each moisture content was assessed by sowing 50 seeds in two replicates of 25 seeds. Seeds of *G. kola*, *T. superba*, *T. ivorensis*, *K. anthotheca*, *E. angolense* and *M. altissima*, were incubated at 30ºC on a gel of 1% water- agar in 90mm plastic Petri dishes or in plastic germination boxes (measuring 17.3cm x11.3cm x 6.0 cm). For *Garcinia afzelii*, germination tests were carried out in heat sterilized river sand in plastic bowls. Germination was recorded at 2 day intervals and seeds were counted as germinated when radicle and plumule attained at least 1cm and free from visual fungal infection or deformation (Rawat, 2005). Moisture content of desiccated samples were determined gravimetrically by weighing about 5 grams in three replicates in the case of *G. afzelii*, *T. superba*, T. *ivorensis*, *K. anthotheca*, *E. angolense* and *M. altissima*. For *G. kola* with large seeds about 10 gram sample in three replicates was used for moisture content tests. These were based on the recommendation by Gosling (2007). Samples were dried in an oven at 103ºC for 17 hours. Dried samples were weighed again and moisture contents expressed as a percentage of fresh weight (f.wt.) (ISTA, 1999).

3.8 Prediction of Drying Periods from Mean Drying Curves of the Species

Drying curves were plotted in order to predict the drying period for the seed species (Rao *et al.,* 2006). Twenty grams (20g) of each of the following species with initial moisture content; *Terminalia ivorensis* (12%), *Terminalia superba* (13%), *Khaya anthotheca* (5%) and *Entandrophragma angolense* (6%), were sampled for this trial. The species were humidified (rehydrated) to raise their moisture contents before drying. In the rehydration process, seeds were placed in porous bags made of mosquito netting above water in desiccators. The desiccators were placed at 25-27 ºC for about 72 hours. The seeds were placed in a monolayer (not more than one seed deep) to enable all seeds absorb moisture equally from the atmosphere within the desiccators. Rehydrated seeds in porous bags made of mosquito netting were placed in desiccators containing self-indicating blue silica gel (1kg of seeds for 1kg of silica gel). Daily renewal of the silica gel was observed to ensure rapid drying of seed. The desiccators were kept at 25-27 ºC and samples of each species were drawn daily to determine the moisture content by the oven- drying method. The data were plotted on graphs with percent moisture content on the y-axis and drying time on the x-axis. The mean drying curves were used to predict drying times for the species as recommended by Rao *et al*. (2006).

The initial moisture contents of the samples were determined by the oven-drying method. The final moisture content that is required for the storage of the species was selected. Horizontal lines from the initial and desired moisture contents were drawn on the vertical y-axis across the drying curves. The days on the x-axis, corresponding to the points of intersection with the drying curve for each of the moisture contents, were noted. The difference between the two points on the x-axis indicated the drying times required to achieve the desired moisture content of the samples.

3.9 Desiccation and viability trials on *Garcinia kola* seeds using electrical conductivity measurement

Electrical conductivity of seed leachate was measured using the CM100. Multiple Cell Conductivity Meter 2.18 manufactured by Reid and Associates of South Africa (Figure 3.3). The device has three main components:

a.) a plastic soaking tray that contains 100 compartments or cells each with about 4ml capacity

b.) a multi-electrode head with 100 pairs of specially designed electrodes conducted to an electrical system, and

c.) a seed analyzer computer programme that analyzes the measurements of the current or resistance across each electrode pair. When the electrode head system is placed on the tray, one pair of electrode is submerged in each cell. An applied voltage exerts a uniform electrical potential across the electrode pair, and the computer translates the amperage into conductivities. Prior to adding the seeds, each tray was filled with 3ml of deionized water to receive the leachate as well as ensuring the full submersion of the electrodes. The soaking tray was washed and rinsed with doubledistilled water prior to each test run to remove ionic impurities. Seeds of *Garcinia kola*, desiccating in silica gel were drawn at two day intervals and cut into the weights of 0.16g, 0.31g, 0.37g, 0.46g, 0.59g and 0.66g and dropped into cells 1, 2, 3, 4 and 5 of the conductivity meter tray.

The conductivity readings were used to assess the vigour levels of the species as they are desiccated (Steere *et al*., 1981; Anon, 1983). Measurement voltage applied during the experiment was 6.00 V with a reference temperature of 25 ºC and a measurement interval of 10 seconds.

100CELL ELECTRICAL CONDUCTIVITY METER

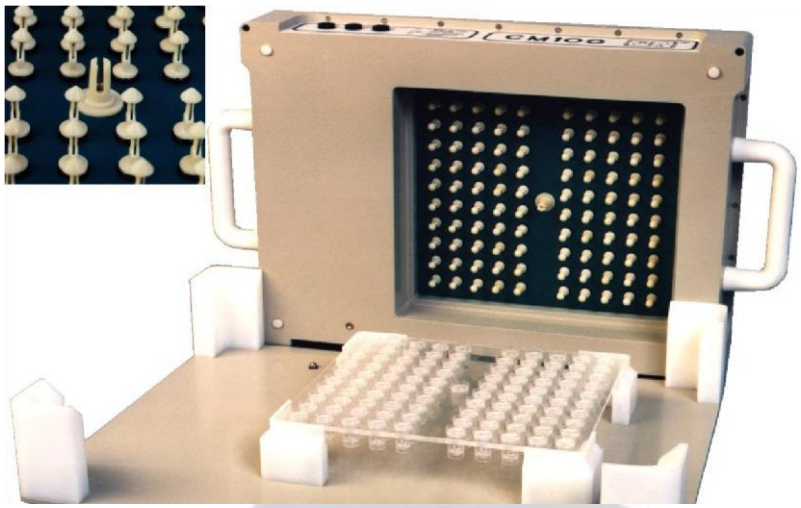


FIGURE 3.3: A CM100 Multiple Cell Conductivity Meter showing a removable sample tray containing 100 cells and a multi-electrode head with 100 pairs of platinum electrodes.

3.10 Temperature Regimes, Substrates and Germination

3.10.1 Using constant temperature incubators:

Constant temperature incubators at 5, 10, 15, 20, 25, 30, 35, and 40ºC were used to germinate seeds of *Garcinia kola*, *Terminalia superba*, *T. ivorensis*, *Khaya anthotheca*, *Entandrophragma angolense* and *Mansonia altissima*. Seeds were sown in sand placed in plastic germination boxes, on agar in plastic boxes and on seed testing paper (STP) in Petri dishes under 8/16 hrs lighting regime (ISTA, 1999; AOSA, 1992). *Garcinia afzelii* was germinated in a plant house with an ambient temperature of 27-30 ºC in heat sterilized sand in plastic germination bowls. Germination capacities in the species were determined using four replicates of 25 seeds.

In another trial samples of *Terminalia superba* seeds were treated with K_2SO_4 (1g/l)

+ GA_3 (200mg/l) and set at 5 to 35 ºC for germination on agar. In the case of *Garcinia kola*, seeds were also divided into seed parts (sections/fragments/ portions) as done by Geeta *et al*. (2006) apart from whole seeds as treatments. Two replicates of 20 seeds parts were used for each germination test. The seed sections used were:

- Half of distal portion (25% of the seed)
- Distal section (50% of the seed)
- Distal end chipped off (80% of the seed)
- Proximal portion (50% of the seed)
- Half of proximal portion (25% of the seed)
- Proximal end chipped off (80% of the seed)
- Middle section (50% of the seed)
- Half of middle section (25% of the seed)

3.10.2. Dormancy breaking trials using chemicals

The following dormancy breaking chemicals were dissolved in distilled water and applied to whole seeds and seed parts/segments/fragments of *Garcinia kola* and *Terminalia ivorensis* as treatments:

(a). Gibberellic acid (GA_3) (500mg/ l)

(b). Gibberellic acid (GA_3) (1000mg/ l)

(c). Potassium Nitrate (KNO_3) (2g / l)

(d). Potassium Nitrate (KNO_3) (1g /l)

(e). Citric acid (2g / l)

(f). Citric acid (1g / l)

(g). GA_3 + $KNO_{3)}$ (500mg + 1g / l)

Glass Petri dishes and plastic sandwich boxes were used as germination containers while agar and heat sterilized river sand were used as the germination media. All the seeds sown were incubated in seed germinators set at the specified temperatures with 95% relative humidity. Germination was recorded at 2 days intervals and seeds were counted as germinated when radicle and plumule attain at least 1cm and free from visual fungal infection or deformation. In sand, a seed was considered as germinated when plumule attained at least 1cm height above the sand surface (Rawat, 2005). Three replicates of 20 seeds were used for each germination test. The chemicals listed as treatments were also applied to decoated seeds from fully matured (brown) seeds (FMB) (moisture content 15%), immature green seeds (IMG) (moisture content 28%), matured seeds green (MSG) (moisture content 19%) and mature brown whole seed (MBWS) of *Terminalia ivorensis*. Glass Petri dishes and plastic sandwich boxes were used as germination containers and agar served as germination media. All the seeds sown were incubated in seed germinators set at the specified temperatures with 95% relative humidity. Germination was recorded at 2 day intervals and seeds were counted as germinated when radicle and plumule attained at least 1cm.

3.4.3 Temperature and germination trials using the thermogradient plate

Terminalia superba, *T. ivorensis* and *Khaya anthotheca* were germinated on the Grant 2-way thermogradient plate (Figure 3.4)- a bi-directional incubator with a controlled light environment (with 64 temperatures combinations), to make a comprehensive comparison of their germination responses to alternating and constant temperatures within a range of 5 to 40 ºC (Manger, 1999). Seeds of *T. superba* and *K. anthotheca* were germinated on a gel of 1% water- agar in 90mm (9cm) plastic Petri dishes. Each Petri dish contained approximately 40ml agar as recommended by Manger (1999). In the case of *T. ivorensis* the substrate used in the germination assessment was seed testing paper (STP). Each Petri dish contained three moistened seed testing papers which were moistened every other day in the higher temperature regions.

			H1(40/5)	**H5(40/25)**	**H6(40/30)**	**H7(40/35)**	**H8(40/40)**
H2(40/10)	**H3(40/15)**	**H4(40/20)**					
G1(35/5)	**G2(35/10)**	**G3(35/15)**	**G4(35/20)**	**G5(35/25)**	**G6(35/30)**	**G7(35/35)**	**G8(35/40)**
F1(30/5)	**F2(30/10)**	**F3(30/15)**	**F4(30/20)**	**F5(30/25)**	**F6(30/30)**	**F7(30/35)**	**F8(30/40)**
E1(25/5)	**E2(25/10)**	**E3(25/15)**	**E4(25/20)**	**E5(25/25)**	**E6(25/30)**	**E7(25/35)**	**E8(25/40)**
D1(20/5)	**D2(20/10)**	**D3(20/15)**	**D4(20/20)**	**D5(20/25)**	**D6(20/30)**	**D7(20/35)**	**D8(20/40)**
C1(15/5)	**C2(15/10)**	**C3(15/15)**	**C4(15/20)**	**C5(15/25)**	**C6(15/30)**	**C7(15/35)**	**C8(15/40)**
B1(10/5)	**B2(10/10)**	**B3(10/15)**	**B4(10/20)**	**B5(10/25)**	**B6(10/30)**	**B7(10/35)**	**B8(10/40)**
A1(5/5)	**A2(5/10)**	**A3(5/15)**	**A4(5/20)**	**A5(5/25)**	**A6(5/30)**	**A7(5/35)**	**A8(5/40)**

Figure 3.4: Layout of the arrangement of treatments on the thermogradient plate. The letters **A1** to **H8** represent the Petri dishes (treatments) and the figures in parentheses are the 64 temperatures combination in ºC experienced by each treatment during the experimental (germination) period.

Two temperature gradients ranging from 5 to 40 ºC were used. The first gradient, progressing from left to right on the thermogradient plate in the dark, was alternated every 12 hours with the second progressing from front to back with light. During the experimental period, dried out agar in Petri dishes located at the hot end of the plate were replaced periodically as recommended by Manger

(1999). Similarly, where test seed paper was used as substrate those at the hot end were moistened everyday due to excessive drying. Germination was recorded at 2 day intervals and seeds were counted as germinated when radicle and plumule attain at least 1cm and free from visual fungal infection or deformation after Rawat (2005). Seeds were germinated on the thermogradient plate for 30 days after which the ungerminated samples were transferred to the 25 ºC incubator for 10 days to see if they would germinate at this temperature. Thereafter, the cutting test was conducted on seeds that were still ungerminated to record seeds that were viable but had failed to germinate.

3.11 Water sorption isotherms of species

The relationship between seed moisture content and equilibrium relative humidity (eRH) was established to construct water sorption isotherms also known as moisture-release curves for the species, to estimate at which moisture content seeds can be dried in a given climate as recommended by Stubsgaard and Poulsen (1995). This is essential for developing optimal seed storage protocols (Merritt *et al*., 2003). Seeds were equilibrated over a series of Lithium Chloride (LiCl) solutions, ranging in relative humidity from 12 to 93% and silica gel with 3% relative humidity at 20ºC. The relative humidity conditions were obtained by dissolving specified weights of

LiCl granules in 100ml of distilled water (MSBP, 2002). Once prepared, the solutions were allowed to equilibrate in sealed containers for at least 24 hours. Storage glass jars were quarter-filled with the prepared LiCl solutions and appropriately labeled as shown in Table 1 as recommended by Gold and Hay (2007).

Table 3.1: Relative humidity series prepared using differing concentrations of Lithium Chloride to equilibrate seed samples at 20ºC.

Weight of LiCl granules (g)	* Silica gel	90	80	70	60	50	40	30	20	10	5
RH (%) generated at 20°C	3	12	13	18	24	33	44	60	80	90	93

* Well- dried Silica gel will generate about 3-4% RH at 20 ºC

Seed samples to be equilibrated were placed in open dishes above the LiCl solutions on plastic supports. To hasten the equilibration of seed samples of *Garcinia kola*, each seed was cut into two pieces due to their large sizes. The glass jars were sealed and seeds allowed to attain equilibrium. To determine when equilibrium point has been reached, seed sample weights were monitored for

weight loss/gain of seeds until there was no further change.
When seeds equilibrated (reached constant weights), samples were drawn for moisture content determination gravimetrically by drying the seeds at 103 ºC for 17

hours (ISTA, 1999). The values of moisture contents were then plotted against relative humidity to construct moisture sorption isotherms (moisture-release curves) for the species (Daws *et al*., 2004; Merritt *et al*., 2003).

3.12 Calculation of mean germination time, seed vigour index, and speed of germination:

- ✓ Mean germination time (MGT), a measure of the spread of germination of seeds was calculated according to Labouriau (1983); Yoursheng and Sziklai (1985) as follows:

  $$MGT\ (t) = \frac{\sum ni.ti}{\sum n} \quad (days)$$

  where: t = mean germination time in days

  ni = number of germinated seeds during a given time interval

  n = total number of germinated seeds.

- ✓ Seed vigour index values (VI) were calculated by multiplying the germination percentage with seedling length (Abdul-Baki and Anderson, 1973).

- ✓ Speed of germination was derived by taking the germination count at weekly intervals up to 103 days and computed according to the method proposed by Maguire (1962).

3.13 Statistical procedures

The experimental design adopted was the Complete Randomized Block Design (CRD**).** Analysis of variance (ANOVA) was used to determine significance in the data obtained using the Genstat statistical package. Mean separation was done after the ANOVA using standard error of the difference (s.e.d).

1.0 INTRODUCTION

In sub-Saharan Africa, forests are still disappearing at a rate of nearly one percent a year (FAO, 2003), despite the many reforestation and conservation programmes. Deforestation affects the daily lives of millions of people. Alarmingly, it is estimated that about 2000 tropical tree species in this region are considered to fall into the categories of being 'near-threatened' to 'critically endangered', as determined by The World Conservation Union (IUCN, 2008) (and by UNEPWCMC (2001). Raising trees and preserving their seeds are means of supporting reforestation, combating desertification, safeguarding the environment and conserving biodiversity (Grainger 1993). Planting trees is globally considered an effective measure to protect the climate and combat climate change. Trees not only bind the greenhouse gas carbon dioxide, they also counteract desertification and soil erosion (Grainger 1993). Trees act as "carbon sinks" and absorb carbon from the air and convert it into plant material. Trees sequester this carbon in roots, trunks, stems and leaves while they grow, and in wood products after they are harvested. That means planting trees, whether in a rural or an urban setting, reduces carbon in the atmosphere (Dudley *et al*., 1995).

This is an enormous challenge that requires the planting of large numbers of adapted species (Sacande and Pritchard, 2004). High quality tree seeds are required for both reforestation and the in- and ex- situ conservation of forest genetic resources (Schmit, 2000).

Regeneration of the forest was initially by natural regeneration but emphasis shifted to artificial regeneration in the fifties (Olatoye, 1968). About one million hectares (2.47 million acres) in the tropics are planted in tree seedlings each year, but only a small portion of the seedlings are indigenous (Bonner, 1992). According to Engels

and Ditlevson (2004), preference has been given to fast exotic growing species for large scale industrial plantations. Brookman-Amissah (1973), observed that very little information was available on the handling, dormancy breaking methods, and optimum conditions and temperatures for storage and germination of indigenous species. This was probably one of the reasons why plantations were set up using mostly exotic species whose seed biology and seed handling procedures were known. In the latter half of the last decade, however, interest has shifted from large industrial plantation to smaller plantings of indigenous multipurpose species. This change in interest has been accelerated by the awareness of germplasm conservation needs, as many multipurpose trees are on the list of endangered species- including 41 fuel wood species in Africa alone (Palmberg, 1981). Many national programmes now favour and prioritize social forestry/ agroforestry projects over industrial plantations.

These conditions have created a large demand for seeds of indigenous species, a demand that is difficult to meet because of lack of basic information on these species (Bonner, 1992). According to Sacande *et al*. (2004) insufficient baseline information about the potential of indigenous species and the availability of seeds and seedlings are major constraints for the deployment of locally adapted tree species. Access to seeds and seedlings, are in general, associated with seed handling and storage problems, which limit the use of many potentially high value indigenous species in tree planting and conservation programmes (Sacande *et al*.

2004)

In Ghana, the Presidential Special Initiative on Forest Plantation Development launched in September 2001, aimed at planting 20,000 hectares of forest annually throughout the country up to the year 2020, with fast growing indigenous and exotic

tree species (Cobbinah *et al.*, 2001). Delivering this national priority programme means that millions of seedlings, healthy and in good time, to meet planting requirements will be required. Thus, the need for large quantities of viable indigenous seeds and an improvement in germination percentage and germination vigour.

It has been estimated that more than 70% of tree species in tropical ecosystems produce recalcitrant seeds, which are difficult to collect, process and store (Ouedraogo *et al*., 1999). Many of these seeds are sensitive to both desiccation and low temperatures and consequently, do not tolerate being dried to a low moisture content and cannot be stored at low temperature for long periods of time (Roberts, 1973). These are major problems for humid tropical forest tree species, where more than 70% of them have seeds with recalcitrant or intermediate seed storage behaviour (Dulloo *et al*., 2004). Consequently, the use of much high value indigenous tropical forest tree species in afforestation and conservation programmes is hindered by problems associated with seed handling and storage (Stubsgaard, 1992).

Engels and Ditlevsen (2004), have observed that studies on tropical forest tree seeds in general also remain more complex compared to those on annual crops as a result of dormancy problems and large variations in seed longevity, compounding, the handling problems. Consequently, a lot of seeds die over the period between collection and sowing, and thus they are rarely used in planting programmes (even though the demand is increasing). They cannot appropriately be stored for the conservation of the species (Stubsgaard, 1992).

If indigenous species are to be used, however, in afforestation and conservation programmes in Ghana, it is important to generate knowledge on their physiology

and identify the appropriate seed handling and storage techniques for them. To this end, basic studies on seed production, collection and handling need to be devised for a range of species for which demand for planting material is increasing (Sacande *et al*, 2004; Omondi, 2004).

Seven important indigenous species in Ghana namely; *Garcinia kola* (Tweapea), *Garcinia afzelii* (Nsokodua), *Terminalia superba* (Ofram), *Terminalia ivorensis* (Emire), *Khaya anthotheca* (Dubini), *Entandrophragma angolense* (Edinam) and *Mansonia altissima* (Aprono) have been selected for the study.

G. afzelii, *T. superba*, *T. ivorensis*, *K. anthotheca*, *E. angolense* and *M. altissima*, have all been described as vulnerable, while *G. kola* has been described as vulnerable with decreasing population trend according to the IUCN Red List of Threatened Species (IUCN, 2008). They therefore require urgent conservation attention. Growing demand for seed banks and reforestation programmes underlines the need for better theoretical and practical knowledge of the behavior of such seeds. Desiccation screening provides a first line management tool to determining seed storability. Besides, it is useful in the determination of high moisture limits for seed survival (Omondi, 2004). Quantifying and predicting desiccation tolerance and desiccation sensitivity is a prerequisite to optimize ex situ long-term preservation methods (Leprince, 2003). There are many gaps in our understanding of tropical seeds, and seed testing is one of them (Bonner, 1992).

The study is aimed at evaluating the effect of drying on seed viability of the seven indigenous species i.e. (1). *G. kola*, (2). *G. afzelii*, (3). *T. superba*, (4) *T. ivorensis*, (5). *K. anthotheca* (6). *E. angolense*, and (7). *M. altissima* meant for afforestation and conservation programmes in Ghana. The specific objectives of the study are:

(i). to find out how drying affects the viability of the seed species selected, (ii). to determine the optimum temperature regimes and the best substrates for the germination of the species, and (iii). to determine the appropriate methods for breaking dormancy of the species if necessary.

2.0 LITERATURE REVIEW

2 1. Major groups of seed species

Seeds are divided into two major groups of species with respect to deterioration and storage possibilities (Stubsgaard 1992). Roberts (1973), introduced the terms ‘‘orthodox’’ and ‘‘recalcitrant’’ to distinguish between these two types of seed storage behaviour. However, a third category of seed storage behaviour has recently been identified and termed as “intermediate’’ (Ellis *et al*., 1990) because a group of species has been identified which shows seed storage behaviour intermediate between the orthodox and recalcitrant categories (Hong and Ellis., 1992). Alternatives to Robert’s terminology have been suggested from time to time. For example, it has been argued that the term “ poikilohydric ’’ should replace “ orthodox” to describe seeds that can be maintained in equilibrium with ambient relative humidity for long periods and that “ homoiohydric ’’ should replace “ recalcitrant ” to describe those that do not tolerate desiccation (Berjak *et al.*,1990).

2.1.1 Orthodox Seeds (Desiccation- tolerant seeds)

Orthodox seeds are seeds that acquire desiccation tolerance during development, can dry to low water contents (generally less than 5%), and retain viability in the dry state for predictable periods (Pammenter and Berjak, 2000). Seeds of orthodox species can survive complete removal of water (Walters and Towill, 1998) and can usually be stored for long periods by drying and cooling; the actual period depends mainly on the temperature and moisture content during storage (Roberts and King, 1980). Over typical ambient conditions, the relationship is such that the longevity approximately doubles with each 5-6°C fall in temperature, or 1% reduction in moisture content (Ellis and Roberts, 1980).

This relationship, known as Harrington's rule, is generally applicable for temperatures between 0 and 40°C and moisture contents between 5 and 14%. This relationship emphasizes the importance of low moisture content and temperature in extending the longevity of seeds (McDonald and Copeland, 1996). Generally, orthodox seeds undergo a period of drying during their maturation and are shed at low water content which is in equilibrium with the prevailing relative humidity (Finch-Savage, 1992; Pammenter and Berjak, 2000). The equilibrium water content at any particular relative humidity is determined by seed composition, but all orthodox seeds can withstand dehydration to 5 percent , even when maturation drying is not completed prior to shedding (Pammenter and Berjak, 2000). At low temperatures and moisture contents, it is predicted that the seeds of many orthodox species will remain viable for hundreds of years. Such predictions are supported by reports of ancient seeds retaining the ability to germinate after many centuries (Hay, 2003).

According to Roberts and King (1980), all the seeds of agricultural and horticultural crop species which are annual or biennial show orthodox seed behaviour and thus there is no fundamental constraint to storing seeds of this type for long periods. The International Board for Plant Genetic Resources (IBPGR, 1976) gave general recommendations for the designs and management of long-term stores for genetic conservation of orthodox seed. The IBPGR recommended that orthodox seeds should be stored in sealed containers at 18°C or less, and at moisture content of 5-7%. Under these conditions it is expected that the majority of orthodox species will not show any significant decline in viability for a century, or perhaps much longer (Roberts and Ellis, 1977).

2.1.2 Recalcitrant seeds (Desiccation sensitive / Desiccation- intolerant seeds)

Recalcitrant species differ from the orthodox in two ways: (i). their seeds die if they are dried below certain limits, and (ii). the seeds of some tropical species of this group also die if the temperature falls below certain limits (Stubsgaard, 1992), usually below about 10-15ºC (Hong *et al.*,1996) which is often described as chilling injury (Roberts and King,1980). Recalcitrant seeds do not undergo a period of maturation drying during their development and as a consequence, they are shed from the parent plant at high water content (Roberts, 1973). They remain desiccation sensitive both during development and after they are shed (Berjak and Pammenter, 1997). Because recalcitrant seeds are sensitive to desiccation and low temperatures, they have a relatively short life span during storage. It is, generally, impossible to store them from harvest till the next suitable sowing; even at optimal moist conditions, survival of seeds of tropical species in this group is typically limited to a few weeks or months (Stubsgaard, 1992).

When fresh recalcitrant seeds begin to dry, viability is first slightly reduced as moisture is lost, but begins to decline considerably at a certain moisture content termed the "critical moisture content" (King and Roberts, 1980) or "lowest safe moisture content"(Tompsett, 1984). If drying continues further, viability is eventually reduced to zero (Hong *et al.*, 1996). Critical levels of moisture content vary greatly among species (Chin, 1988) and even among cultivars and seed lots (King and Roberts, 1979). They may also vary with methods of drying (Pritchard, 1991). The values of the "lowest safe moisture content" vary between extremes of 23% for cocoa (*Theobroma cocao*) Mumford and Brett (1982) and 61.5% for *Avicennia marina* (Farrant *et al*., 1986). There appear to be two main types

of species with recalcitrant seeds: those from aquatic habitats and some large – seeded trees (Roberts *et al*., 1984). These are, however, not diagnostic features of recalcitrant seed storage behavior (Roberts *et. al.,* 1984). Moreover, seed storage behaviour may differ among species within a genus. For example, Tompsett (1992) has shown that the large fleshy seeds of the species of *Araucaria* from South America are recalcitrant, whereas the smaller seeds of many *Araucaria* species from Western Pacific are orthodox.

Recalcitrant seeds are metabolically active after shedding **(**Hay, 2003). Internally, a recalcitrant seed is actually a seedling rather than a seed (Thomsen and Stubsgaard 1998**).** With regard to the desiccation intolerance of recalcitrant seeds, Farrant *et al*., (1988) suggest this is because germination events have begun, and the further these events progress, the more sensitive the seed becomes to desiccation. Due to their relatively large size and nature of the outer coverings, they dry slowly in nature, as well as under usual dehydrating regimes used in the laboratory (Hay, 2003).

2.1.3 Intermediate seed storage behavior

For the majority of species, it was previously a relatively simple matter to classify seeds storage behaviour as either orthodox or recalcitrant in accordance with Robert's definitions. However, evidence began to accumulate that these two categories did not account satisfactorily for all observations on seed storage behavior (Hong and Ellis, 1996). For example, Teng and Hor (1976), showed that seeds of both star fruit (*Averrhoa carambola*) and papaya (*Carica papaya*) withstood desiccation to around 1012% moisture content and could be stored successfully in hermetic containers at these levels of moisture contents. In contrast to orthodox seeds, however, they lost viability much more rapidly

in air-dry storage at 0°C than at warmer temperatures of 12-21°C. Similarly, Tompsett (1984), showed that the longevity of seeds of *Araucaria columnaris* was increased in air-dry storage by reduction in storage temperature or moisture content, but only within limited ranges, because viability was reduced with desiccation below about 12% moisture content. In both cases then it was clear the seeds could be stored in certain air-dry environments successfully but their behaviour did not satisfy the definition of orthodox seed storage behaviour provided by Roberts (1973).

The essential feature of intermediate seed storage behaviour is that the negative relation between seed longevity in air-dry storage and moisture content is reversed at values below those in equilibrium (at 20°C) with about 40-50% RH (Hong and Ellis, 1996). Seeds of *Coffea arabica* (Hong and Ellis, 1992), rubusta coffee (co*ffea canephora*) (Hong and Ellis, 1995), oil palm (*Elaeis guineensis*) (Ellis *et al*., 1991), several *Citrus* species (Hong and Ellis, 1995) and neem (*Azadirachta indica*) (Sacande *et al*., 2000) among others have all been mentioned to exhibit intermediate storage behaviour.

The critical levels of moisture content of intermediate seeds at which more rapid loss in viability occurs during hermetic storage or desiccation damage to germination varies considerably with species, degree of maturity and method of seed extraction and handling (Hong and Ellis, 1996). In general, seeds which are extracted at maturity tolerate desiccation moisture contents in equilibrium with about 40-50% RH, i.e. about 10% moisture content for arabica coffee, and 7% moisture content for certain *Citrus* spp (Hong and Ellis, 1996). Another feature of intermediate seeds of tropical origin is the fact that the longevity of dry seeds (7-10 % M.C.) is reduced with reduction in storage temperature

below about 10°C (Hong and Ellis, 1992**)**. Seed maturity also affects desiccation tolerance in intermediate seeds (Vertucci *et al*., 1994). For example, seeds of arabica and robusta coffee extracted from fruits of intermediate maturity (yellow) were able to tolerate greater desiccation than those from either red or immature (green) fruits (Hong and Ellis, 1995). The method of seed extraction and handling may also influence desiccation tolerance. For example, seeds of arabica coffee imbibed at 30°C for 3-10 days showed greater sensitivity to desiccation (Ellis *et al*., 1991). It is suggested that seed processing methods involving high seed moisture contents, e.g. soaking, fermentation, moist storage in cold water (e.g. *Zizania*) will tend to reduce subsequent desiccation tolerance and seed longevity in intermediate seeds (Hong and Ellis, 1992). Intermediate seeds of species of tropical origin die more rapidly when the temperature is lowered below about 10°C. In some cases, temperatures just below 0°C kill whole seeds immediately (Ellis *et al*., 1991).

2.2 Factors influencing desiccation sensitivity in recalcitrant seeds

Desiccation tolerance in recalcitrant seeds increases during seed development on the mother plant, however, unlike orthodox seeds, maturation drying to low moisture contents does not occur (Finch-Savage 1992), and fresh recalcitrant seeds have high levels of moisture contents at maturity (shedding), between, for example, 36% for rubber **(**Chin *et al*., 1981) and 90% for choyote (or chayote) (*Sechium edule*) (Ellis, 1991). Considerable differences in moisture content can be detected among tissues within particular recalcitrant seed. For example**,** Grabe (1989) reported that, with the exception of durian (*Durio zibethinus*) and jackfruit (*Artocarpus heterophyllus*), the storage tissues of recalcitrant seeds are always at lower moisture content than the embryonic axis.

Desiccation of excised embryos or embryonic axes has considerable practical potential for the in *vitro* conservation of recalcitrant embryos, since embryos are able to survive desiccation to lower moisture contents than whole seeds (Chin, 1988). For example, fresh seeds (36% moisture content) of *Hevea brasiliensis* tolerated desiccation to 20% moisture content but no seeds survived further desiccation to 15% moisture content (Chin *et al*., 1981). However, 50-80% of their excised embryos (55% moisture content) survived desiccation to 14% moisture content when cultured *in vitro* **(**Normah *et al*., 1986).

Reports of survival of excised embryos or embryonic axes to a lower moisture content than their intact seeds are numerous (Finch-Savage, 1992; Chandel *et al*., 1995). While there are several reports that fast drying allows intact recalcitrant seeds to survive desiccation to lower moisture contents than slow drying (Pritchard, 1991), Finch-Savage (1992) showed that drying rate does not affect the desiccation sensitivity of whole seeds of *Quercus robur*. However, fast drying allowed excised embryos of *Araucaria hunsteinii*, *Hevea brasiliensis*, *Landolphia kirkii*, *Quercus robur* and *Quercus rubra* to survive desiccation to lower moisture contents than similar embryos dried more slowly within intact seeds (Pammenter *et al*., 1991; Pritchard, 1991; Normah *et al*.,1986). Fu *et al*. (1993) reported that drying excised embryonic axes by silica gel or an aseptic air current allowed excised embryonic axes to survive desiccation to a lower value than that achieved by the vacuum drying method. For example, although the vacuum drying method provided more rapid drying, no excised embryonic axes of *Artocarpus heterophyllus* survived desiccation to 44% moisture content, while the excised embryonic axes dried with an aseptic air flow

and silica gel tolerated desiccation to 26 and 16% moisture content, respectively (Fu *et al.*, 1993).

2.3 Mechanism of desiccation tolerance in seeds

Mechanism conferring desiccation tolerance on maturing seed have been inferred from numerous studies that use hormonal mutants endogenous manipulation of water levels and hormone activities, and exogenous application of hormones to excised embryo (Kigel and Galili ,1995). A number of processes or mechanisms have been suggested to confer, or contribute to desiccation tolerance. Different processes may confer protection against loss of water at different hydration levels, and the absence or ineffective expression of one or more of these could determine the relative degree of desiccation sensitivity (Berjak and Pammenter, 1997). Mechanisms that have been implicated to date include the following: intracellular physical characteristics (such as vacuolation and reserve deposition, integrity of the cytoskeleton, and nuclear architecture); intracellular de-differentiation; "switching off "of metabolism; presence and efficient operation of antioxidant systems; accumulation and roles of putatively protective molecules (such late embryogenic accumulation/abundant proteins (LEA's), sucrose and certain oligosaccharides, or galactosyl cyclitols); deployment of certain amphipathic molecules; an effective peripheral oleosin layer around lipid bodies; the presence and operation of repair mechanisms during rehydration (Fansworth, 2000; Berjak and Pammenter, 1997).

2.3.1 Intracellular physical characteristics

2.3.1.1 Vacuolation and reserve deposition

One of the major requirements for cells of plant material to tolerate desiccation is an ability to withstand the mechanical stress associated with volume reduction. Berjak and Pammenter (1997), reported that reduction of the aqueous vacuolar volume , by shrinkage, breaking up of one large vacuole to several smaller ones, or by their becoming filled with insoluble reserve material, is one of the factors that would contribute to increased mechanical resilience of cells to dehydration , and a high degree of vacuolation can lead to lethal mechanical damage upon dehydration. This aspect was examined by Farrant *et al.* (1997) for (i) *Avicenia marina*, a highly recalcitrant seed which can withstand very little dehydration either before or after they are shed; (ii)

Aesculus hippocastanum, a temperate recalcitrant species and (iii) *Phaseolus vulgaris* , a typical orthodox seed that attains a low water content prior to shedding and is long-lived in this condition.

Avicenia marina seeds lose no water during development, and are as sensitive to dehydration before shedding as after abscission (Farrant *et al*., 1992). These seeds, at best, are unable to survive water contents lower than 0.5 g g $^{-1}$ (33% wmb). The vacuoles ultimately occupy almost 60 percent on the average of the volume across the cells of all axis tissues, and 90 percent of the cotyledonary cells when mature. At no stage do either the axial or cotyledonary vacuole contain insoluble reserves (Berjak and Pammenter, 2004).

Seeds of *A. hippocastanum* naturally undergo a measure of dehydration during development, accompanied by an increase in relative desiccation tolerance (Tompsett and Pritchard, 1998). The mature seed are more desiccation tolerant than those of *A. marina*,

being able to withstand dehydration to water contents in the range of 0.42 to 0.25 g g^{-1} (30 to 20 percent wmb). Vacuoles ultimately constitute only a small fraction of the intracellular volume, particularly in the axis cells at maturity. The cotyledonary cells contain many large, starch–filled plastids and protein bodies and are considerably less vacuolated than those of *A. marina* (Berjak and Pammenter, 2004).

In *P. vulgaris* seeds, which are othodox and able to tolerate low water contents, vacuolar volume is reduced to an insignificant proportion in axis cells, and vacuoles in the cotyledonary cells accumulate an amorphous, presumably insoluble, material. The differential degree of vacuolation and insoluble reserve deposition among the three species, in both developing and mature seeds, correlates with their degrees of desiccation sensitivity **(**Farrant *et al*., 1997).

2.3.1. 2 Integrity of the cytoskeleton

The cytoskeleton, the major components of which are microtubules and microfilaments, is an integrated intracellular support system as well as imposing organization on the cytoplasm and the nucleus (Berjak and Pammenter, 2004). The authors reported that in the hydrated state, there is an extensive microfilamentous network in the cells of orthodox seeds which becomes dismantled as the seeds are increasingly dehydrated. In desiccation-tolerant seeds, orderly reassembly of the elements of the cytoskeleton accompanies imbibitions, but once the water content falls to damagingly low levels in desiccation-intolerant seeds, the microfilaments are not reassembled when the seeds are subsequently rehydrated. According to Berjak and Pammenter (2004), the resultant lack of the intracellular support and structural organization afforded by the cytoskeleton would

obviously be a major damaging factor upon rehydration of recalcitrant seeds. Thus the failure of the cytoskeleton to reassemble following deleteriously low levels of dehydration would have physiological as well as structural consequences in the cells of desiccation-sensitive seed tissues (Berjak and Pammenter, 2004).

2.3.1.3 DNA, chromatin, and nuclear architecture conformation

Maintenance of the integrity of the genetic Deoxyribonucleic acid (DNA) material in the desiccated condition in orthodox seeds, and/ or its rapid repair when seeds are rehydrated, is considered to be a fundamental requirement for desiccation tolerance (Osborne and Boubriak, 1994). DNA assumes different conformational states depending on water activity- it is considered that as water is lost (i.e. water activity is lowered) such conformational changes will occur (Osborne and Boubriak,1994). According to Berjak and Pammenter (2004), even though little is known about the effects of dehydration on the DNA, chromatin, and nuclear architecture in desiccation- sensitive seeds, their stability in the dehydrated state clearly must be a prerequisite for desiccation tolerance They concluded that , maintenance of the integrity of the nucleus as a whole, and the genome in particular, may be imperfectly expressed, or the ability for this may even be totally lacking, in recalcitrant seeds.

2.3.2 Intracellular de -differentiation

Vertucci and Farrant (1995), defined de- differentiation as a process by which intracellular structures are simplified and minimized and this accompanies the onset of maturation drying in orthodox seeds. During this process, mitochondria and plastids loose internal structure, and the endo-membranes such as the rough endoplasmic reticulum(ER) become

substantially reduced and the cisternae or Golgi bodies diassociated. These changes lead to minimization of metabolic activity, including respiration and membrane synthesis and processing (Vertucci and Farrant, 1995).

An examination of the quantitative and qualitative status of mitochondria in seeds of *Avicenia marina, Aesculus hippocastanum* and *Phaseolus vulgaris* showed that the proportion of cell volume occupied by these organelles was highest in *A. marina*, which is very desiccation sensitive, and substantially less for A. h*ippocastanum* ,which is less recalcitrant. In *P. vulgaris* (which is typically orthodox), mitochondria occupied a significantly smaller proportion of the cell volume, preceding the onset of maturation drying (Farrant *et al*., 1997). There were also marked differences in the structural complexity of the mitochondria among these species. *A. marina* and A. *hippocastanum*, had well- developed cristae and a structure that was generally typical of an active, hydrated plant tissue; while in *P. vulgaris* the mitochondria were almost completely de- differentiated even at tissue water contents comparable to those of the recalcitrant species at shedding (Farrant *et al*., 1997). Berjak and Pammenter (2004), concluded from the above observations made from mitochondria examination from the three plants that, retention of organelles in the highly differentiated state is a major factor in the desiccation sensitivity of recalcitrant species, whereas the ability for ordered de-differentiation is, in fact, a prerequisite for seed survival in the dehydrated state.

2.3.3 "Switching off'' of metabolism

An indication of metabolic "switch off" is the cessation of DNA replication and the arrest of most of embryo cells in the G_1 phase (prereplication; DNA in the undoubled

2C form) with the onset of maturation drying (Brunori, 1967). Rogerson and Matthews (1967), observed that a sharp decline in sugars proceeds the fall in respiratory rate, and they suggested these are essential events permitting orthodox seeds to withstand loss of water during maturation drying. This is in keeping with the observations of Farrant *et al*., (1997) indicating that mitochondria become substantially de- differentiated prior to maturation drying in the orthodox seeds of *Phaseolus vulgaria*. According to Berjak and Pammenter (2004), dehydrated orthodox seeds are effectively ametabolic, not simply because no water is available, but because of a controlled shutdown of activity and dismantling of structures proceeding or accompanying maturation drying.

The authors further reported that marked de-differentiation does not occur in any of the recalcitrant embryos of a variety of species examined to date, and that respiration rates remain high. Farrant *et al*. (1997), however, observed that the mitochondria of recalcitrant embryos of both *Avicenia marina* and *Aesculus hippocastanum* remains highly differentiated. The mitochondria occupy a greater proportion of cell area in the more desiccation- sensitive species, *A. marina*.

2.3.4 Presence of efficient operation of antioxidant system

Antioxidants are a diverse group of otherwise unrelated chemicals that combine with free radicals (or other) chemicals that release free radicals that would otherwise attack and oxidise molecules in cells or tissues.

Hendry (1993) and Leprince *et al*. (1993) reported that a range of antioxidants processes operate in orthodox seeds. According to Vertucci and Farrant (1995), it is particularly in

the water content range from 0.25 to 0.45 g g^{-1} (20-30 percent, wmb), that unregulated metabolic events resulting in the first wave of free-radical generation are likely to occur. Thus antioxidant systems (i.e. free-radical scavenging systems) are maximally effective during maturation drying of orthodox seeds (Berjak and Pammenter, 2004). Reviews of metabolic damage associated with dehydration of recalcitrant seeds have highlighted the idea that free-radical generation may be a major injurious factor (Berjak and Pammenter, 1997; Come and Corbineau, 1996), particularly because protective mechanisms appear to become impaired under conditions of water stress (Smith and Berjak, 1995).

Rapid formation of free-radicals and decreasing activity of antioxidant systems have been reported as occurring during dehydration of seeds of temperate recalcitrant species *Quercus robur* (Finch- Savage and Blake, 1994). Lipid peroxidation which is a major consequence of uncontrolled free-radical generation, with the ultimate accumulation of a stable free- radical in the embryonic axes, has, been shown to accompany dehydration of seeds of some temperate recalcitrant species (Finch- Savage and Blake, 1994).

Free-radical formation has been reported to accompany viability loss in seeds of the highly recalcitrant, tropical species *Shorea robusta* (Chaitanga and Naithani, 1994). According to Berjak and Pammenter (2004), damage ascribable to uncontrolled freeradical generation occurs during dehydration in the recalcitrant seeds of a range of species that show differing degrees and manifestations of non-orthodox behavior. It was concluded that not only are free radicals produced as a consequence of water stress in desiccation-sensitive seeds, but that antioxidant systems also are ineffective at curbing them and therefore these factors

must be seriously considered as constituting one of the major causes of desiccation sensitivity (Berjak and Pammenter, 2004).

2.3.5 Accumulation of putatively protective molecules:

2.3.5.1. Late Embryogenic Accumulating (LEA's) / Abundant Proteins): The synthesis of a set of hydrophilic, heat- resistant proteins termed LEAs (Galau *et al*., 1986) is associated with the acquisition of desiccation tolerance in developing orthodox seeds (Reid and Walker-Simmons, 1993). Their synthesis appears to be associated with the high abscisic acid (ABA) levels that peak during the later stages of seed development (Kermode, 1990). The characteristics of LEAs and the conditions under which they appear have led to suggestions that they function as protectants perhaps stabilizing subcellular structures in the desiccated condition (Dure, 1993), although Walters *et al*. (1997) have proposed that they operate by buffering water loss so that cells stay at the critical water potential. The position of LEA's (or dehydrin- like proteins as they may be termed) in the non-orthodox seeds appears at first to be anomalous, as some species do not express these proteins while others express them to variable extents (Gee *et al*., 1994) . Seeds of *Avicenia marina*, which are extremely desiccation sensitive, appear not to express LEA's (Farrant *et al*., 1992). In contrast, seeds of *Zizania palustris* (North America wild rice), which are recalcitrant (Vertucci et al., 1994) but show differential response to dehydration depending on temperature (Kovach and Bradford, 1992) do express this type of protein (Bradford and Chandler, 1992). Dehydrin- like proteins were shown to be expressed in a range of temperate, recalcitrant species (Gee *et al*., 1994), but the absence of such proteins in

correlation with low ABA levels was found to characterize the mature, recalcitrant seeds of 10 tropical, wetlands species (Farrant *et al*., 1997).

The authors however observed the presence of dehydrin – like proteins in other temperate and tropical recalcitrant (nonwetland) species, and suggested that their occurrance may be habitat–related, perhaps also providing protection against low-temperature stress. Reviewing the above accounts, Berjak and Pammenter (1997), commented that it seems clear that the ability, or lack thereof, to express LEA's or dehydrin- like proteins on its own cannot be taken as an indication that the seeds of a particular species can or cannot withstand dehydration. They concluded that desiccation tolerance must be the outcome of the interplay of more than one (and probably many) mechanisms or processes.

2.3.5.2 Sucrose, oligosaccharides or galactosyl cyclitols

The accumulation of non- reducing sugars , particularly of the raffinose series (Leprince *et al*., 1990) and/or galactosyl cyclitols (Obendorf , 1997), has been implicated in the acquisition and maintenance of the desiccated state in orthodox seeds, generally in two ways. The first of these is the so-called "Water Replacement Hypothesis" (Crowe *et al*., 1992). On dehydration, specific sugars are suggested to replace the water normally associated with membrane surfaces, thereby maintaining the correct lipid head –group spacing and so preventing liquid crystalline-to-gel phase transition in the lipid bilayer. The second proposal concerns vitrification of the aqueous, otherwise referred to as glassy state formation (Obendorf, 1997). On the loss of water, sucrose and certain oligosaccharides or galactosyl cyclitols, form high-viscosity, amorphous, super-saturated solutions. The presence of glasses because of their high viscosity, in effect

impose a stasis (slowing or cessation) on intracellular activity, reducing the deleterious effects of deranged metabolism, protecting macromolecules against denaturation and perhaps preventing or minimizing liquid crystalline-to-gel phase transitions in the membrane lipid bilayer of membranes.

Walters *et al*. (1997) have suggested a quite different role for sugars in that they are associated with LEA's and in this form control the rate of water loss. According to Koster and Leopold (1988), a further advantage of the formation of oligosaccharides, and the subsequent reduction of monosaccharide content of cells, is the removal of immediately-available respiratory substrates. This is relevant in terms of the damaging reactions that can occur as orthodox seeds pass through critical moisture ranges that favour unbalanced metabolism, during maturation drying. According to Berjak and Pammenter (1997), whatever the role(s) of sucrose and oligosaccharides or galactosyl cyclitols in orthodox seeds, seeking parallels for desiccation-sensitive seeds is simply inappropriate as some of the few recalcitrant seed species that have been assayed for sucrose and other oligosaccharides do produce these compounds (Finch-Savage and Blake, 1994). The metastable glassy state occurs at water contents in seeds well below the lethal limit (Obendorf, 1997). According to Pammenter *et al*. (1991), when recalcitrant seeds are dehydrated under ambient conditions, they lose viability at relatively high water contents – in the region of 0.7g (or more) water per g dry mass { 40 percent, wmb }, which are far higher than those required for glass formation to occur (William and Leopold, 1989).

Berjak and Pammenter (2004), agreed that the one involvement of sugars in the variable desiccation sensitivity of recalcitrant seeds might be via the mechanism suggested by

Walters *et al*. (1997) for maturing orthodox seeds, *viz*. the modulating effect of sugar/LEA complexes on dehydration rate. Very marked variability occurs in the rate at which recalcitrant seeds of different species lose water under the same conditions (Berjak and Pammenter, 1997) and it is possible that the significance of sugars and LEA's in the embryos of recalcitrant seeds of some species lies in the modulation of the drying rate by complex formation (Farrant *et al*., 1989)

2.3.6 Development of certain amphipathic molecules

Golovina and Hoekstra (1997), have suggested that there is partitioning of endogenous amphipathic molecules (amphipaths) e.g. flavonoids into membranes upon water loss preventing the formation of the gel phase in the desiccated state and this may be a prerequisite for desiccation tolerance (Hoekstra *et al*., 1992). On rehydration, the amphipaths have been shown to partition back into the cytoplasm. These authors suggested that the partitioning of amphipathic molecules into the bilayer substantially lowers the water content at which the membrane lipids undergo a change from liquid crystalline phase to the gel phase, and thus maintains the integrity of membranes in the dry state in desiccation tolerant organisms. According to Berjak and Pammenter (2004), if the partitioning of amphipaths into membranes is established as a universal phenomenon occurring during dehydration of orthodox seeds, it is possible that they are absent or, if present, incompletely functional or nonfunctional in desiccation-sensitive seeds.

2.3.7 The ability of damage repair on rehydration

Several authors have shown both direct and indirect evidence that repair mechanisms do come into play when dry orthodox seeds are rehydrated (Bray, 1995; Smith and Berjak, 1995). Ultrastructural studies on maize seed provided evidence supporting the fact that there was a mitochondria repair after seed rehydration (Berjak and Villars 1972). Studies on rye seeds by Elder *et al*. (1987) showed that even in the dry state there is progressive deterioration of the DNA as a result of endo- and exonuclease activity during storage, which cannot be repaired until the seeds are rehydrated. Much of the evidence for the operation of repair processes during rehydration comes from osmopriming experiments on low vigour seeds. This process involves controlled rehydration to the end of phase II, which achieves a hydration level that facilitates repair but precludes germination proper (Bray, 1995). In addition the author has shown that replacement of damaged rRNA occurs, and lesions in the DNA and protein-synthesizing systems are repaired, during priming. Smith and Berjak (1995) reported that free radical generation continues in airdried orthodox seed during storage and the ensuing damage must be repaired on rehydration , arguing strongly for the presence and efficient operation of antioxidant systems. During dehydration of desiccated- sensitive seeds, however, such systems have been shown to fail (Hendry *et al*., 1992; Laprine *et al*., 1992) and are assumed to remain ineffective when water is once again provided (Come and Corbineau, 1996). Following rehydration of the highly recalcitrant seeds of *Avicenia marina*, no DNA repair is possible once 22 percent of the originally present water has been lost , suggesting a very inadequate DNA repair system compared with orthodox seeds (Boubriak *et al*., 2000).

2.4 Levels of recalcitrance in seeds

The application of the single term ‘recalcitrant’ to species showing a wide range of post shedding behaviour has led to considerable confusion as there has been an increasing tendency to describe any species that does not show strictly orthodox behaviour as recalcitrant. When the term ‘‘recalcitrance’’ (Roberts, 1973) was first introduced, it was applied in the context of seeds that could not be stored in the air-dry condition. The implication is that such seeds cannot be dehydrated to equilibrium with ambient relative humidity and stored in that condition. However, it has become apparent that the desiccation sensitivity and storage behaviour of ‘recalcitrant’ seeds differ considerably among species (Berjak and Pammenter, 1994).

It has thus been suggested that there is a continuum of recalcitrant seed behaviour , individual species being categorized as highly, moderately or minimally recalcitrant depending on their degree of desiccation sensitivity, hydrated storage lifespan and sometimes, chilling sensitivity (Farrant *et al*., 1988). Ellis *et al*. (1991) have since defined a category of seeds described as having storage behaviour that is intermediate between recalcitrant and orthodox, in that they will tolerate dehydration down to low water contents, but become chilling sensitive in this state. Berjak and Pammenter (1994), recommends that to understand the desiccation responses and seed storage behavoiur of seeds, where possible, future publications on ‘unorthodox’ seeds should include as much information as possible about the properties of the seeds that are pertinent to their characterization.

2.5 Desiccation tolerance /sensitivity as a quantitative feature

Desiccation tolerance/sensitivity has traditionally been regarded as a qualitative feature: a tissue either survives drying or it does not. Walters (1999), however prefers the idea that desiccation tolerance/sensitivity be expressed quantitavely considering the many functions of water in living cells and consequently the numerous stresses that must result when water is not available to fulfill its roles. The moisture level at which various dehydration stresses occur in living cells vary. For example, osmotic excursions (i.e. when water is not available to fill up the volume within cells) are observed at water potentials < -3MPa (Walters, 1999). Changes to membrane structure occur at water content of about 0.20g H_2O/g dw or water potential close to -12MPa (Vertucci and Farrant, 1995).

According to Walters (1999), identification of the various dehydration stresses that occur upon water removal has not received much attention in the literature and there is even less information on the water contents or water potentials at which these stresses are observed in various tissues. The term "critical water content" is used to define the limit to which seed tissues can be dried without apparent damage. Thus, the critical water content marks the level of dehydration stress that the tissue can tolerate (Pammenter *et al*., 1991). The critical water content of seed embryonic axes decrease as they mature. This has been observed in numerous species- orthodox and non- orthodox (Farrant and Walters, 1999). At the early stages of dry matter accumulation, axes of both types of seeds are damaged at water contents as high as 3.0 g H_2O/g dw, and the critical water content declines as seeds mature to about 0.3 g H_2O /g dw in recalcitrant seeds and almost 0g H_2O /g dw in orthodox seeds (Walters, 1999).

2.6 Rate of dehydration affect desiccation tolerance of recalcitrant seeds

Desiccation tolerance in seeds depends on the species, development stage and drying conditions, especially the water removal rate (Liang *et al*., 2000). Slow drying rate can induce tolerance to desiccation in immature orthodox seed or somatic embryos (Black *et al.* 1999; Sanhewe and Ellis, 1996*)* or induce tolerance at high drying temperature in orthodox seeds (Rosa *et al*., 2005). Slow drying in orthodox seeds is an event occurring naturally at the end of maturation stage and in some cases is a prerequisite for germination, as observed in pea (Rogerson and Matthews, 1977) and corn seeds (Herter and Burris, 1989).

In contrast, recalcitrant seeds do not go through drying at the end of maturation stage and apparently do not acquire tolerance to desiccation , probably because the seeds start germination shortly after maturation and at this stage events associated with germination vary among species (Pammenter and Berjak, 1999; Farrant *et al*., 1993).According to these authors, some seeds sensitive to dehydration can tolerate some water loss and the amount varies with the rate at which the water is removed. Fast drying has been reported to allow the tissues of several recalcitrant seeds to achieve greater desiccation tolerance (Pritchard and Manger, 1998; Pritchard, 1991).

Under slow drying conditions, seed tissues have to spend a longer period of time at the intermediate water contents, at which aqueous – based deleterious processes occur. Thus fast drying may minimize such deleterious effects associated with the dehydration of the metabolically active tissues (Pritchard and Manger, 1998; Berjak and Pammenter, 1997). Thomsen (2000) observed that drying rate is a factor that needs to be investigated, as the minimum safe moisture contents are dependent on drying rate, and this needs to be

specified in the recommendations for storage conditions of a species together with moisture content and temperature.

2.7 Electrical conductivity as an indicator of orthodox or recalcitrant seed physiology

Non-viable or deteriorated seeds have been reported to leak more solutes when placed in water than viable or vigorous seeds (Leopold, 1980; Simon and Harun, 1972). Aged, damaged, or non-functional cellular membranes (Simon and Harun, 1972; Murphy and Noland, 1982) and cellular rapture caused by imbibition damage (Duke and Kakefuda, 1981) have been suggested as major causes. The solutes leaked from seeds of various species include free amino acids (Harman and Granett, 1972), proteins (McKersie and Stinson, 1980), sugars (Takayanagi and Murakami, 1969), and phenolics (Samad and Pearce, 1978). Ions and inorganic compounds of potassium, phosphate, and magnesium are also leaked (McKersie and Stinson, 1980; Samad and Pearce, 1978; Simon and Harun, 1972). The rate of leakage is not the same for each substance. Thus Mckersie and Stinson (1980) record that in the first 2 hours scarified Lotus seed lose 13% of their potassium and 9% soluble protein, but only 3.6% sugar, 3.1% of phosphate and 1.4% of amino acids.

Measurement of the conductivity of electrolytes has been adopted as a laboratory procedure to assess solute leakage for different crop seeds (Anon, 1983). Several studies have shown good agreement between conductivity readings, germination and emergence with several crop species (Steere *et al*., 1981) especially when conductivity is expressed per gram of seed (Siddique and Goodwin, 1985), while other studies have yielded conflicting results (Hepburn *et al*, 1984). A possible explanation for these inconsistent results is the large

number of factors that influence conductivity measurements. These include the integrity of the testa (Duke and Kakefuda, 1981; Samad and Pearce, 1978) or pericarp (Wann, 1986), the instrument used for measurement (Hepburn *et al*.,1984), seed moisture content (McDonald and Wilson, 1980), seed weight (Siddique and Goodwin, 1985; Hepburn *et.al*., 1984) and incubation temperature (Tao, 1978).

Time course curves of leakage from dehydrated seeds show initial rapid leakage rates associated with the imbibition of water (Parrish and Leopold , 1977 ; Simon and Harun 1972). It has been proposed that this phase of rapid leakage represents a period of membrane reorganization associated with dehydration, and that subsequent linear leakage rates represent steady-state diffusion of solutes through organized membrane (Simon, 1974). It has also been suggested that water uptake by desiccation-tolerant seeds reinstates the original structure of the celluler membrane, whereas the membranes of desiccation-sensitive seeds that have been dehydrated are unable to reform completely (Mckersie and Stinson, 1980). If dehydration stress increases the amounts of solutes leaked may be detectable in response to dehydration, and these changes should be associated with loss of viability (Becwar *et al*. 1982). In silver maple seeds and areca palm embryos, which are desiccation-sensitive tissues at maturity, an increase in leakage and decrease in viability occurred below a critical moisture content; 40% in silver maple seeds and 55% in areca palm embryos (Becwar *et al*., 1982). The authors stated that their results are consistent with the concept that membranes of desiccation-sensitive seeds are damaged by dehydration below a critical moisture content and do not become effective barriers to solute leakage during imbibition. McKersie and Stinson (1980) interpreted the increased leakage from seeds in the desiccation-sensitive state to indicate an alteration in membrane in

response to dehydration. Simon (1974) suggested that the membrane alterations in seed at low moisture contents involved a lipid phase change from a lamellar to a hexagonal structure.

2.8 Water sorption properties of seeds

Seed water content is crucial to long-term survival of stored seeds, as it affects the rate of metabolic and deteriorative reactions (Vertucci and Roos, 1990). There appears to be critical water content above and below which seed ageing rates increase (Walters, 1998). This optimum water content, which maximizes seed longevity at a given temperature, is species specific. It appears to occur at a constant relative humidity for all species (Walters and Engels, 1989). Therefore, understanding the relationship between the storage temperature, relative humidity and seed water content is essential for developing optimal seed storage protocols (Merritt *et al*., 2003).

Most seeds are hygroscopic, adsorbing or desorbing water until they are in equilibrium with the storage environment (Merritt *et al.*, 2003). Water sorption isotherms describe the relationship between relative humidity and the equilibrium seed moisture content at a given temperature (Reuss and Cassells, 2003). The exact nature of this relationship varies between different species as it is influenced by seed composition (Vertucci and Leopold 1987) and temperature (Eira *et al.*, 1999). According to Vertucci and Roos (1993) and Walters (1998), isotherms of orthodox seeds generally have the reverse sigmoidal shape, indicative of three regions of water binding or tissue water interactions. Desiccation intolerant species have the monotonic shape or a simple rising curve (Eira *et al.*, 1999;

Vertucci and Leopold 1987). Eira *et al*. (1999) have reported that the shape of isotherms of coffee seed tissues was intermediate to the reverse sigmoidal shape observed for orthodox seeds and the monotonic shape observed for desiccation intolerant plant tissues. Thus coffee seeds are now considered to have storage behavior intermediate between recalcitrant and orthodox seeds (Hong and Ellis, 1995; Ellis *et al*., 1991). Isotherms are also useful for analyzing seed water-binding properties (Merritt *et al*., 2003). The strength and nature of water binding in seeds is considered to influence the rate of deteriorative reactions (Vertucci and Roos, 1990). Therefore, water-binding characteristics may be a factor in the variation in seed longevity between different species, although any relationships between seed water-binding properties and storage behavior are yet to be resolved (Eira *et al.*, 1999).

Isotherms constructed at several temperatures also provide insights on the strength of association of water in seeds (Sun *et al*., 1997). Sorption isotherms can be used to estimate to which moisture content seed can be dried in a given climate (Thomsen and Stubsgaard, 1998).

2.9 Germination Media (Substrates)

For seed germination testing, filter papers, blotters, agar, sand or soil are recommended (AOSA, 1992). Each germination medium has its own property and suitability for different species (Smith *et al*., 2002). Soil and compost have serious deficiencies from the point of view of the seed tester. Their composition is subject to considerable variation and this affects their moisture retention capacity. They may contain pathogens which can reduce the germination of seeds tested (Ellis *et al*., 1985). Sand too is bulky but, if prepared well

it can be a satisfactory germination substrate but the laboratory must be designed and equipped to handle it. If seeds have been chemically treated in some way (e.g. by fungicide or insecticides), testing in sand, agar or on paper can result in abnormal germination (stunted growth is a particularly common symptom). In this case it may be necessary to test on compost where such effects are minimized.

In the tropics, paper towels and sand are used for small and large seed germination test, respectively, in the ASEAN Forest Tree Seed Centre, Thailand. Sand is the standard germination medium used for germination tests for all species in the National Tree Seed Program in Tanzania (Msanga, 1998). Sand is probably the most suitable medium for tropical tree seed germination due to its availability, low cost, capacity to hold moisture, and suitability for large seeds (Smith *et al*., 2002). Over the last few years, evidence has been accumulating to suggest that the germination medium has importance beyond merely being a medium for water retention and for transmission of light (Bosy and Aarssen, 1995). When the seeds of eight species were placed on the surface of an agar substrate, or 2mm below the surface, in a variety of orientation, some surprising results were observed. Five of the eight species showed less germination when planted with the with radicle end pointing downward, two showed no differential response to orientation, while one species responded poorly to burial and germinated best when on agar surface (Bosy and Aarssen,1995). These authors speculated that uneven exposure to gravity, oxygen, or light may have been responsible for these effects. No significant effect of different soil types (loamy soil; washed, sterile river sand; or non -sterile river sand) was seen on the

emergence of *Ceiba pentandra*, *Leucaena leucocephala*, *Gmelina arborea* and *Tectona grandis*, although *T. grandis* germination was orientation sensitive (Agboola *et al*., 1993). Paper is the most widely used of all substrates. Seeds are either tested on top of paper or between sheets of paper. Three types of paper are used-blotting paper, filter paper, and paper towels - usually in the form of sheets or dics (Ellis *et al*., 1985). The paper should be porous (to enable water to be held), but strong (to maintain structure when wet), free of fungi and bacteria, and should not contain any toxic substances which would injure germinating seedlings (Ellis *et al*., 1985).

2.10 Temperature and the germination of seeds.

Temperature influences both the percentage of germination and rate of germination of seeds, and it is one of the most critical factors affecting seed germination (Smith *et al*., 2002). Seeds of each species have optimal temperatures for attaining maximum germination; most species however can reach their maximum germination at an alternating temperature regime of an 8-hour light at 30°C and a 16-hour darkness at 20°C (AOSA, 1992).

Alternating temperatures are preferred to constant temperatures because they can overcome shallow seed dormancy and enhance uniform germination. When alternating

temperatures of 30°/20°C, which are prescribed for most tree seed germination (AOSA, 1992) are not available, a constant temperature of 25°C can substitute for them. For most tropical tree seeds, room temperature of 25 to 30°C in the tropics will be quite suitable for maximum germination (Smith *et al*., 2002). The temperature effect can be modified by light as well as by moist chilling treatment (Wang, 1987). Liengsiri and Hellum (1988) noted that while six different provenances of *Pterocarpus macrocarpus* showed different germination characteristics, maximum final germination could be attained for all the sources using alternating temperatures of 30°/25°C (8/16hr). Corbineau and Come (1986) reported that the optimal temperature for the germination of the recalcitrant species *Shorea roxburghii* and *Hopea odorata* was 30 to 35°C. Differences were also noted between the lower temperature limits for seed germination in the two species, and these were different from those of the seedlings. Optimum germination temperatures for *Prosopsis argentina* and *P. alpataco* were shown to be 35°C, with the minimum temperature being 15°C and the maximum 40°C (Villagra, 1995) ; other studies have indicated somewhat lower temperature optima (e.g. 25°C for *P. chilensis* and *P. flexuosa),* although, the lower temperature limit of 15°C seems to be common throughout (Catalan, 1992). Seeds of *Ochroma lagopus* are simulated by very high temperatures, possibly attributed to an association with fire in the natural habitat (Vazquez-Yanes and Orozco –Segovia, 1993).

Mori *et al*. (1990) investigated seed germination and seedling growth of several Malaysian species including *Shorea assamica* , *S. parviflora, Bombax valetonii* and the exotic *Acacia auriculiformis*. The authors observed that performance was linked to the day/night temperature regimes of their respective ecotypes. Seeds of *Manihot glaziovii* are known

to be deeply dormant, possibly as a result of coat-imposed dormancy. Drennan and van Staden (1992), observed that, while incubation of seed at 25°C gave 70 percent germination after 14 days, temperature of 35°C resulted in 98 percent germination, but only if seed were subjected to a temperature shift to 25°C after 21days. Exposure to the ethylene- producing compound, ether, resulted in over 90 percent germination within 14 days, over the temperature range of 20 to 30°C. Temperatures of 35°C and 40°C were inhibitory to germination and, unless seeds were subjected to a temperature shift, no improvement in germination was seen in the presence of ether.

2.11 Species information

2.11.1 *Garcinia kola*

Garcinia kola (Heckel) belongs to the Guttiferrae (Clusiaceae) family (Geeta *et al*., 2006). There are more than 600 species in this genus, of which there are only 16 in West Africa (Steentoft, 1988). *Garcinia. kola* occurs in the wet and semi-deciduous forest zones (Irvine, 1961). The species is a multi- purpose fruit tree producing fruits, seeds, roots and stem which are extensively used in Ghana, Nigeria and other West African countries for dental care in the form of chewing-sticks (Adu-Tutu *et al*., 1979). *Garcinia kola* which is also known as 'bitter cola' is highly used for its medicinal purposes because of its anti-

viral, anti-inflammatory, anti-diabetic, bronchio-dilator and antihepotoxic attributes. Fruit extracts from *G. kola* have proven effective at stopping Ebola virus replication in laboratory test (Anon, 1999). The trees are often felled to facilitate both removal of bark and harvest of chew-sticks, contributing to the increasing scarcity of the species in West Africa. The natural regeneration of the species is poor, and seedlings are uncommon and slow- growing (Gyimah, 2000; Abbiw, 1990), making the species now close to commercial extinction (IUCN, 2004; Hawthorne, 1995).

G. kola is listed as one of the priority species for immediate conservation action in the sub-Saharan forest genetic resource, SAFORGEN programme, and is therefore one of the focus species that is being investigated through the recently established African Tree Seed Research Network (Sacande *et al*., 2004).

Fruiting in the tree commences in July and ends in October (Keay, 1998). The fruits are processed to extract oval-shaped seeds. The fruits are 5 to 10cm in diameter and contain one to four seeds. The average fresh seed weighs about 10grams and is covered by a thin leather-like testa around the endosperm (Gyimah, 2000). The embryo is linear in position and is not well differentiated from the endosperm and cotyledons. The cotyledons do not open up during germination (Ng, 1992). According to Nzegbule and Mbakwe (2001), cultivation of this fruit tree is limited because of poor seed germination. In nursery trials, the seed attained 75% germination over 18 months (Taylor, 1960), illustrating that G. *kola* seed exhibits a high degree of dormancy.

2.11.2 *Garcinia afzelii*

Garcinia afzelii is a species of small to medium tree (about 18m in height) in the Guttiferrae (Clusiaceae) family. It has a short bole and bushy spreading crown It is endemic to the tropical forest of West Africa mostly in the Cameroon and Ghana (AduTutu *et al*., 1979). The fruits are brownish-yellow, to 2 .5 cm in diameter and contain 2– 4 seeds embedded in an acidulous pulp which is edible and much relished (Burkill, 1985). The species is widely exploited as a source of chew stick and is listed as 'vulnerable' (IUCN, 2008). The leaves and flowers are used for their antibacterial properties (Waffo *et al*., 2006). As with other *Garcinia species*, like *G. cambogia* and *G. virgata*, the species can be exploited and used in cosmetic preparations since the crude extract from it has been found to be a free radical scavenger that exhibits significant anti-oxidant effect (Waffo *et al*, 2006). In taking an African-wide overview of medicinal plant resources, Cunningham (1994), mentioned *Garcinia afzelii* as being among those species in the highest threat category and requires urgent conservation attention.

2.11.3 *Terminalia superba*

It belongs to the Combretaceae family (Irvine, 1961). The tree flowers between August and September and fruits between October and November (Irvine, 1961). Its fruit is two-winged and bears a single seed. Seed propagation is by the whole fruit and germination of fleshly collected fruits can be as high as 90% (Gyimah, 1984). This high germination capacity of the seeds, however, is drastically reduced a few months after harvesting under ambient or room temperature (Gyimah, 1984). According to Schaeffer (1990), seeds of tropical genus *Terminalia* are identified as short-lived and recalcitrant and that both

chemical and physical seed coat dormancy has been recognized as well. The FAO (1988) had reported earlier that seeds of the species do not store well under ambient temperature or at high moisture level. Seeds of good initial quality can be stored satisfactorily at a temperature of 4°C, provided a low humidity can be maintained (airtight containers, room with controlled humidity) (FAO, 1988).

2.11.4 *Terminalia ivorensis*

This tree is also a Combretaceae (Irvine, 1961). The species is used as building poles, roofing shingles and for medicinal purposes (Irvine, 1961). Somade (1985), described the seed storage behavior of *Terminalia ivorensis* as orthodox. Long term storage of the species has been possible at the IITA Genebank (Ng *et al*., 1993). The seeds of *Terminalia ivorensis* germinate with difficulty and its inability to germinate is caused probably by the seed coat because this is thick and lignified (Corbineau and Côme, 1994). Extracted embryos germinated much better than un-cut seeds. However, the extracted embryos germinated well only if they have reached proper stage of development prior to collection (Corbineau and Côme, 1994).

Gibberellic acid (GA_3) at high concentrations (10^{-3}M) significantly facilitated the germination of the complete seeds, but this stimulating effect is possible only if the seeds are sufficiently matured (FAO, 1993). Scarification for 3 hours in Sulphuric acid was not sufficient to ensure a satisfactory germination of the seed, but it had positive effect when complemented by soaking in a cellulose solution ($1.25gl^{-1}$). After such a combined treatment, the use of $GA_3 10^{-3}$M was very efficient (FAO, 1993). Willan (1985) and

Agboola (1998), also reported that chemical dormancy apparently occurs in *T. ivorensis* and using ($ZnSO_4$) and alternating temperatures (34°C/24°C), 93% germination was obtained in 41days compared to 27% at a constant temperature of 30°C, both under continuous light.

2.11.5 *Khaya anthotheca*

This species belongs to the Meliaceae family (Irvine, 1961). Its common name is White Mahogany and occurs in lowland evergreen forest. The species is heavily exploited, particularly in East and West Africa and serves as a source of Africa mahogany, used in cabinet and furniture making, veneer, paneling, boat building and joinery (Irvine, 1961). The species it is listed as vulnerable (IUCN, 2002). The trees flower at the end of the dry season or beginning of the rainy season mainly in November, and the fruits mature from January to March (Irvine,1961). When the seeds are matured the fruits will begin to split open. The capsules are dried in the sun until they split and seeds are extracted manually. The seeds are light brown, flat and surrounded by a narrow wing. There are 2000-4000 seeds/kg (Joker, 2003). According to the Regional Soil Conservation Unit (RSCU 1992), it is not possible to dry and store *Khaya anthotheca* seed, due to its short viability and storing seed for longer than 3 months is not recommended under ambient conditions. Joker (2003), however reported that seeds of the species are tolerant to desiccation and should be dried down to a low moisture content (5-7%) and stored in airtight containers. The author further reported that studies on optimal storage temperature indicate that the seeds may be chilling sensitive and that storage at 15°C is better than 5 or -18°C, but results are unclear. Hong *et al*. (1998) classified the seed storage behavior of the species as intermediate.

2.11.6 *Entandrophragma angolense*

This species is also a member of the Meliaceae family (Irvine.1961). Its common name is the African mahogany. It occurs in the rain forest, deciduous forest and the transitional formations of tropical Africa. The commercial exploitation of this timber has resulted in the large-scale extinction of matured individual trees throughout its range. The timber is used for exterior and interior construction, furniture making and flooring. The fruit is five-valved capsule containing numerous winged seeds (Irvine, 1961). According to Olatoye (1968), seed storage behavior of the species is orthodox, but the seed is shortlived at room temperature. Viability can however be maintained for 6 years in cold storage. Tompsett (1994) observed that the seeds tolerate desiccation to 5% moisture content and 76% germination is produced following 150 days subsequent hermatic storage at 2°C.

2.11.7 *Mansonia altissima*

This species is from the family Sterculiaceae (Irvine 1961). It occurs in Benin, Cameroon, Congo, Cote d'Ivoire, Ghana and Nigeria. The species is considered vulnerable (IUCN, 2002) due to excessive exploitation (Hawthorne, 1995). It is protected by law in Cote d'Ivoire where it is considered a priority for in-situ conservation and in Ghana export of the species in log form is banned (FAO, 1984).

4.0 RESULTS

4.1.1 Equilibrium Relative Humidity (eRH) of Seed Samples

Equilibrium relative humidity (eRH) of seed samples measured (using a Rotronic AWVC-DIO instrument at 20ºC) when they were received fresh at the laboratory and after various days of desiccation in silica gel or being kept in moistened vermiculite (control) are presented in Table 4.1.

The eRH measured at 20ºC and moisture content of *Garcinia kola* seeds when they were received fresh at the laboratory in the United Kingdom were 96.9% and 58.0%, respectively. The eRH and moisture content of the species after 30 days of desiccation in silica gel were 57.7% and 20.0%, respectively. *Garcinia kola* seeds kept in moistened vermiculite as control for 30 days had eRH and moisture content of 96.4% and 56.0%, respectively.

The eRH at 20ºC and moisture content of fresh *G. afzelii* when they were received at the laboratory were 93.3% and 38.2%, respectively. The eRH and moisture content of the species after 11 days of desiccation in silica gel were 28.0% and 13.5%, respectively. G. afzelii kept in moistened vermiculite as control for 11 days had eRH and moisture content of 91.3% and 33.1%, respectively.

In the case of *Terminalia superba*, the eRH (%) measured at 20ºC and moisture content were 61.5% and 14.0%, respectively when seeds were received at the laboratory. After 14 days of desiccation in silica gel, the eRH and moisture content of the sample were 7.2% and 3.5%, respectively. *Terminalia superba* seeds kept in moistened vermiculite as control had an eRH and moisture content of 96.9% and 30.0%, respectively.

For *Terminalia ivorensis*, the eRH measured at 20ºC and moisture content were 70.5% and 18.5%, respectively when seeds were received at the laboratory. After 14 days of desiccation in silica gel, an eRH and moisture content of 13.0% and 3.6% respectively were recorded. *Terminalia ivorensis* seeds kept in moistened vermiculite as controls recorded an eRH of 95.6% and seed moisture content of 33.3%, respectively.

For *Khaya anthotheca*, the eRH measured at 20ºC and moisture content were 54.7% and 14.2%, respectively when seeds were received at the laboratory. After 11days of desiccation in silica gel, the eRH and moisture content were 10.1% and 2.2%, respectively. *Khaya anthotheca* seeds kept in moistened vermiculite as control had eRH and moisture content of 93.3% and 37.5%, respectively.

For *Entandrophragma angolense*, the eRH measured at 20ºC and moisture content were 34.9% and 6.4%, respectively when seeds were received at the laboratory. After 8 days of desiccation in silica gel, the eRH and moisture content were 4.9% and 2.2%, respectively. *E. angolense* seeds kept in moistened vermiculite as control had eRH and moisture content of 98.9% and 14.9%, respectively.

For *Mansonia altissima*, the eRH measured at 20ºC and moisture content were 68.0% and 13.4%, respectively when seeds were received at the laboratory. After 11 days of desiccation in silica gel, the eRH and moisture content were 25.3% and 6.9%, respectively. *Mansonia altissima* seeds kept in moistened vermiculite as control had eRH and moisture content of 94.0% and 46.0%, respectively.

Table 4.1: Equilibrium Relative Humidity (eRH) of Seed Samples measured using a Rotronic AWVC-DIO at 20ºC when they were received fresh at the laboratory and after various days of desiccation in silica gel or kept in moistened Vermiculite (Control).

Seed Species	Period of desiccation in silica gel (Days)	eRH of seed samples dried in silica gel (%)	Moisture content of seed samples dried in silica gel (%)	Period of being kept in moistened vermiculite (Days)	eRH of seed samples kept in moistened vermiculite (%)	Moisture Content of seed samples kept in moistened Vermiculite (%)
Garcinia kola	30	57.7 (96.9)	20.0 *(58)	30	96.4 (96.9)	56 *(58)
Garcinia afzelii	11	28 (93.3)	13.5 *(38.2)	11	91.3 (93.3)	33.1 *(38.2)
Terminalia superba	14	7.2 (61.5)	3.5 *(13.8)	14	96.9 (61.5)	30.0 *(13.8)
Terminalia ivorensis	14	13.0 (70.5)	3.6 *(18.5)	14	95.6 (70.5)	33.3 *(18.5)
Khaya anthotheca	11	10.1 (54.7)	2.2 *(14.2)	11	93.3 (54.7)	37.5 *(14.2)
E. angolense	8	4.9 (34.9)	2.2 *(6.4)	8	98.9 (34.9)	14.9 *(6.4)
Mansonia altissima	11	25.3 (68.0)	6.9 *(13.4)	11	94.0 (68.0)	46.2 *(13.4)

Figures in brackets are the Equilibrium Relative Humidity (eRH) (%) of seed samples when they were received fresh at the laboratory while figures in brackets with asterisks are the seed sample moisture contents (%) when they were received fresh at the laboratory.

4.1.2 Seed Dimension and Weights of Species

Seed dimensions and seed weights of the species based on 50 individuals measured are presented in Table 4.2**.** The data are mean values (± SD).

Garcinia kola: Mean length, breadth and width of *Garcinia kola* seed were 35.4 ± 4.5 mm, 20.1 ± 1.8 mm and 18.3 ± 2.3 mm, respectively. The mean seed weight obtained for the species was 8.9 ± 2.3 g.

Garcinia afzelii: Mean seed length and width of *G. afzelii* were 14.6 ± 1.8 and 8.9 ± 1.2 mm, respectively. Mean seed weight recorded for the species was 0.53 ± 0.03g.

Terminalia superba: The mean seed length, breadth and width recorded for *T. superba* were 20.3 ± 2.7, 6.6 ± 0.9 and 5.6 ± 0.5 mm, respectively. The mean seed weight obtained for *T. Superba* was 0.12 ± 0.02 g.

Terminalia ivorensis: The mean seed length, breadth and width recorded were 22.1±1.8mm, 9.5 ±0.8mm and 5.8±0.6 mm, respectively. The mean seed weight obtained for *T. ivorensis was* 0.16 ± 0.02 g.

Khaya anthotheca *and* ***Entandrophragma angolense***: Mean seed weight of 0.26 ± 0.05 g and 0.46 ± 0.08 g respectively were obtained for *K. anthotheca* and *E. angolense*. Seed dimension data was not obtained for the two species because the seeds do not possess distinct length and width.

Mansonia altissima: The mean seed length, breadth and width recorded for *M. altissima* were 11.5 ± 0.9 mm, 7.5 ± 0.7 mm and 7.2 ± 0.7 mm, respectively. The mean seed weight obtained for *Mansonia altissima* was 0.21 ± 0.02 g.

Table 4.2: Seed dimensions and seed weights of species. 50 individuals were measured.

The data are mean values (± SD)

Seed Species	Length(mm)	Breadth (mm)	Width (mm)	Weight/Seed (g)
Garcinia kola (whole seed)	35.4 ± 4.5	20.1 ± 1.8	18.3 ± 2.3	8.90 ± 2.31
Garcinia afzelii	14.6 ± 2.5	-	8.9 ± 1.2	0.53 ± 0.02 -
Terminalia superb	20.3 ± 2.7	6.6 ± 0.9	5.6 ± 0.5	0.12 ± 0.02
Terminalia ivorensis	22.1 ± 1.8	9.5 ± 0.8	5.8 ± 0.6	0.16 ± 0.02
Khaya anthotheca	-	-	-	0.26 ± 0.05
Entandrophragma angolense	-	-	-	0.46 ± 0.08
Mansonia altissima	11.5 ± 0.9	7.5 ± 0.7	7.2 ± 0.7	0.21 ± 0.02

4.1.3 Initial Moisture Contents of whole seed and seed components

The initial moisture contents of whole seed and seed components of the species are presented in Table 4.3. The average initial fruit, seed, mesocarp and seed coat moisture content obtained for *G. kola* were 86.6%, 58.0%, 93.2% and 79.4%, respectively. In the case of *G. afzelii*, the initial fruit, seed, mesocarp and seed coat moisture content obtained were 65.0%, 35.2%, 73.8% and 61.2%, respectively. The seed, decoated seed and seed coat moisture content of *Terminalia superba* were 14.0%, 15.6% and 7.3%, respectively. *Terminalia ivorensis* recorded 18.5%, as seed moisture content, 19.3% for decoated and 8.3% as seed coat moisture. *Khaya* anthotheca registered values of 14.2%, 11.6% and 27.2% as seed, decoated seed and seed coat moisture content, respectively. For *Entandrophragma angolense*, the seed, decoated seed and seed coat recorded average moisture content of 6.4%, 5.1% and 9.5%, respectively. The average initial

seed, decoated seed and seed coat moisture contents for *Mansonia altissima* were 13.4%, 12.3% and 15.2%, respectively.

Table 4.3: Initial moisture content of whole seed and seed components of the species.

Initial Moisture Content (%)					
Species	**Fruit**	**Seed**	**Mesocarp**	**Decoated**	**Seed**
seed		**coat**			
G. kola	86.8	58.0	93.2	_	79.4
G. afzelii	65.0	35.2	73.8	_	61.2
T. superba	_	14.0	_	15.6	7.3
T. ivorensis	_	18.5	_	19.3	8.2
K. anthotheca	_	14.2	_	11.6	27.2
E. angolense	_	6.4	_	5.1	9.5
M. altissima	_	13.4	_	12.3	15.2

4.1.4 Desiccation and germination trials on *Garcinia kola*

Results of the desiccation and germination trials carried on the species are presented in Tables 4.4 to 4.6. Whole seeds of *Garcinia kola* with initial moisture content of 58.0% had a germination percentage of 80.0%. When seeds were desiccated in silica gel (SG) or under shade (SD), seed viability declined gradually with decreasing moisture content (Table 4.4). At moisture contents of 52.1% and 40.3% (SG) germination significantly ($p < 0.001$) reduced to 75% and 60% respectively. Seed germination at moisture content of 30.2% was 52% and this drastically declined to 13 % at a moisture content of 27.5%. After 20 days drying in silica gel, the moisture content had reduced to 24.3%, and none of the seed germinated, (0%). For seed dried under shade (SD), seed germination at moisture contents of 50.1% and 43.0% significantly reduced to 75% and 64%, respectively. Germination at moisture content of 30.8% was 50% which significantly reduced to 3% at seed moisture level of 26.1 % after 20 days of drying.

Table 4.4: Germination of whole *Garcinia kola* seed dried to various moisture contents in silica gel and under shade at ambient temperature condition (28-30ºC)

Period of drying in Silica Gel (Days)	Seed Moisture Content (%)	Seed germination (%)	Period of drying under shade (Days)	Seed Moisture Content (%)	Seed germination (%)
0	58.0	80	0	58.0	80
2	55.3	77	2	56.2	78
4	52.1	75	4	54.3	75
6	48.4	72	6	50.1	75
8	44.8	65	8	46.8	73
10	40.3	60	10	43.0	64
12	36.4	54	12	38.0	57
14	33.3	52	14	35.1	55
16	30.2	52	16	32.2	55
18	27.5	13	18	30.8	50
20	24.3	0	20	26.1	3
SED	2.13	2.34	SED	2.18	2.41
CV	3.5%	3.9%	CV	3.1%	4.3%

Proximal sections of *G. kola* with initial moisture content of 58.0% had 85.0 % germination. When the seed portions were desiccated in silica gel or under shade, seed viability declined gradually with decreasing moisture content. At moisture contents of 50.4 % and 40.2 % (SG) germination significantly ($p < 0.001$) reduced to 72 % and 60 %, respectively. Seed germination at moisture content of 31.0% was 53% and this drastically declined to 10 % at a moisture content of 26.6%. For shade drying (SD), seed germination at moisture contents of 53.9 % and 42.4 % significantly ($p < 0.001$) reduced to 76% and 64%, respectively. Germination at moisture content of 31.3. % was 52%

and this reduced to 10% at seed moisture level of 25.5 % after 18 days of drying (Table 4.5).

Table 4.5: Germination of proximal sections of *Garcinia kola* seed dried to various moisture contents in Silica gel and under shade at ambient temperature condition (28-30 ºC).

Period of drying in silica Gel (Days)	Seed moisture content (%)	Seed germination (%	Period of drying under shade(Days)	Seed moisture content (%)	Seed germination (%

0	58.0	85	0	58.0	. 85
2	53.5.	77	2	55.8	79
4	50.4	72	4	53.9	76
6	46.7	70	6	50.1	73
8	43.5	66	8	46.0	68
10	40.2	60.	10	42.4	64
12	35.3	56	12	38.1	59
14	31.0	53	14	35.6	55
16	26.6	10	16	31.3	52
18	-	-	18	25.5	10
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SED	2.21	2.25	SED	2.32	2.35
CV	2.7%	3.1%	CV	3.8%	4.1%

Distal sections of *G. kola* with initial moisture content of 58% had 95% germination (rooting). When the seed portions were desiccated in silica gel or under shade, seed viability declined gradually with decreasing moisture content. At moisture contents of 50.1 % and 40.3% (SG) germination (rooting) significantly ($p < 0.001$) reduced to 80 % and 62 %, respectively. Rooting at moisture content of 31.2% was 55% and this drastically declined to 15 % at a moisture content of 26.4%. For sections dried under shade (SD), rooting at moisture contents of 53.4% and 42.3 % significantly ($p < 0.001$) reduced to 82% and 65%, respectively. Rooting at moisture content of 31.3. % was 55% and this significantly reduced to 10% at seed moisture level of 26.6 % (Table 4.6).

Table 4.6: Germination of distal sections of *Garcinia kola* seed dried to various moisture contents in silica gel and under shade at ambient temperature condition (28-30 ºC)

Period of drying in silica gel (Days)	Seed moisture content (%)	Seed germination (%)	Period of drying under shade(Days)	Seed moisture content (%)	Seed germination (%)

0	58.0	95	0	58.0	. 95.0
2	53.7	85	2	56.1 53.4	87.0
4	50.1	80	4	50.0 46.0	82.0 78.0
6	46.3	72	6	42.3 38.0	77.0 65.0
8	43.0	65	8	35.0 31.3	64.5 57.5
10	40.3	62	10	26.6	55.0
12	35.0	60	12	----------	10.0
14	31.2	55	14	2.29	----------
16	26.4	15.0	16	1.9%	2.11
18	22.4	10.0	18		2.1%
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SED	2.45	2.33	SED		
CV	3.3%	2.5%	CV		

4.1.5 Desiccation and germination trials on *Garcinia afzelii*

Garcinia afzelii seeds with initial moisture content of 31.5% had 100% germination. When seeds were desiccated in silica gel or under shade, seed viability declined gradually with decreasing moisture contents. Seed dried in silica gel (SG), with 26.7% and 25.0% moisture content had 94% and 88% germination percent, respectively, Seed viability drastically declined to 43% at 23.3% moisture content and a complete loss of viability at 9.2%. Seeds with 27.6% and 25.3% moisture after drying under shade gave 94% and 92% germination, respectively. Below a moisture content of 25.3% seed viability drastically and significantly ($p < 0.001$) declined to 43% at 23.6% moisture content and a complete loss of viability at 11.0% seed moisture (Table 4.7).

Table 4.7: Germination of *Garcinia afzelii* seeds dried to various moisture contents in silica gel and under shade at ambient temperature condition (28-30 ºC)

Period of drying in silica gel (Days)	Seed moisture content (%)	Seed germination (%)	Period of drying under shade (Days)	Seed moisture content (%)	Seed germination (%)
0	31.5	100	0	31.5	. 100
2	28.8	96	2	29.2	98
4	26.7	94	4	27.6	94
6	25.0	88	6	25.3	92
8	23.3	43	8	23.6	43
10	21.0	18	10	21.7	18
12	16.1	12	12	17.5	13
14	14.4	10	14	15.5	11
16	11.3	8	16	12.4	9
18	9.2	0	18	11.0	0
	SED = 1.23	SED = 2.37		SED = 1.15	SED = 2.33
	CV = 3.3%	CV = 3.6%		CV = 2.8%	CV = 3.4%

4.1.6 Desiccation and germination trials on the other species

Terminalia superba seeds with initial moisture content of 16% had a germination percentage of 78.5 ± 5.0% (4.8). Seed desiccated in silica gel to 5% moisture content had a germination percentage of 77.5 ± 2.9%. Desiccation to 14%, 11% and 8% resulted in 78.8 ± 2.5 %, 80.0 ± 4.1 and 80.5 ± 2.9% germination, respectively. The control experiment showed that *T. superba* seeds at 24% moisture content had 75.8 ±

2.3% germination as shown in Table 4.8

Table 4.8: Germination % (± SD) of *T. superba* seeds after desiccation to different moisture content

Treatment	Drying time(days)	Seed moisture content (%)	Germination (±SD %)
Drying in silica gel	0	16	78.5 ± 5.0
	1	14	78.8 ± 2.5
	2	11	80.0 ± 4.1
	3	8	80.5 ± 2.9
	4	5	77.5 ± 2.9
Control (Vermiculite)	0	20	77.5 ± 3.8
	1	23	77.3 ± 4.3
	2	Not determined	78.0 ± 6.2
	3	24	75.8 ± 2.3
	4	21	75.5 ± 3.1

Entandrophragma angolense seeds with initial moisture content of 13% recorded a germination of 73.3 ± 2.5%. Seed desiccated in silica gel down to 4% moisture content also had a germination of 77.5 ± 2.9%. Seed desiccated for 1 to 4 days had 10%, 8%, 5% and 4% moisture content with viability being 75.0 ± 0%, 80.3 ± 2.5% , 77.3 ± 2.5% and 77.5 ± 2.9, respectively. The control experiment showed that *E. angolense* seeds at 19% moisture content germinated to 70.4 ± 2.3% as shown in Table 4.9

Table 4.9: Germination % (± SD) of *E. angolense* seeds after desiccation to different moisture content.

Treatment	Drying time(days)	Seed moisture content (%)	Germination (± SD %)

Drying in silica gel	0	13	75.3 ± 2.5
	1	10	75.0 ± 0
	2	8	80.3 ± 2.5
	3	5	77.3 ± 2.5
	4	4	77.5 ± 2.9
Control (Vermiculite)	0	15	73.0 ± 0
	1	18	73.5 ± 2.1
	2	Not determined	73.3 ± 0
	3	19	70.4 ± 3.4
	4	17	75.3 ± 2.6

Khaya anthotheca seeds with initial moisture content of 14% had a germination of 76.3 ± 2.5% (Table 4.10). Seed desiccated in silica gel to 4% moisture content gave 77.5 ± 2.9% germination. Desiccation of the seed to 11%, 8% and 6% moisture content resulted in 77.5 ± 2.9%, 83.5 ± 5.0% and 80.6 ± 2.5% germination, respectively. The control experiment showed that *K. anthotheca* seeds kept in moistened vermiculite for 3 days had 23% moisture content and germination percentage of 73.4 ± 2.3 as shown in (Table 4.10).

Table 4.10: Germination % (± SD) of *K. anthotheca* seeds after desiccation to different moisture contents.

Treatment	**Drying time(days)**	**Seed moisture content (%)**	**Germination (± SD %)**

Drying in silica gel	0	14	76.3 ± 2.5.
	1	11	77.5 ± 2.9
	2	8	83.5 ± 5.0
	3	6	80.6 ± 2.5
	4	4	77.5 ± 2.9
Control (Vermiculite)	0	18	73.4 ± 2.3
	1	22	73.0 ± 2.8 77.0
	2	Not determined	± 1.5 73.4 ±
	3	23	2.3
	4	21	75.2 ± 3.4

Mansonia altissima seeds with initial moisture content of 13% had 68.8 ± 2.5% germination. Seed desiccated in silica gel down to 4% also had 71.3 ± 2.5% germination (Table 4.11). Seed desiccation to 10%, 7% and 6% resulted in 70.0 ± 4.1%, 71.3 ± 2.5% and 71.3 ± 2.2% germination, respectively. The control experiment showed that *Mansonia altissima* seeds placed in moistened vermiculite increased in moisture content from 16 at 20% after 4 days. Viability however did not change much and was still within the 68% range at 68 ± 1.8% as (Table 4.11).

Table 4.11: Germination % (± SD) of *Mansonia altissima* seeds after desiccation to different moisture contents.

Treatment	**Drying time(days)**	**Seed moisture content (%)**	**Germination (± SD %)**

Drying in silica gel	0	13	68.8 ± 2.5
	1	10	70.0 ± 4.1
	2	7	71.3 ± 2.5
	3	6	71.3 ± 2.2
	4	4	71.3 ± 2.5
Control (Vermiculite)	0	16	68.3 ± 1.9
	1	19	68.0 ± 2.6
	2	Not determined	65.8 ± 3.4
	3	21	65.0 ± 2.4
	4	20	68.0 ± 1.8

4.1.7 Prediction of drying periods from mean drying curves of species

Results of the drying curve experiments are shown in Figures 4.1 to 4.4**.** At the end of the humidification (rehydration) period, moisture contents of the species were found to be: *Terminalia superba* 21.4%, *Terminalia ivorensis* 23.5%, *Khaya anthotheca* 23.3% and *Entandrophragma angolense* 23.3%, Seed moisture content declines steeply during the initial stages of drying in silica gel and as seed moisture content reduced the rate of seed moisture losses also reduced.

At an ambient temperature of (26-30°C) *Terminalia superba* seed sample (20g in weight) of initial moisture content 21.4%, lost moisture rapidly to 15.3% at the end of the second day of drying in an equal weight of silica gel. Moisture content was 11.8% after the third day of drying. Seed moisture slowly reduced to equilibrium moisture of 3.6% after 10 days of drying. From Figure 4.1, it could be shown that the time (days) to dry 20 grams

T. superba seeds of initial moisture content 21.4% to 4.0% is 6 days as indicated by lines from the drying curve to the time axis (x-axis) i.e. 7 days minus 1 day = 6 days.

At an ambient temperature of (26-30°C), *Terminalia ivorensis* seed sample (20g in weight) of initial moisture content 23.5%, had seed moisture rapidly dropped to 16.3% at the end of second day of drying. Moisture content was 11.3% after the third day of drying. Seed moisture slowly dropped to equilibrium moisture content of 4.0 after 9 days of drying. From Figure 4.2**,** it could be shown that the time (days) to dry 20g *T. ivorensis* seeds of initial moisture content 23.5% to 4.0% is 7 days as indicated by lines from the drying curve to the time axis (x-axis) i.e. 8 days minus 1 day = 7 days.

At an ambient temperature of (26-30°C), *Entandrophragma angolense* seed sample (20g in weight) of initial moisture content 20.8%, had seed moisture dropped rapidly to 15.5% at the end second day of drying in an equal weight of silica gel. Moisture content was 10.8% after the third day of drying. Seed moisture slowly reached equilibrium moisture content of 5.2% after 9 days of drying. From Figure 4.3**,** it could be shown that the time (days) to dry 20 grams *E. angolense* seed of initial moisture content 20.8% to 5.3% is 6 days as indicated by lines from the drying curve to the time axis (x-axis) i.e. 7 days minus 1 day = 6 days.

At an ambient temperature of (26-30°C), *Khaya anthotheca* seed sample (20g in weight) of initial moisture content of 23.3%, had seed moisture rapidly dropped to 16.3% after the second day of drying in an equal weight of silica gel. Moisture content was 10.6% after the third day of drying. Seed moisture slowly reached equilibrium level of 4.4% after 9 days of desiccation. From figure 4.4**,** it could be shown that the time (days) to dry 20 grams of *K. anthotheca* seed of initial moisture content of 23.3% to 4.4%

is 8 days as indicated by lines from the drying curve to the time axis (x-axis) i.e. 8 days minus 1 day = 7 days. i. *T. superba*

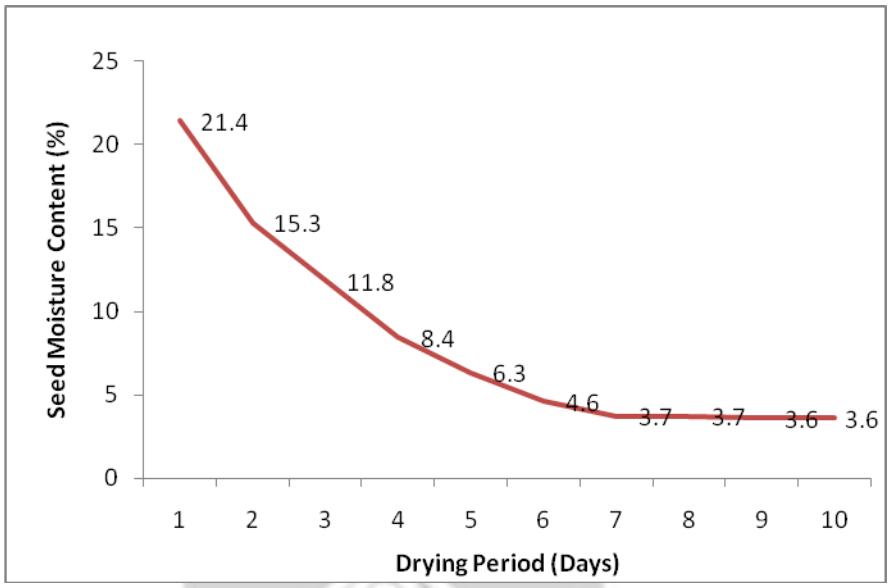

Figure 4.1: Drying curve of *Terminalia superba* seed. 20g sample of seed was dried in silica gel in a ratio of 1: 1 at an ambient temperature (26-30°C).

ii. *T. ivorensis*

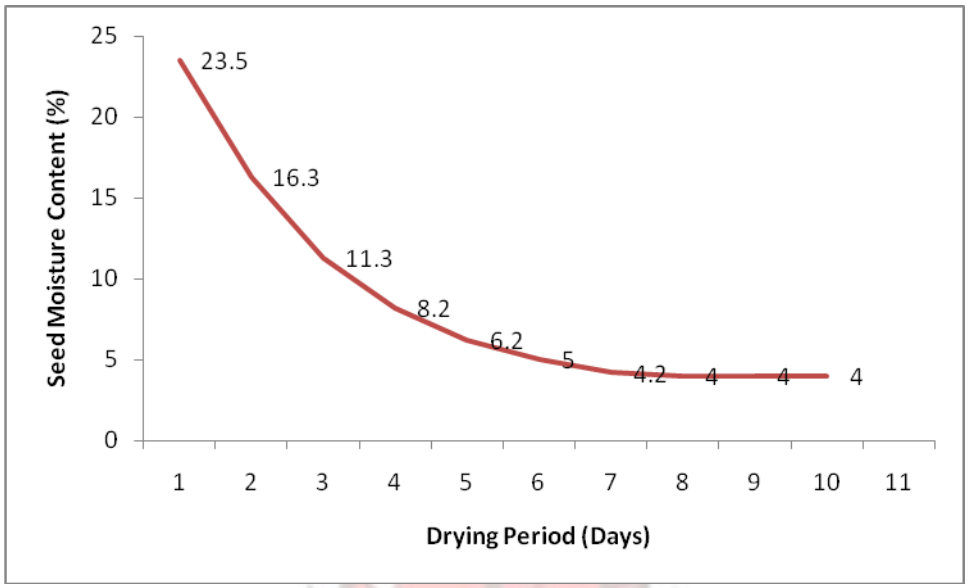

Figure 4.2: Drying curve of *Terminalia ivorensis* seed. 20g sample of seed was dried in silica gel in a ratio of 1: 1 at an ambient temperature (26-30°C).

iii. *E. angolense*

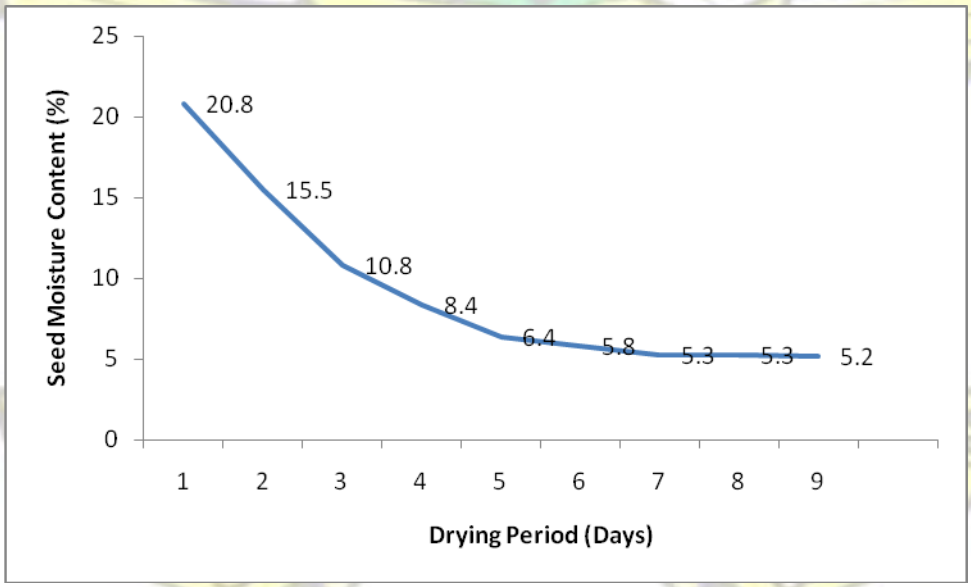

Figure 4.3: Drying curve of *Entandrophragma angolense* seed. 20g sample of seed was dried in silica gel in a ratio of 1: 1 at an ambient temperature (26-30°C).

iv. *K. anthotheca*

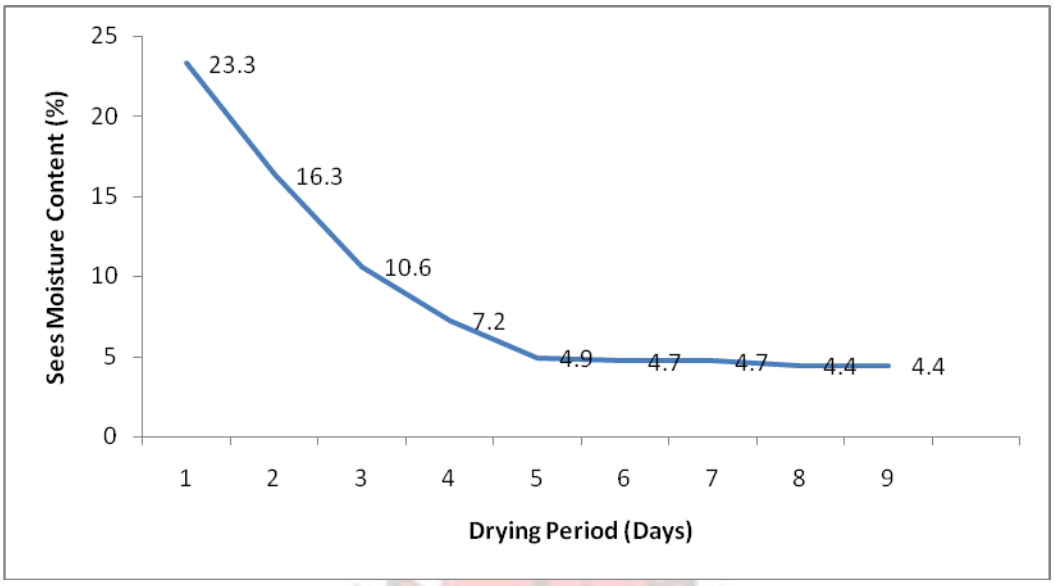

Figure 4.4: Drying curve of *Khaya anthotheca* seed. 20g sample of seed was dried in silica gel in a ratio of 1: 1 at an ambient temperature (26-30°C)

4.2 Germination pattern observed in *Garcinia kola* seed and seed sections

Germination in whole seed of *Garcinia kola* began with the emergence of the primary root (PR) at the distal end of the seed followed by the appearance of a shoot from the proximal end. Subsequently, prior to leaf differentiation, an adventitious root (AR) originated from the base of the shoot. The primary root (PR) disintegrated later and eventually the adventitious root took over as the main root system of the plant. Germination of Distal Section (DS) and Half Distal Section (HDS) of *G. kola* started with the primary root emerging at the distal end of the seed section. No emergence of shoot was observed from any part of the seed section. Subsequently, the primary root degenerated later leading to the termination of any further development.

The Proximal Section (PS) and Half Proximal Section (HPS) of *G.kola* showed a similar germination pattern. There was the appearance of a shoot from the proximal ends of the

sections followed by the appearance of adventitious roots (AR) from the bases of the shoots. No primary root emerged from the cut ends of the seed sections. Like the whole seed, the Proximal Section (PS) and Half Proximal Sections (HPS) developed normal seedlings.

No emergence of root or shoot was observed when the Middle Section (MS) and Half Middle Section (HMS) of *G. kola* were set for germination.

Germination of Distal End Cut (DEC) fragments of *G. kola* initiated with the appearance of a shoot from the proximal end. Subsequently, a robust and vigourous adventitious root emerged from the base of the shoot. In very few cases a primary root (PR) emerged from the distal end of the seed section which had been severed. Like the primary roots which appeared in the other seed fragments of the species, this soon degenerated and eventually the adventitious root took over as the main root system of the young seedling.

Germination of the Proximal End Cut (PEC) fragment of *G. kola* also started with the appearance of a primary root (PR) from the intact distal end followed by the emergence of a shoot at the cut proximal end. There was a subsequent appearance of a robust and vigorous adventitious root from the base of the developing shoot and soon there was the degeneration of the primary root and eventually the adventitious root took over as the main root system of the plant.

Germination of Distal and Proximal Ends Cut (DPEC) section began with a shoot emerging at the cut proximal end. This was followed by the appearance of an adventitious root from the base of the shoot. In some cases, the distal cut end of the seed fragment gave rise to a primary root which soon degenerated whilst the adventitious root took over as the main root system of the plant. A mass of jelly-like substance covered the two cut ends of the seed portions prior to root and shoot emergence.

(Figures 4.5 and 4. 6).

FIGURE 4.5: Panel A, germinating seed of *G. kola* showing a primary root (PR) at the distal end and a shoot with an adventitious root at the proximal end. **Panel B,** germinating half proximal section (HPS) of *G.kola* seed showing a shoot with an adventitious root. **Panel C,** germinating distal sections (DS) of *G. kola* seed showing the primary roots (PR). **Panel D**, germinating proximal sections of *G. kola* seed showing shoots and adventitious roots (AR) at the bases of the shoots.

FIGURE 4.6: Panel A1, proximal end cut (PEC) of *G. kola* seed showing a primary root at the intact distal section and an emerging shoot with an adventitious root at the cut proximal end. **A2,** a distal end cut (DEC) section of the seed showing an emerging shoot with an adventitious root from the intact proximal end. **Panel B**, half distal sections (HDS) of *G.kola* seeds showing emerging roots from the distal ends

4.3 Temperature Regimes, Substrate and Seed Germination:

4.3.1 *Garcinia kola*

Tables 4.12 and 4.13 summarise time (days) of first germination, mean germination time (days) and germination percentages recorded when whole seed and seed fragments of *Garcinia kol*a were sown on agar at different temperatures ranging from 20-35ºC.

Germination temperatures and the type of material used for germination interacted to give significant differences ($p < 0.001$) in days to first germination, germination percentages and mean germination times.

The fastest 1st germinations in days were recorded when Half Proximal Section(HPS), Proximal Section(PS) and Proximal End Cut(PEC) seed pieces were used as germinating materials at 25 and 30 ºC. These were significantly faster than ($p < 0.001$) 1st germinations recorded at the other temperatures using other germinating materials. The longest time in days for 1st germination was recorded when whole seed (WS) of

G. kola was used as germination material at 20 ºC

Germination percentages of Half Proximal Section (HPS), Proximal Section (PS) and Proximal End Cut (PEC) seed pieces set at 25, 30 and 35 ºC over the period were significantly higher than germination percentages recorded for similar germination material at 20 ºC.

Temperatures at which the germination materials were incubated as well as chemical pre-treatment did not influence the germination pattern observed in the various germination materials of *Garcinia kola*.

Table 4.12: Germinating *Garcinia kola* seed and seed parts at 20 and 25 ºC on agar over a period of 20 weeks with no chemicals

	20°C			25°C			
Germinating material	**1st Germ.(Days)**	**Germ (%)**	***MGT(Days)**	**1st Germ. (Days)**	**Germ (%)**	***MGT (Days)**	Observations
Whole Seed	59	35	149	45	45	125	Normal Seedlings
Distal/Half Distal Section	-	-	-	-		-	Rooting only
Middle / Half Middle Section	-	-	-	-	-	-	No emergence of root or shoot observed
Proximal Section	35	63	93	26	73	83	Normal Seedlings
Half of Proximal Section	30	65	89	23	83	78	Normal Seedlings
Distal end chipped off	41	50	97	33	63	87	Normal Seedlings
Proximal end chipped off	38	68	96	30	75	84	Normal Seedlings
Distal and Proximal ends cut off	49	30	127	43	40	119	Normal Seedlings, heavy mass of jelly-like substance covers cut ends of seed portions
s.e.d	1.08	2.7	1.42	1.08	2.7	1.42	
cv	3%	4.5%	1.4%	3%	4.5%	1.4%	

*MGT is Mean germination time in days.

Table 4.13: Germinating *Garcinia kola* seed and seed parts at 30 and 35 °C on agar over a period of 20 weeks with no chemicals

	30°C				**35°C**			
Germinating material	**1st Germ. (Days)**	**Germ (%)**	***MGT (Days)**		**1st Germ. (Days)**	**Germ (%)**	***MGT (Days)**	Observations
Whole Seed	43	55	113		49	45	127	Normal Seedlings
Distal /Half Distal Section	-	-	-		-	-	-	Rooting only
Middle /Half Middle Section	-	-	-		-	-	-	No emergence of root or shoot observed
Proximal Section	24	80	75		29	70	87	Normal Seedlings
Half of Proximal Section	21	80	71		27	75	82	Normal Seedlings
Distal end chipped off	32	65	83		34	55	91	Normal Seedlings
Proximal end chipped off	30	83	81		30	73	87	Normal Seedlings
Distal and Proximal ends cut off	36	43	112		44	30	124	Normal Seedlings , heavy mass of jelly-like substance covers both cut ends of seed portions.
s.e.d	1.08	2.7	1.42		1.08	2.7	1.42	
cv	3.0%	4.5%	1.4%		3.0%	4.5%	1.4%	

*MGT is Mean germination time in days.

4.3.2 Chemical pre-treatment of distal sections of *Garcinia kola* seed and temperature on root emergence:

The interaction effect between chemical treatment and rooting temperature on rooting percentage of distal sections of *G. kola* seed (Figure 4.7) showed that there were significant differences between treatments ($p<0.001$). In all chemical pre-treatments, rooting was lowest at 20°C and highest at 30°C. At 30°C, rooting percentage recorded for treatment GA_3 (500mg/l) was significantly higher than all the other treatments. This was followed by the treatment GA_3 (1000mg/l) which was also significantly higher than all the other treatments at 30°C. At 25°C, the highest rooting percentage was recorded for the treatment GA_3 (500mg/l). This was significantly higher than all the other treatments at 25°C. At 25°C, there were no significant differences between rooting percentages recorded for treatments Citric acid(2g/l), GA_3(1000mg/l) and KNO_3(1g/l). Rooting percentage in the Control (No chemical pre-treatment) experiments at all temperatures was significantly lower than rooting percentages recorded for any of the other treatments.

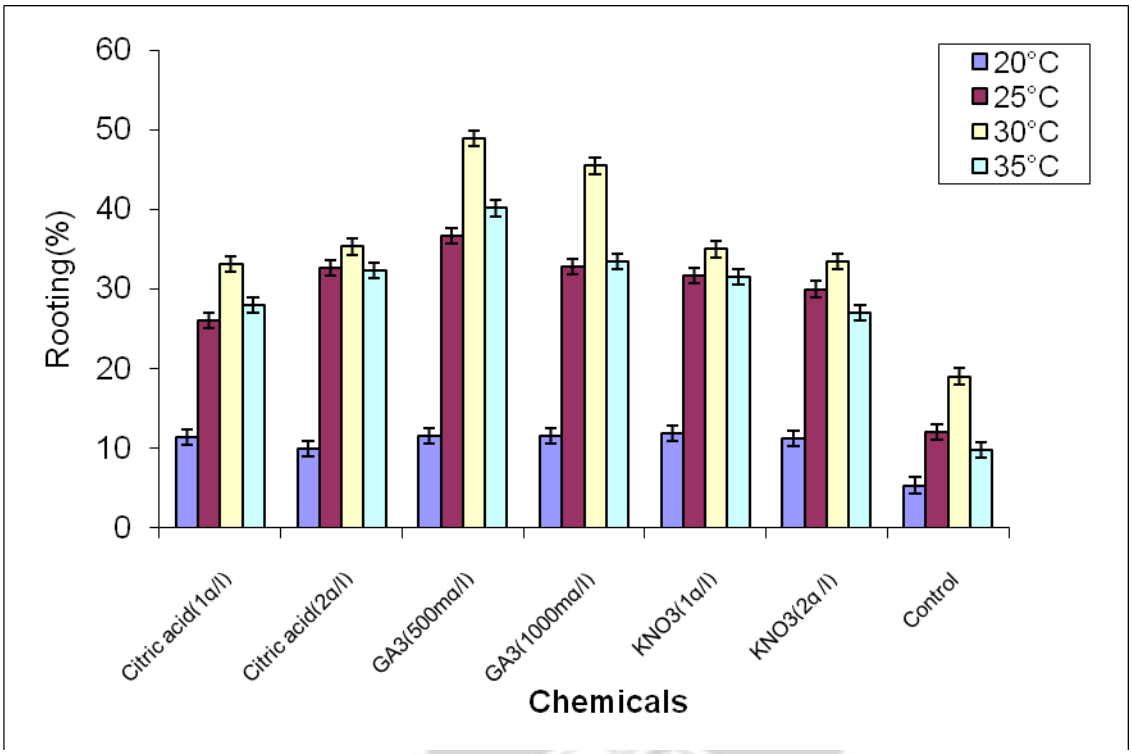

FIGURE 4.7: Effect of chemical pre-treatment of distal sections of *G. kola* seed and temperature on rooting. Bars represent two standard error of the difference.

4.3.3 Responses of Proximal Sections, Half Proximal Sections, Proximal End Cut Sections and Distal End Cut Sections of *Garcinia kola* seed to germination temperatures.

The interaction effect between seed sections and germination temperatures on germination percentages of Proximal Sections (PS), Half Proximal Section (HPS), Proximal End Cut Section (PEC) and Distal End Cut Section (DEC) of *Garcina kola* (Figure 4.8) indicated that there was significant interaction between seed sections and germination temperatures ($p<0.001$). All the seed sections germinated least at 20°C and performed best at 25 and 30°C. Germination of seeds sections at 35°C was significantly lower than at 25 and 30°C (Figure 4.8)

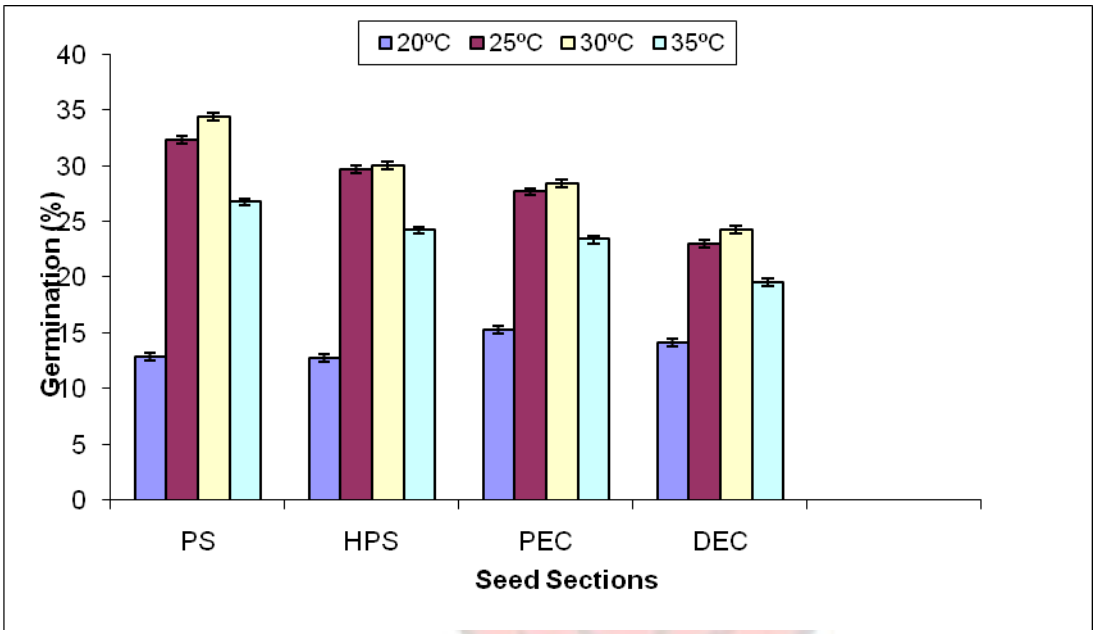

FIGURE 4.8: Responses of seed sections of *G. kola* to germination temperatures of 20- 35°C. Bars represent two standard error of the difference.

PS = Proximal Section HPS = Half of Proximal Section

PEC = Proximal End Cut DEC = Distal End C

4.3.4 Germination temperature and period of germination on cummulative germination percentage of proximal sections of *Garcinia kola* seed

The interaction effect between germination temperatures and germination period on cummulative germination percentages of proximal sections of *Garcina kola* (Figure 4.9) indicated that there was significant interaction between germination temperatures and period of germination ($p<0.001$). Throughout the 8 weeks that germination was recorded, cumulative germination was significantly higher at 25 and 30°C than at 35°C. Cumulative germination at 20°C was significantly lower than what was recorded at the other temperatures throughout the period (Figure 4.9).

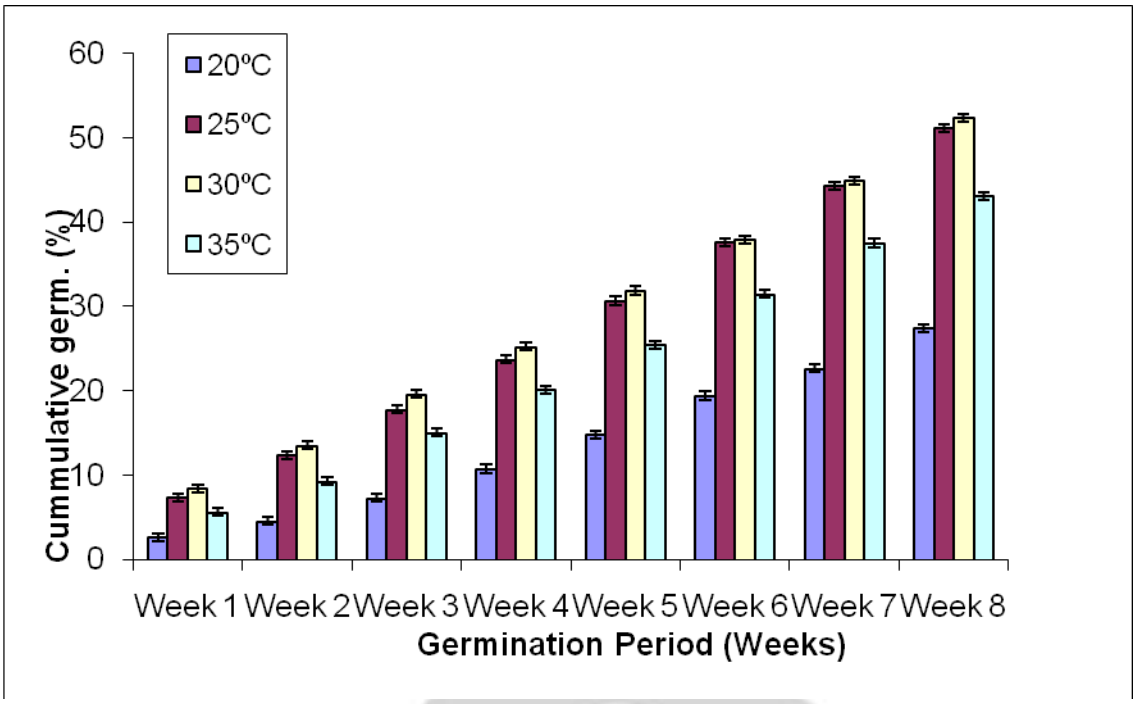

FIGURE 4.9: Cummulative germination of proximal sections *G. kola* seed at 20-35°C for a period of 8 weeks. Bars represent two standard error of the difference.

4.3.5 Responses of distal end chipped off sections of *Garcinia kola* seed to germination temperature and chemical pre-treatment

The interaction effect between germination temperatures and chemical pre-treatment on germination percentages of distal end chipped off sections of *Garcina kola* (Figure 4.10) indicated that there were significant interaction between germination temperatures and chemical pre-treatment ($p<0.001$). For all chemical pre-treatments used, germination percentages recorded at 25 and 30°C were significantly higher than those recorded at 35°C. In all cases, germination at 20°C was significantly lower than at all other temperatures. At 20°C, samples pre-treated with GA_3 (500mg/l), GA_3 (1000mg/l) and KNO_3 (2g/l) performed significantly better than the rest of the chemical pretreatments. The control experiment (where no chemicals were used on seed parts) performed

significantly poorer in terms of germination percentages at all temperatures compared to the other treatments.

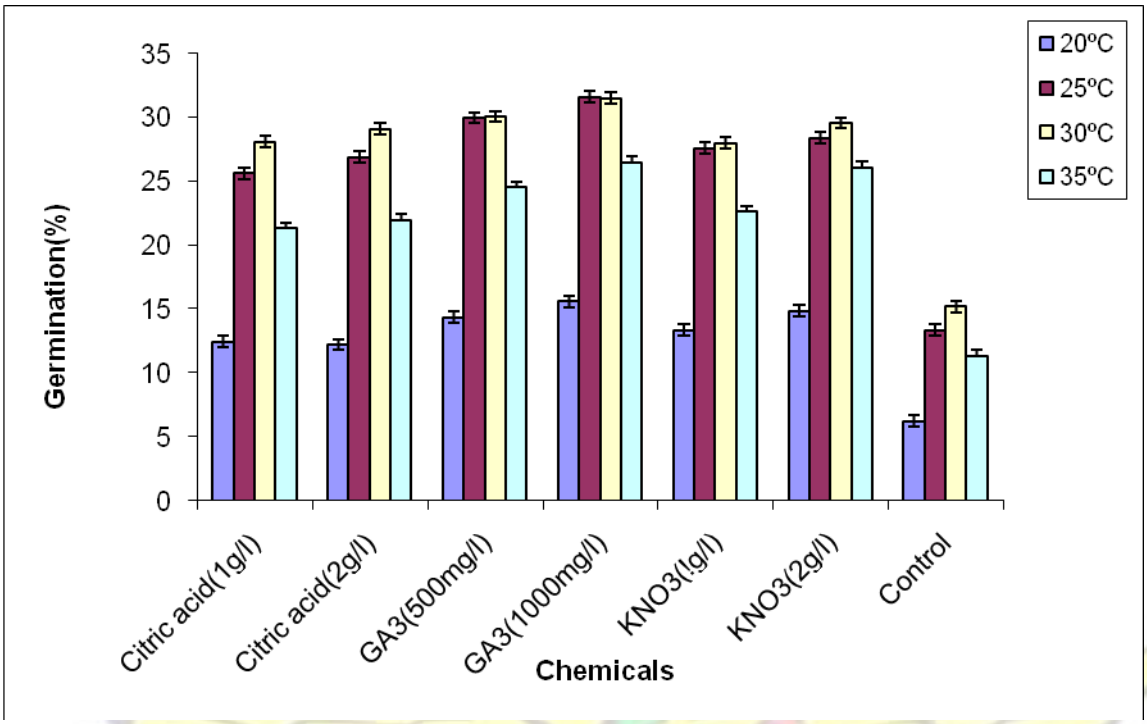

FIGURE 4.10: Chemical pre-treatment of distal end chipped off sections of *G. kola* seed and germination at different temperature regimes (20°- 35°C. Bars represent two standard error of the difference.

4.4 *Garcinia afzelii*

4.4.1 Seed moisture contents of *Garcinia afzelii* in relation to seedling dry weight and seed vigour index.

The effect of reduction in seed moisture content on seed vigour index of *Garcinia afzelii* is presented in Table 4.14. Seed vigour (VI) index was not significantly different from each other at seed moisture content between 31.5 and 27.6% even though it was numerically highest at 29.2% (VI=1513). Seed vigour indices at seed moistures from

25.3% and below were significantly lower ($p < 0.001$) than those recorded at higher moisture contents. Seed vigour index at moisture at 25.3% was significantly higher ($p < 0.001$) than VI figures recorded at lower seed moisture contents. Seedling dry weight (SDW) was significantly influenced by seed moisture content. SDW was significantly ($p < 0.001$) higher at seed moisture content 27.6% than at all the other moisture contents. SDW at moisture content 29.2% was significantly higher than SDW at seed moisture content 31.5%. SDW at seed moisture contents lower than 24.4% were significantly lower than SDW at higher seed moisture content (Table 4.14).

TABLE 4.14: Effect of drying on seed moisture content, germination speed, seedling dry weight and seed vigour index of *Garcinia afzelii* seeds.

Drying period (days)	Fruit moisture content (%)	Seed moisture content (%)	Seed germination speed	Seedling dry weight(g)	Seed vigour index
0	51.5	31.5	0.1625	0.2178	1485
2	43.5	29.2	0.1625	0.2302	1513
4	41.9	27.6	0.1475	0.2408	1490
6	36.9	25.3	0.1375	0.2253	1214
8	32.9	24.4	0.0725	0.2163	559
10	30.8	21.7	0.0350	0.2140	208
12	28.6	17.5	0.0275	0.2053	142
14	23.4	15.5	0.0200	0.1855	113
16	15.0	12.4	0.0150	0.1713	84
18	13.9	11.0	0.0000	0.0000	0
			Rep = 4 df = 30 s.e.d = 0.0585 Probability < 0.001	Rep = 4 df = 30 s.e.d = 0.004439 Probability < 0.001	Rep = 4 df = 30 s.e.d = 42.76 Probability <0.001

4.5 Effect of germination media, temperature and chemicals on germination of *Terminalia superba*

4.5.1 Germination media and chemicals

The interaction effect between germination media and chemical pre-treatment on germination percentages of *Terminalia superba* (Figure 4.11) indicated that there was a significant interaction between germination media and chemical pre-treatment ($p<0.001$). *Terminalia superba* seeds which

received chemical pre-treatment of K_2SO4(1g/l) + GA_3(+200mg/l) and set to germinate on agar or on seed testing paper germinated to a significantly higher percentage compared to seeds which were pretreated but germinated in soil. Seeds that received no chemical pre-treatment (control) and were germinated on agar gave a significantly higher germination percentage than those germinated in soil and on seed testing paper. In all cases, chemical pre-treatment performed significantly better ($p < 0.001$) than the control.

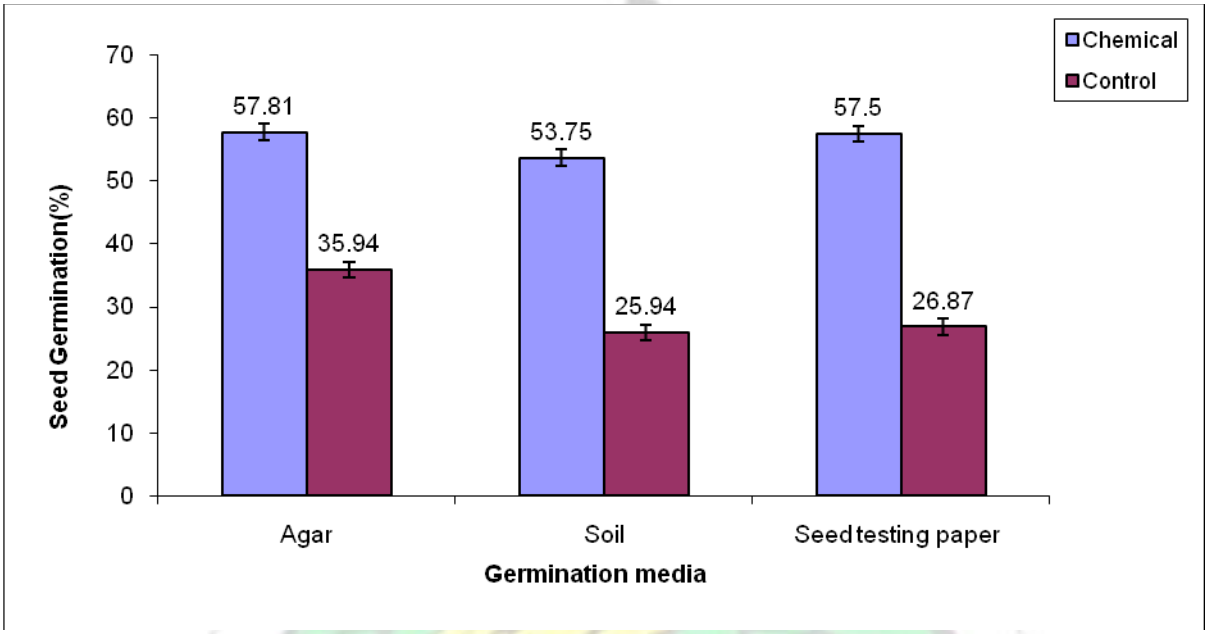

FIGURE 4.11: Effect of germination media and chemical pre-treatment on seed germination (%) of *Terminalia superba*. Bars represent two standard error of the difference.

4.5.2 Temperature and Chemical

The interaction effect between temperature and chemical pre-treatment on the germination percentages of *Terminalia superba* (Figure 4.12) indicated that there was a

significant interaction between temperature and chemical pre-treatment ($p<0.001$). *Terminalia superba* seeds pre-treated with K_2SO4 (1g/l) + GA_3 (+200mg/l) and set at 35°C germinated significantly higher than those incubated at 30 and 25°C. Germination at 25°C was significantly lower than germination at 30°C. At 25°C, zero germination was recorded for the control (no chemical treatment) whilst significantly higher germination percentages were registered at 30 and 35°C.

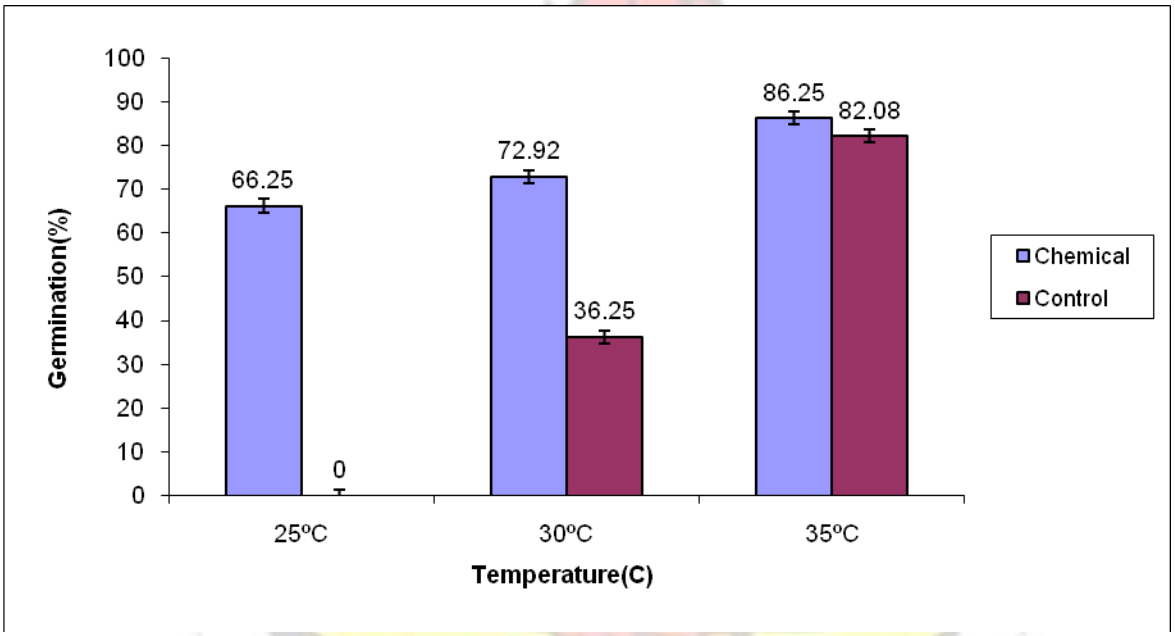

FIGURE 4.12: Effect of temperature and chemical pre-treatment on germination (%) of *Terminalia superba.* Bars represent two standard error of the difference.

4.5.3 Effect of chemical pre-treatment and germination media on mean germination time (MGT) of *Terminalia superba*

The interaction effect between chemical pre-treatment, germination media and temperature indicated that there was significant interaction between the factors ($p < 0.001$) (Table 4.15). There was a significant difference between MGTs as well as

germination percentages at temperatures 25 to 35°C using agar as media. The minimum MGT was recorded at 25°C (19.1days) and the maximum at 30°C (23.4days). MGT at 35°C was significantly lower than at 30°C but germination percentage at 35°C was significantly higher ($p < 0.001$) than at 30°C. Differences in MGTs when soil was used as media were not significantly different from each other at all temperatures, however, germination percentage was significantly higher at 35°C and at 30°C than at 25 °C. In using seed testing paper as germination media, there were no significant differences between MGTs at 25, 30 and 35°C. Similar to the case when soil was used as media, germination was significantly higher ($p < 0.001$) at 35 and 30°C than at 25°C. At 25°C there were significant differences in MGTs using the three germination media.

MGT on agar was the minimum and it also recorded the highest germination even though this germination was not significantly higher than what was recorded at this temperature on seed testing paper. At 30°C, there were no significant differences between MGTs using agar or soil or seed testing paper. Germination was however significantly higher on agar and seed testing paper than in soil. At 35°C, there were no significant differences in MGTs as well as in germination percentages. The control treatment (no chemical) recorded significant differences in MGTs as well as in germination at 30°C using the three germination media. At 35°C, however, MGT on soil was significantly lower than MGT on seed testing paper. Germination in soil was significantly higher ($p < 0.001$) than on agar and seed testing paper.

TABLE 4.15 : Effect of chemical pre-treatment($K_2SO4+GA_3$), germination media and temperature on germination percentage and mean germination time (MGT)(Days) of *Terminalia superba* seed. Figures in brackets are germination percentages.

		Temperature (°C)		
Chemical	**Germination**			

	Media		20	25	30	35
Mean germination time (MGT) (Days)						
	Agar	NG	19.1	23.4	21.9	
			(70)	***(77)***	***(84)***	
	Soil	NG	23.9	23.7		
23.3				***(60)***	***(69)***	
					(86)	
	STP	NG	21.4	22.4	22.3	
			(69)	***(73)***	***(89)***	
Control	**Germination Media**					
	Agar	NG	NG	26.2	17.6	
				(66)	***(77)***	
(15)	***(89***)					
	Soil	NG	NG	29.4	18.0	
	STP	NG	NG	24.6	19.7	
				(***28)***	***(80)***	

---

s.e.d **s.e.d**

(ChemxTempxMedia) for MGT= 0.714; Chem xTemp xMedia for germ % = 2.543

f probability < 0.001

STP = Seed Testing Paper ; NG = No germination recorded

4.6.1 Effect of chemical pre-treatment and temperature on the germination of *Terminalia ivorensis* seeds

The interaction effect between temperature and chemical treatment indicated that there was significant interaction between the factors ($p < 0.001$). In all chemical treatments, germination at 30°C was significantly higher than at the other temperatures. Within the

various chemical treatments, GA_3(1000mg/l) and KNO_3(2g/l) performed significantly better than KNO_3(1g/l) and Citric acid (2g/l). Germination at 40°C was significantly lower than at the other temperatures. Germination at 25 and 35°C were also significantly lower than at 30°C but significantly better than at 40°C (Figure 4.13)

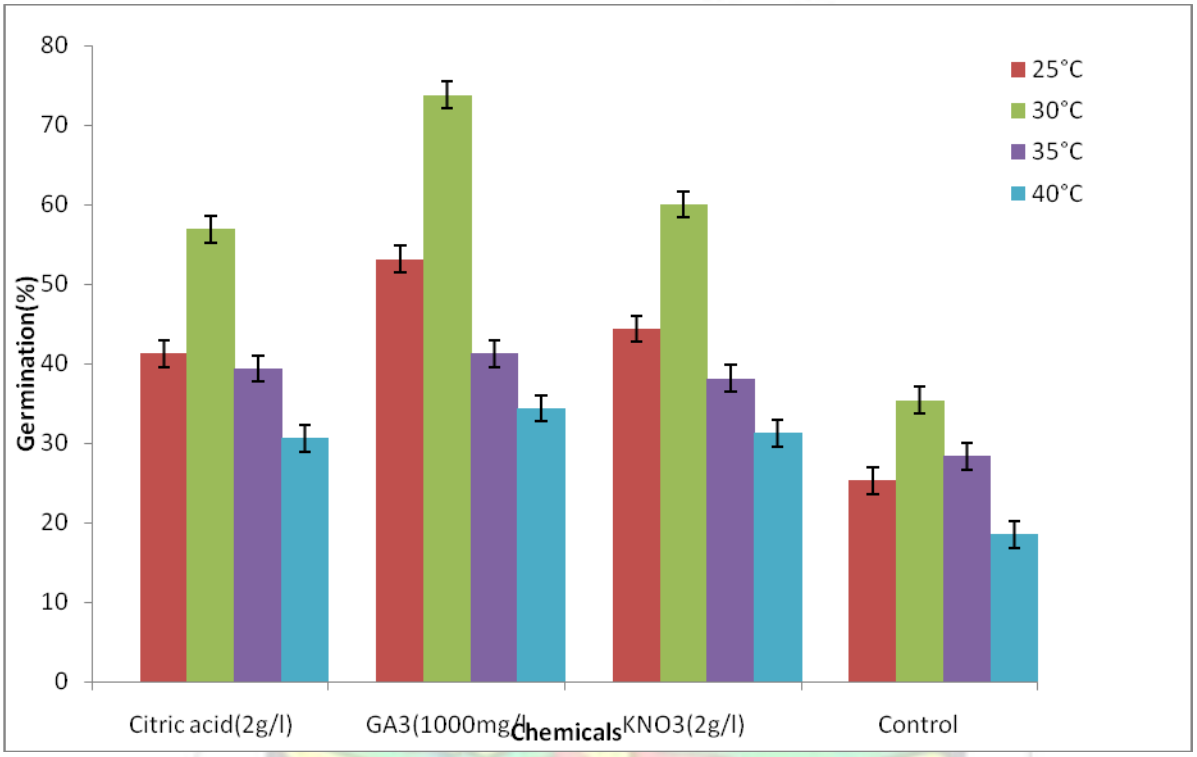

FIGURE 4.13: Chemical pretreatment of *Terminalia ivorensis* seeds and germination at temperatures of 25 to 40°C. Bars represent two standard error of the difference.

4.6.2 Temperature and germination of whole seed and extracted embryo at different maturity stages from seeds of *Terminalia ivorensis*:

There was a significant interaction between germination temperature and germination materials ($p < 0.001$) (Figure 4.14). Fully matured brown seed (FMB) with testa removed and green but matured seed (MSG) also with testa removed germinated to a significantly higher percentage than matured brown whole seed (WSB) at all

temperatures. The highest germination was at 30°C for all the mentioned seed materials. There were significant differences in the germination of decoated fully mature brown seed (FMB), decoated green matured seed (MSG) and matured brown whole seed at all temperatures. Germination percentage of decoated fully matured brown seed (FMB) at 40°C was significantly lower than at the other temperatures except at 20°C. This was also true for whole seed (WSB) at all temperatures apart from 20°C. Decoated immature green seeds (IMG) germinated poorly at all temperatures and did not germinate at all at 20 and 40°C.

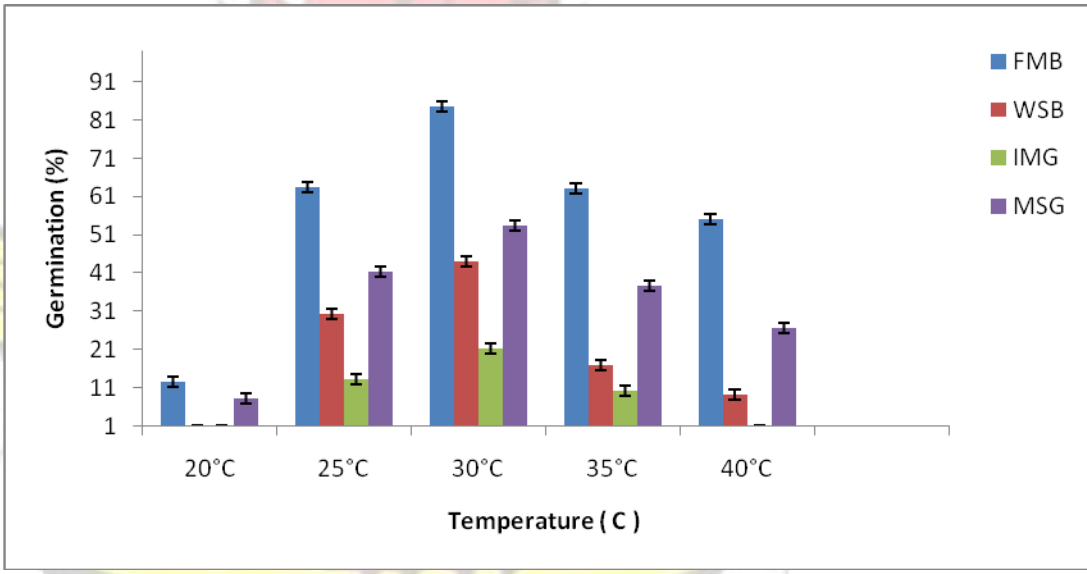

FIGURE 4.14: Temperature and germination materials interaction on the germination of *Terminalia ivorensis*. Bars represent two standard error of the difference

4.7.1 Germination media and germination of *Khaya anthotheca* :

Germination media had a significant effect on the germination percentage of *Khaya anthotheca* (P <0.001). Percent germination of seed germinated on agar and in soil were significantly higher than what was recorded for seed testing paper as shown in Figure 4.15.

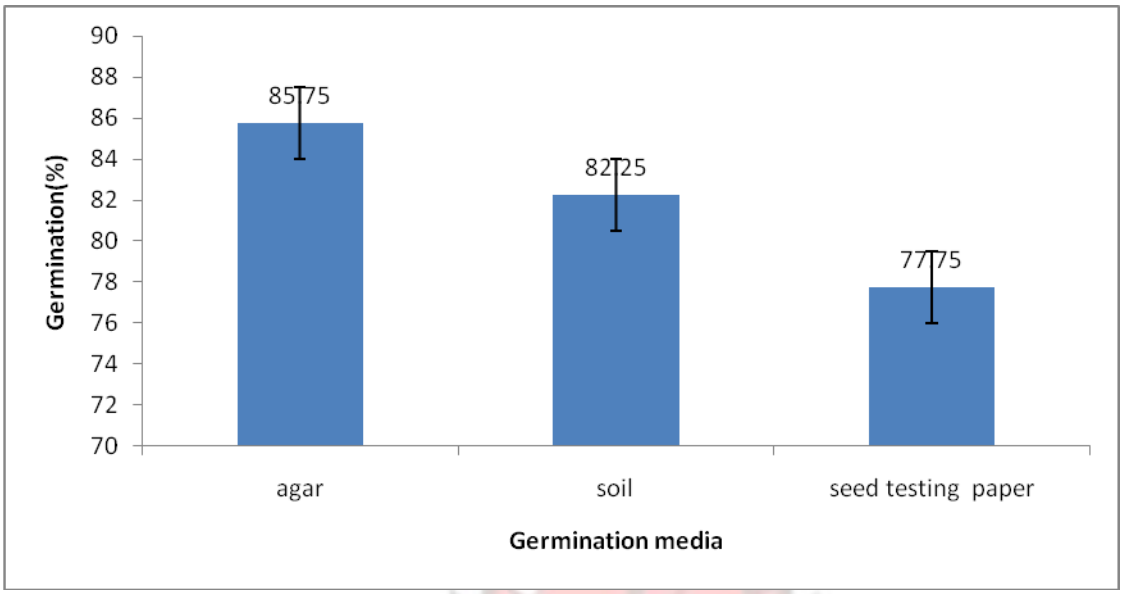

FIGURE 4.15: Germination media and seed germination (%) of *Khaya anthotheca* Bars represent two standard error of the difference

4.7.2 Temperature and germination of *Khaya anthotheca*

Germination percentages recorded at 20, 25, 30 and 35°C for *K. anthotheca* were not significantly different from each other. However, germination percentages at these four temperatures were significantly higher ($p < 0.001$) than what was recorded at 40°C as shown in Figure 4.16.

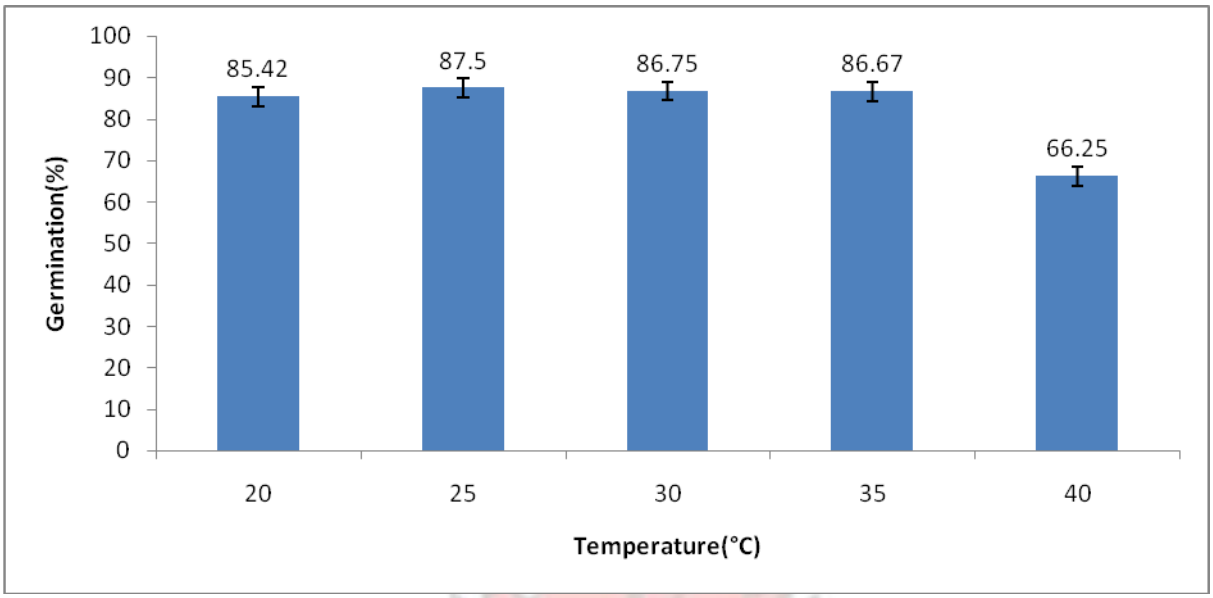

FIGURE 4.16: Effect of temperature (°C) on the germination (%) of *Khaya anthotheca* seed. Bars represent two standard error of the difference

4.7.3 Temperature and germination media on mean germination time (MGT) of *Khaya anthotheca*

The interaction effect between germination media and temperature on mean germination time (MGT) in days (Figure 4.17) indicated that there was significant interaction between the factors. Mean germination time was significantly different at all the temperatures and with all germination media ($p < 0.001$) except at 25°C where mean germination time was not significantly different from each other whether agar, soil or STP was used as media for germination and at 30°C where soil and STP were used as media. The minimum MGT was recorded at 30°C on all the three germination media and at 35°C on soil whilst the maximum MGT was recorded at 20°C. At all temperatures, except 25°C the minimum MGTs were obtained with soil as media for germination.

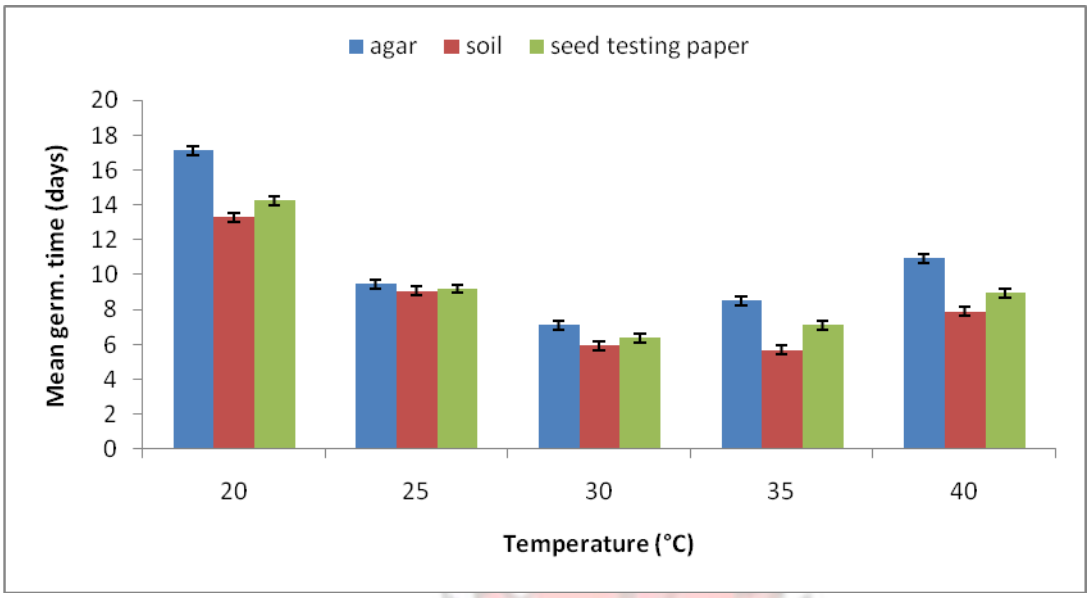

FIGURE 4.17: Germination temperature and media effect on mean germination time MGT (days) of *Khaya anthotheca*. Bars represent two standard error of the difference.

4.8.1 Germination media and temperature on germination of *E. angolense*

The interaction effect between germination media and temperature on germination percentage of *E. angolense* (Figure 4.18) indicated that there was significant interaction between the the two factors ($p < 0.001$). Germination at 25, 30 and 35°C on agar, soil or seed testing paper were significantly higher than at 20°C. At 25°C there were no significant differences in germination percentages in using agar, soil or seed testing paper. This was also true at 30°C where germination on all the three media resulted in the highest germination percentages apart from germination on agar at 35°C. No germination were recorded at 40°C whether on agar, soil or seed testing paper.

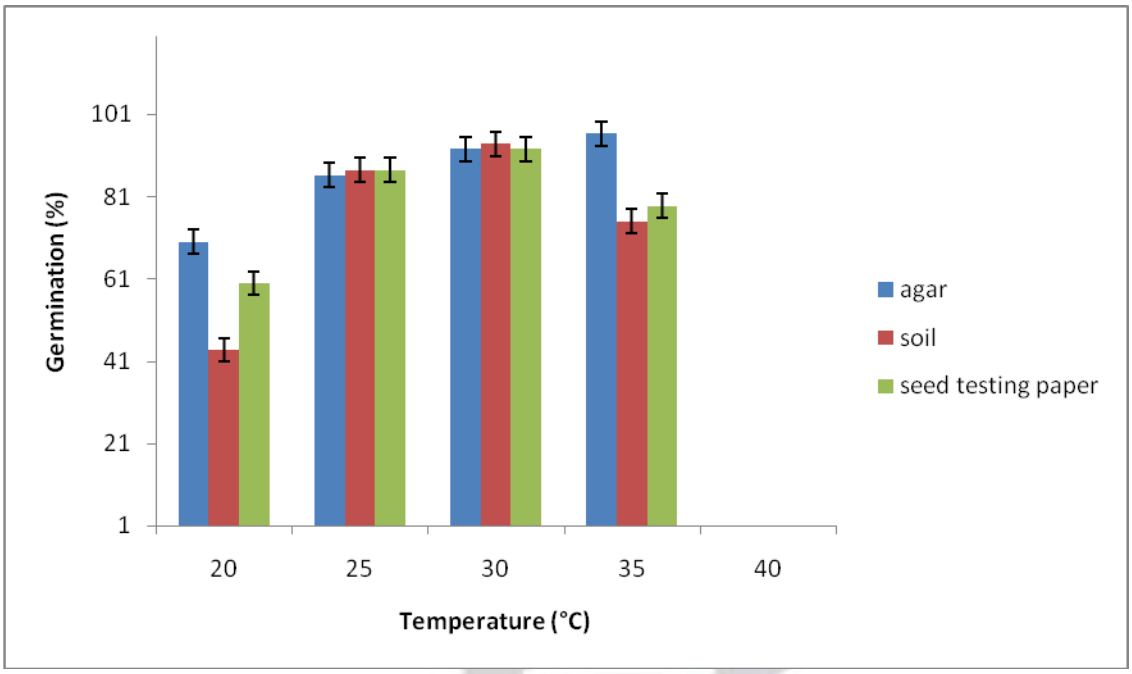

FIGURE 4.18: Germination temperature and media effect on the germination of *E. angolense*. Bars represent two standard error of the difference.

4.8.2 Germination media and temperature effect on mean germination time (MGT) (days) of *E. angolense*

Temperature and germination media interacted to give a significant difference in mean germination time (MGT) of *E. angolense* ($p < 0.001$). The highest MGT was recorded at 20°C on all the three germination media. These were significantly higher than MGT recorded at all the other temperatures and germination media. The lowest MGTs were recorded at 35°C. These were significantly lower than MGTs recorded at the other temperatures. At 35°C, there were no differences in MGTs recorded for the three germination media. At 25°C, no significant differences were between MGTs when agar or soil was used as media. However at 30°C there was no significant difference in MGTs using agar or soil (Figure 4.19).

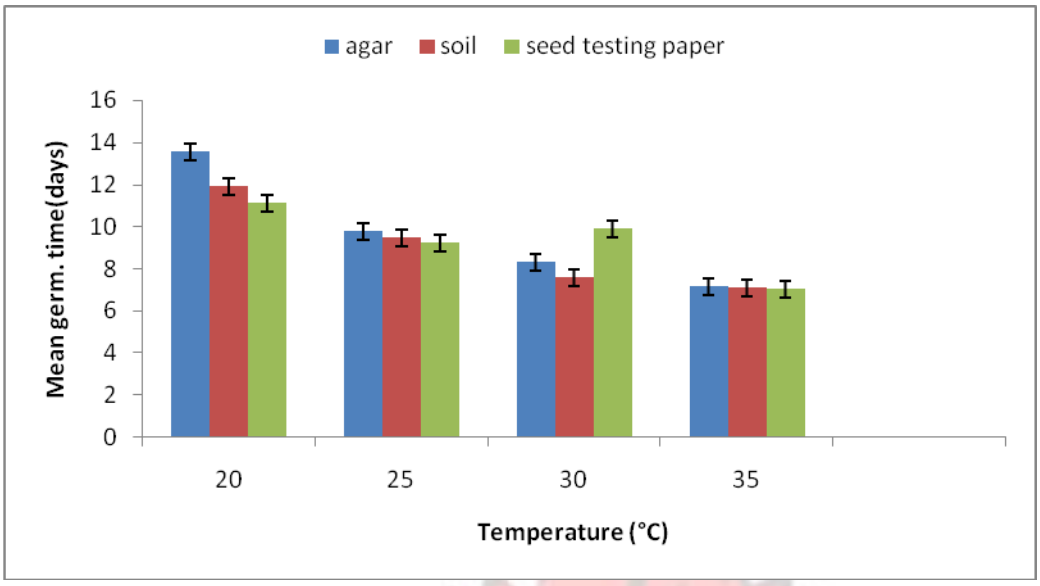

FIGURE 4.19: Temperature and germination media effect on mean germination time (MGT) (days) of *Entandrophragma angolense*. Bars represent two standard error of the difference.

4.9.1 Germination media and temperature on germination of *Mansonia altissima*

There was a significant interaction effects ($p < 0.001$) between temperature and germination media. The highest germination percentage was recorded at 30°C when agar and soil were used as media for germination. These were significantly higher than germination percentage values recorded from the other temperatures and media. At 20, 25 and 30°C germination values on seed testing paper were significantly lower than germination on agar and in soil at these temperatures. *M. altissima* germinated significantly better at 35°C on seed testing paper than on agar. While at 40 °C, zero germination was recorded on agar and in soil, but germination on seed testing paper was comparable to those that germinated at 20°C. At 30°C, there was no significant differences in germination percentages when agar or soil were used as germination media (Figure 4.20)

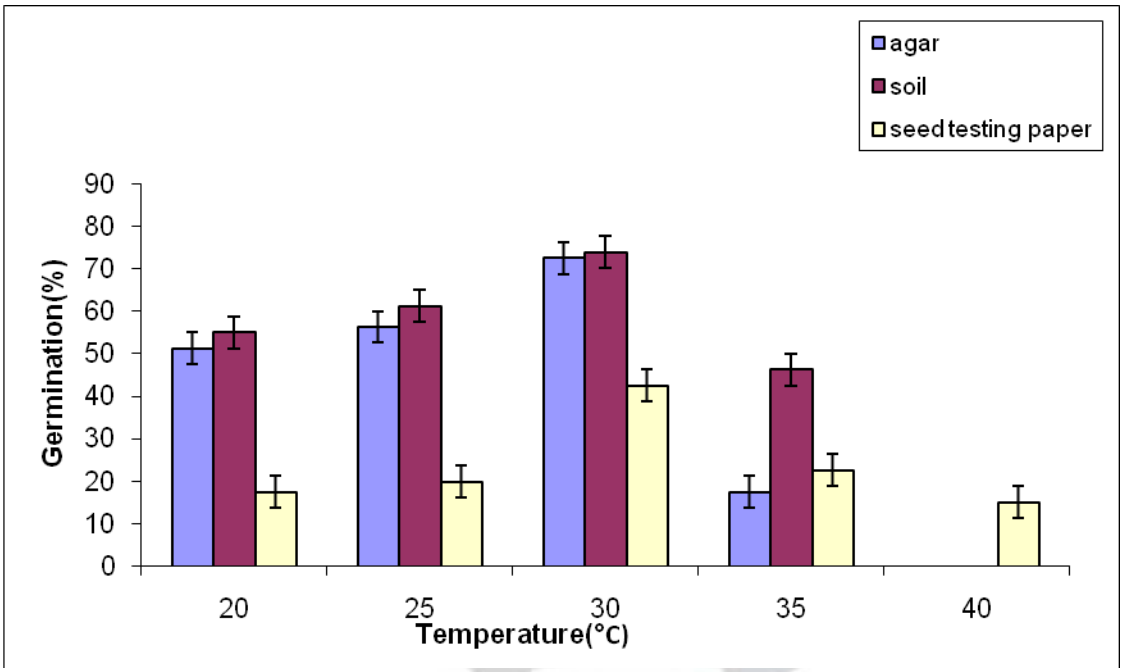

FIGURE 4.20: Germination temperature and media effect on the germination of *Mansonia altissima*. Bars represent two standard error of the difference.

4.9.2 Germination media and temperature on mean germination time (MGT) of *Mansonia altissima*

Figure 4.21 shows the interactive effect of temperature and germination media which resulted in a significant difference in mean germination time (MGT) of *Mansomia altissima* ($p < 0.001$). The highest MGT was recorded at 20°C when seed testing paper (STP) was used as a germination medium. This was significantly higher than MGT values obtained at all other temperatures and on other germination media. The lowest MGTs were obtained at 30°C on agar and in soil as well as at 25°C in soil.

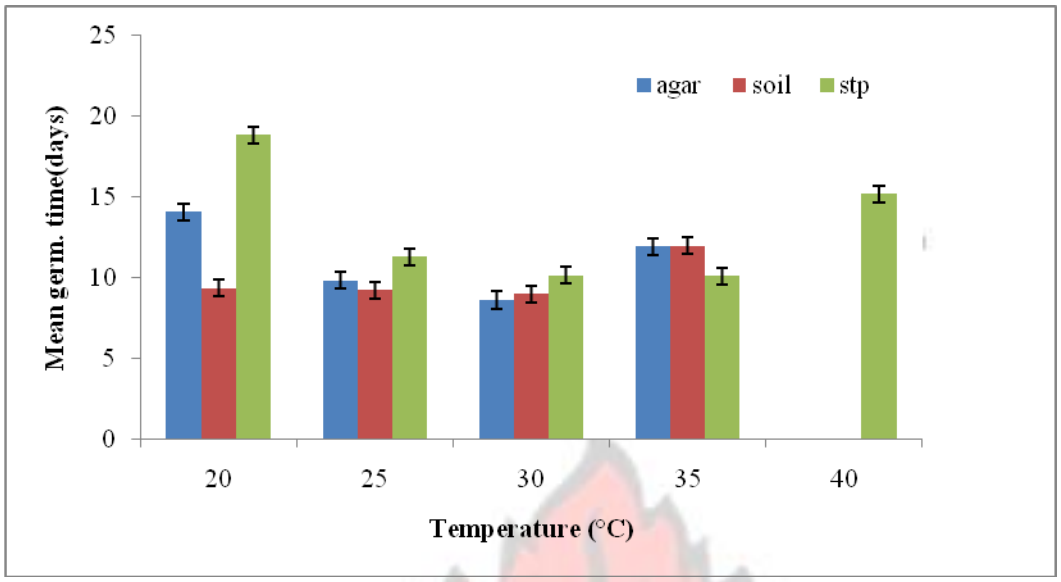

FIGURE 4.21: Temperature and germination media effect on mean germination time (MGT) (Days) of *Mansonia altissima*. Bars represent two standard error of the difference.

4.10 Germination of *Terminalia superba, Terminalia ivorensis* and *Khaya anthotheca on* the two- way thermogradient plate

4.10.1 *Terminalia superba*

No germination occurred on areas of low alternating temperatures and areas of low constant temperatures on the thermogradient plate. Areas with one very low alternating temperature and the other high (but the higher alternating temperature being < 35°C) recorded some levels of germination but these germination levels were low. For instance, there was either no germination or low germination percentages at the following alternating temperatures: 5/30°C (0%), 10/30°C (5%), 15/30°C (20%) and 5/35°C (27.5%) in connection with *Terminalia superba.* Areas with one very low alternating temperature and the other high (but the higher alternating temperature being > than 35°C) recorded relatively high germination percentages. Examples are 40/5°C (75%); 40/10°C

(60%) and 40/15°C (83%). Constant temperatures < 35°C resulted in low germination levels. For instance, germination at 20/20°C was 0% while germination at 30/30°C was 30%. Constant temperatures > 35°C (40/40°C was 45%) resulted in lower germinations for examples compared to constant temperatures 35/35°C which attained 88% germination. Germination at the following alternating temperatures resulted in very good germination percentages: 15/35°C (90%); 15/40°C (90%); 20/35°C (90%); 20/40°C (93%); 30/35°C (88%); 30/40°C (85%); 35/15°C (95%); 35/25°C (85%); 35/40°C (100%); 40/15°C (83%); 40/25°C (95%); 40/30°C (93%) and 40/35°C (83%) (Figure 4.22).

Terminalia ivorensis

Similar to *T. superba*, no germination of *T. ivorensis* occurred at areas of low alternating temperatures and areas of low constant temperatures on the thermogradient plate. The alternating temperature regimes were 5/10°C; 5/15°C; 5/20°C; 10/5°C; 10/15°°C; 10/20°C; 15/5°C; 15/10°C; 20/5°C; and 20/10°C. The constant temperature regimes that resulted in zero germination were 5/5°C; 10/10°C; and 15/15°C. Areas with a very low alternating temperature (< 15°C) and the other high (> 30°C) recorded some levels of germination but these were low. For instance, there were low germination rates at 5/30°C (27%); 5/35°C (20%) and 5/40°C (24%). Constant temperatures below 25/25°C and above 30/30°C gave very poor germination results.

For instance, germination percentages at 20/20°C and 35/35°C were zero percent.

Alternating temperatures: 15/35°C, 20/35°C, 30/35°C, 35/40°C and 40/30°C gave germination percentages above 73%. The constant temperature of 30/30°C recorded the highest germination rate of 87% as shown in Figure 4.23.

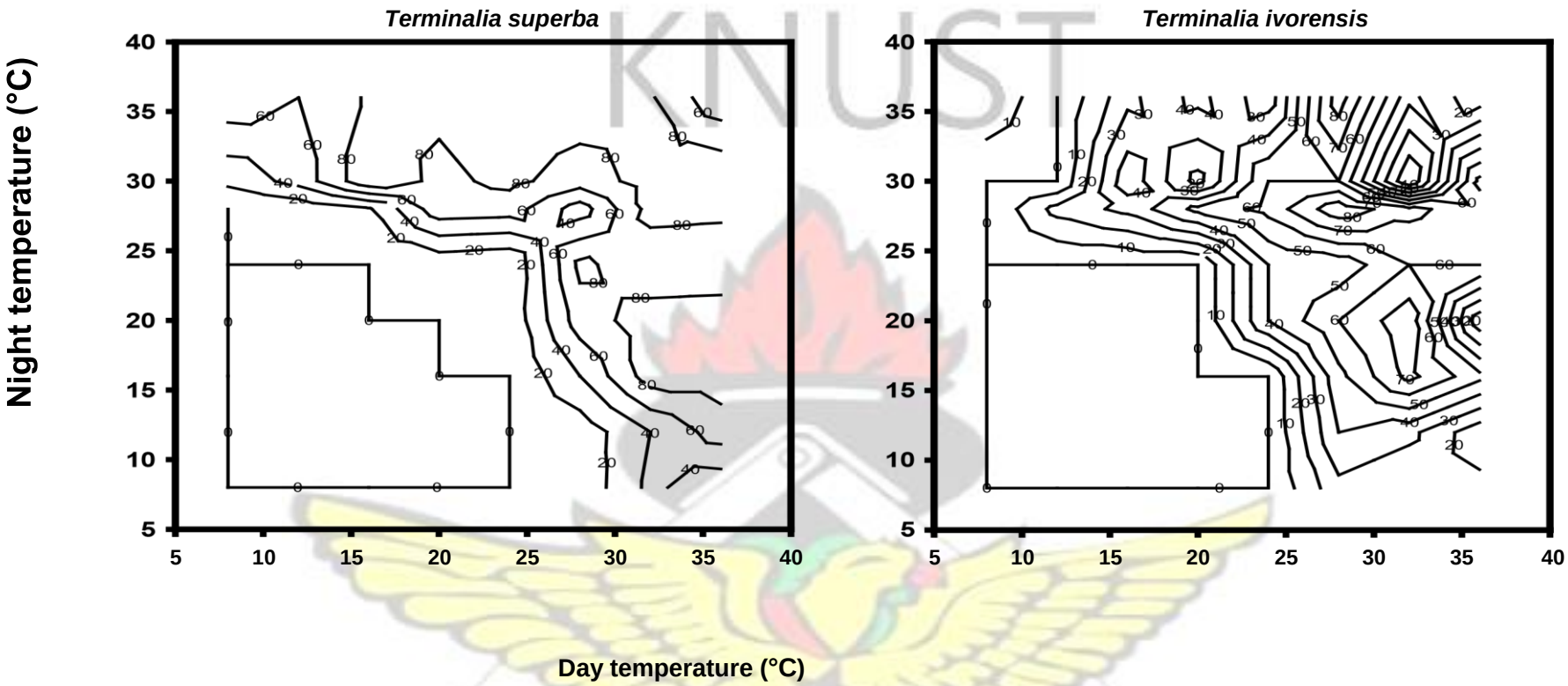

FIGURE 4.22: Presentation of germination of *T. superba* on the thermogradient plate. Areas with common ‘‘contours’’ have similar germination percentages.

FIGURE 4.23: Presentation of germination of *T. ivorensis* on the thermogradient plate. Areas with common ‘‘contours’’ have similar germination percentages

4.10.3 *Khaya anthotheca*

Figure 4.24 shows the germination of *Khaya anthotheca* seeds placed on the thermogradient plate. *Khaya anthotheca* seeds did not germinate at areas of low alternating temperatures as well as on areas of low constant temperatures. For example zero germinations were recorded at the following alternating temperature regimes: 5/10°C; 5/15°C; 5/20°C; 10/5°C; 10/15°C; 15/5°C; 15/10°C; and even at 15/20°C; 15/25°C; 20/5°C and 20/10°C. Zero germination was also recorded at constant temperature regimes of 5/5°C; 10/10°C and 15/15°C.

In, contract to *T. superba* and *T. ivorensis*, *K. anthotheca* gave excellent germinations at regions of very low temperatures (5°C and 10°C alternating with temperatures < 35°C. For intance there was 100% germination at 5/30°C and 97% at 10/30°C.

K. anthotheca seeds also germinated well at the alternating temperatures 20/25°C (100%); 20/30°C (93%) and 20/35°C (100%) as well as at 25/15°C (100%); 25/20°C (97%); 25/30°C (100%) and 25/35°C (93%). Germination of seeds at 30/10°C, 30/15°C, 30/20°C, and 30/ 35°C were 97%, 87%, 100% and 93%, respectively, while those on temperature of 35/10°C and 35/15°C were 97% and 100%, respectively. Regions where any one of the alternating temperatures was > 35°C resulted in zero germination percentages (Figure 4.24).

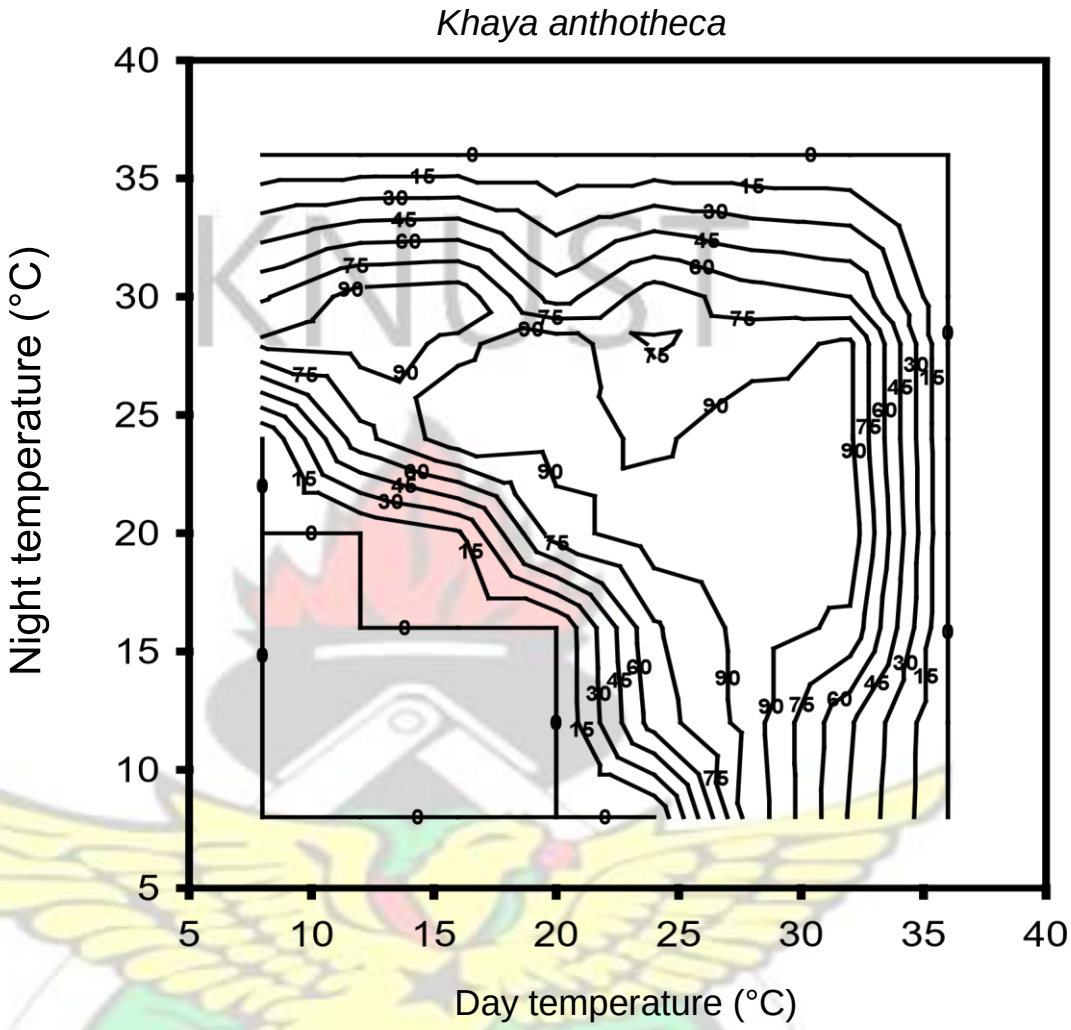

FIGURE 4.24: Presentation of germination of *K. anthotheca* on the thermogradient plate. Areas with common ‘‘contours’’ have similar germination percentages.

4.11 Water Sorption Isotherms

Water sorption isotherms for the various species constructed at 20°C are presented in

Figures 4.25 to 4.30. Water sorption isotherm for *Garcinia kola*, *Terminalia superba*, *Terminalia ivorensis, Khaya anthotheca, Entandrophragma angolense, and Mansonia altissima* seeds show that water content increased with relative humidity in a reverse sigmoidal trend.

4.11.1 Water Sorption in *Garcinia kola* seed

Generally seeds placed at all relative humidities from 3 to 93% lost weight (Table 4.16) during the experimental period which lasted for about 91 days before all the seed samples equilibrated. Samples weight loss was generally slow but this was more pronounced in samples dried under high relative humidity regimes.

The sorption curve shows (Figure 4.25) that when moist *Garcinia kola* seeds are placed at 20°C and 3% RH, the moisture content of the seeds will settle to an equilibrium moisture content of 4.2% and when placed at 93%, the moisture content will settle at 21.8%. At RH of 66% the seed moisture content will settle at 12.2%. Seed deterioration manifested by fungal growth was observed as RH of the drying environment increased. To hasten the equilibration of samples of *G. kola*, seeds were cut into pieces due to their large sizes (Table 4.2). This probably promoted fungal growth on the cut surfaces of seeds drying in relative humidity chambers with high relative humidity. At 90% and 93% RHs which were very moist all seed deteriorated before reaching equilibrium.

Table 4.16: Weight changes of *Garcinia kola* seed samples recorded during the first 30 days of drying under various RH chambers at 20°C

Drying Period(Days)	Relative Humidity(%)											
	93		83		66	44	33		26	15	3	
	Seed Samples Weight(g)											
0			9.526						7.776	7.007	9.857	9.371
3	8.831		9.475	9.427	7.387	7.927	7.605	8.746 8.428	7.495	7.295	6.592 6.057	8.887
6	8.805	8.777	9.368	9.291	7.090	7.392	7.046	8.307 8.146	6.766	6.144	5.388 5.013	8.253
9	8.744	8.689	9.202	9.123	7.050	6.612	6.164	7.910 7.624	5.607	5.358	4.775 4.661	7.509
12	8.608	8.522	9.045	8.942	6.956	5.815	5.552	7.365 7.131	5.227	5.115	4.588 4.524	6.467
15	8.389	8.108	8.849		6.896	5.300	5.214	6.841 6.707	5.076		4.499	5.684
18	8.091		8.643		6.779	5.108		6.453	5.013		4.459	5.313
21	8.065				6.560							5.010
24					6.233							4.950
27					5.805							
30					5.635						4.850	
					5.329							

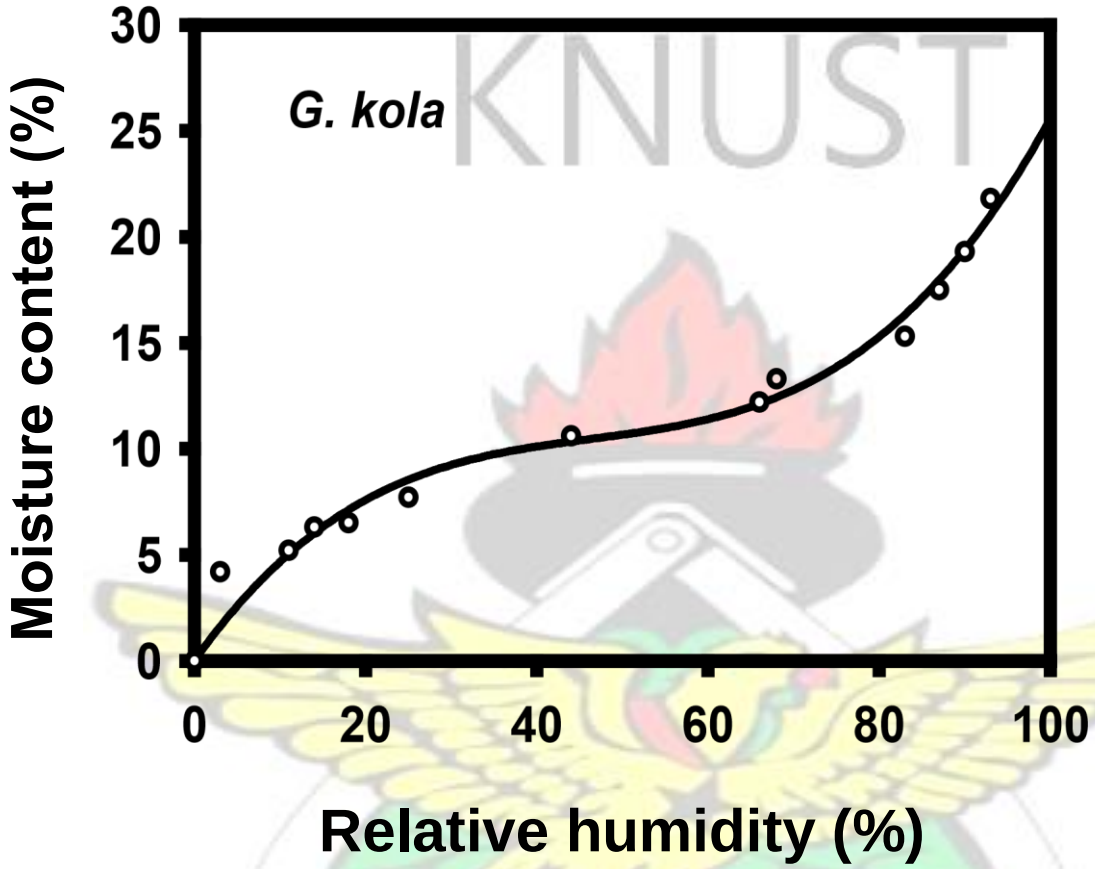

FIGURE 4.25: Water sorption isotherm of *Garcinia kola* seeds showing the relationship between the equilibrium MC and RH at 20°C

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4.11.2 Water sorption in *Terminalia superba* seed

Generally *Terminalia superba* seeds placed at all relative humidities from 18 to 93% gained fresh weight whilst samples placed between 3 and 11% RH lost fresh weight (Table 4.17) during the experimental period which lasted for about 31 days for all the seed samples to reach equilibration.

Table 4.17: Weight changes of *Terminalia superba* seed samples recorded during the first 30 days of drying under various RH chambers 20°C

	Relative Humidity(%)							
	93	**90**	**80**	**60**	**33**	**18**	**11**	**3**
Drying Period(Days)	**Seed Sample Weight(g)**							
0	3.546	4.062	3.946	3.862	4.181	4.190	3.959	4.228
3	3.949	4.506	4.283	4.118	4.310	4.229	3.859	3.940
6	4.191	4.728	4.399	4.168	4.318	4.227	3.822	3.882
9	4.193	4.735	4.399	4.163	4.321	4.227	3.819	3.876
12	4.177	4.715	4.405	4.172	4.325	4.229	3.816	3.871
15	4.211	4.748	4.411	4.178	4.323	4.225	3.812	3.868
18	4.202	4.777	4.423	4.179	4.324	4.225	3.811	3.865
21	4.242	4.808	4.429	4.184	4.326	4.226	3.810	3.865
24	4.247	4.773	4.412	4.216	4.323	4.224	3.809	3.864
27	4.237	4.753	4.422	4.206	4.323	4.224	3.807	3.863

30 4.247 4.757 4.422 4.204 4.324 4.223 3.807 3.863 The sorption curve (Figure 4.26) indicated that when moist *Terminalia superba* seed was placed in an environment with temperature of 20°C and 3%

RH, the moisture content dropped to an equilibrium moisture content of 3.9%. When placed at 20°C and 60% RH, the moisture content settled at an equilibrium moisture content of 16.0% and at 20°C with 93% RH, the moisture content of the seed sample equilibrated at 24.3%. The sorption curve of *T. superba* is divided into three zones. Water content increased rapidly between 3 and 20% RH, followed by a more gradual, linear increase in water content between 20 and about 60% and a final, rapid region of hydration above 80% (Figure 4.26).

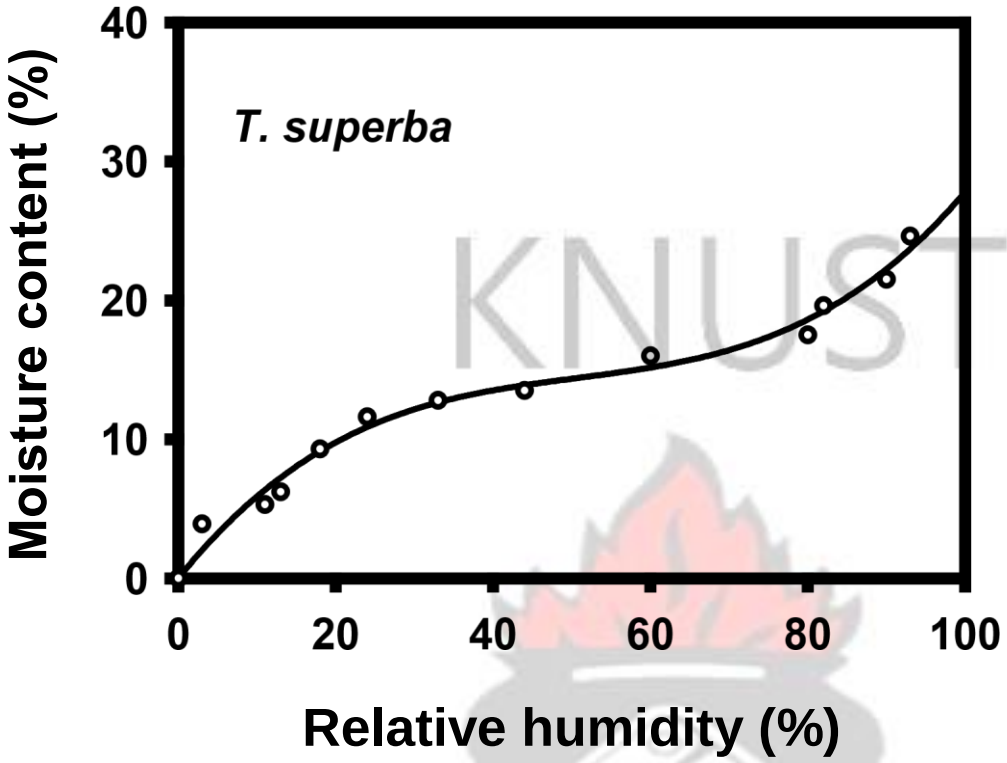

FIGURE 4.26: Water sorption isotherm of *Terminalia superba* seeds showing the relationship between the equilibrium MC and RH at 20°C

4.11.3 Water sorption of *Terminalia ivorensis* seed

Generally *T. ivorensis* seeds placed at all RHs from 44 to 93% gained weight whilst samples placed between 3 and 33% RH lost weight during the experimental period which lasted for about 30 days for all the seed samples to be equilibrated (Table 4.18.)

Table 4.18 Weight changes in *Terminalia ivorensis* seed samples recorded during the first 30 days under various RH chambers at 20°C

Relative Humidity(%)

	93	90	60	44	33	24	11	3
Drying Period(Days)			**Seed**		**Sample**			
	Weight(g)							
0	3.048	3.531	3.643	3.633	3.863	3.676	3.855	3.778
3	3.332	3.808	3.711	3.657	3.793	3.590	3.648	3.571
6	3.443	3.876	3.708	3.650	3.771	3.557	3.584	3.505
9	3.440	3.870	3.708	3.652	3.769	3.554	3.581	3.498
12	3.490	3.871	3.709	3.648	3.765	3.552	3.574	3.494
18	3.462	3.874	3.703	3.644	3.761	3.547	3.567	3.486
21	3.473	3.873	3.703	3.645	3.761	3.545	3.564	3.484
24	3.476	3.861	3.698	3.622	3.756	3.542	3.562	3.480
27	3.481	3.861	3.697	3.640	3.754	3.540	3.560	3.480
30	3.471	3.854	3.694	3.637	3.755	3.538	3.558	3.477

The sorption curve shown by Figure 4..27 established the fact that when the moist *Terminalia ivorensis* seed was placed at the temperature of 20°C and 3% RH, the moisture content dropped to an equilibrium moisture content of 4.4%. When placed at 20°C and 60% RH, the moisture content settled at an equilibrium moisture content of 10.6% and at 20°C with 93% RH, the moisture content of the seed sample at equilibration was 22.9%. Water content increased rapidly between 3 and 13 % RH, followed by a more gradual, linear increase in water content between 13 and about 60% and a final, rapid region of hydration above 80%.

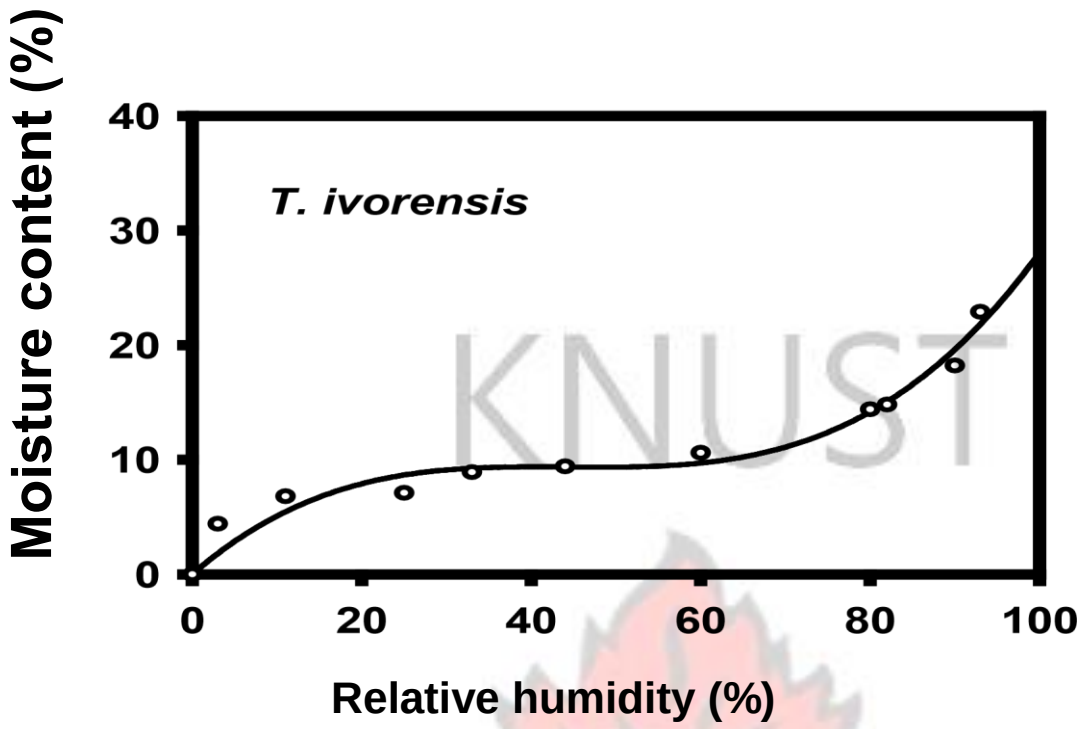

FIGURE 4.27: Water sorption isotherm of *Terminalia ivorensis* seeds showing the relationship between the equilibrium MC and RH at 20°C

4.11.4 Water sorption characteristics of *Khaya anthotheca* seed Seeds of *K. anthotheca* placed in the chamber with RHs from 60 to 93% gained weight whilst samples placed between 3 and 33% RH lost weight (Table 4.19) during the experimental period which lasted for about 15 days for all the seed samples to equilibrate.

Table 4.19 Weight changes of *Khaya anthotheca* seed samples recorded during the first 15 days under various RH chambers at 20°C

Relative Humidity (%)							
93	90	80	60	33	18	11	3

Drying Period(Days)	Seed Sample Weight(g)							
0	1.375	1.315	1.268	1.419	1.323	1.100	1.421	1.252
3	1.444	1.374	1.31	1.447	1.320	1.085	1.3512	1.185
6	1.481	1.397	1.329	1.454	1.320	1.084	1.3461	1.173
9	1.507	1.408	1.336	1.457	1.320	1.084	1.3455	1.166
12	1.511	1.409	1.336	1.457	1.319	1.083	1.3451	1.166
15	1.510	1.409	1.336	1.457	1.319	1.083	1.3451	1.166

When moist *Khaya anthotheca* seed was placed at an environment with temperature 20°C and 3% RH, the moisture content dropped to an equilibrium moisture content of 1.8%. When placed at 20°C and 60% RH, the moisture content rose to an equilibrium moisture content of 9.3%. When placed in chambers at 20°C and 93% RH, the moisture content of the seeds continued to increase to an equilibration of 15.9%. The isotherm curve shows three zones. Water content increased rapidly between 3 and 25 % RH, followed by a more gradual, linear increase in water content between 25 and about 70% and a final, rapid region of hydration above 75-80% (Figure 4.28),

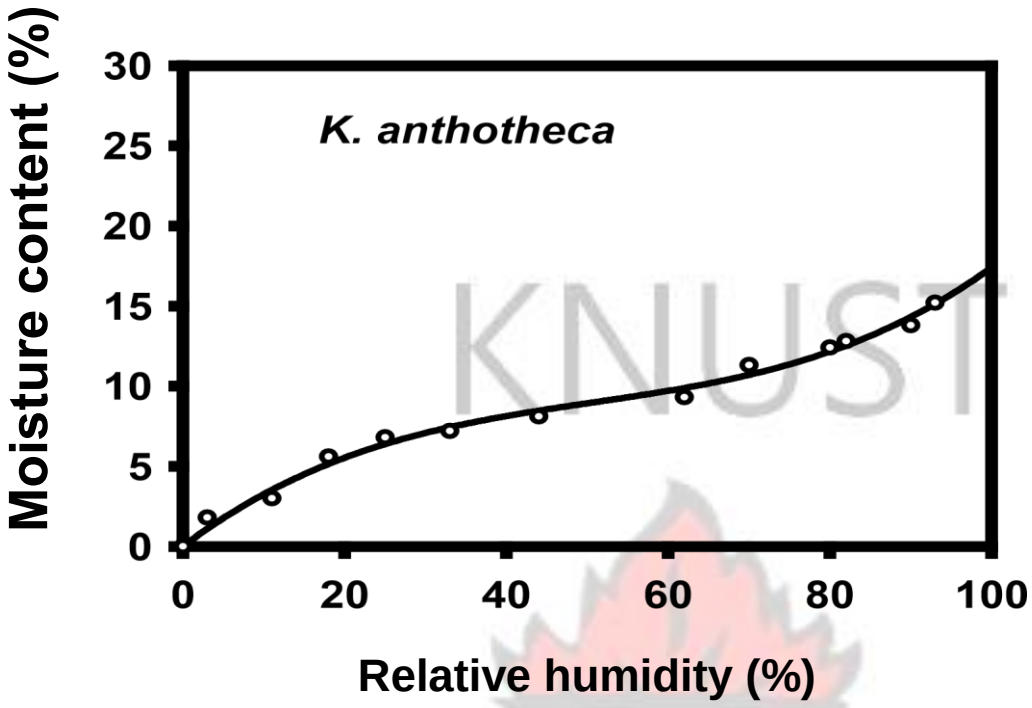

FIGURE 4.28: Water sorption isotherm of *Khaya anthotheca* seeds showing the relationship between the equilibrium MC and RH at 20°C.

4.11.5 Water sorption in *Entandrophragma angolense* seed

Seeds of *E. angolense* which were in chambers with RHs from 70 to 93% gained weight whilst samples placed in chambers with RHs between 3 and 60% lost weight (Table 4.20) during the experimental period which lasted for about 13 days for all the seed samples to equilibrate.

Table 4.20 Weight changes of *E. angolense* seed samples recorded during the first 13 days under various RH chambers 20°C

Relative Humidity (%)

	93	90	80	70	60	18	11	3

Drying

Period(Days)	**Seed**				**Sample**		**Weight(g)**	
0	1.591	1.603	1.482	1.432	1.703	1.572	1.652	1.155
1	1.659	1.648	1.574	1.468	1.696	1.561	1.606	1.467
2	1.751	1.674	1.607	1.485	1.693	1.554	1.598	1.459
3	1.745	1.702	1.605	1.502	1.692	1.555	1.594	1.454
4	1.760	1.709	1.599	1.505	1.692	1.555	1.594	1.452
5	1.785	1.715	1.602	1.507	1.692	1.552	1.593	1.452
6	1.796	1.719	1.603	1.508	1.692	1.552	1.593	1.451
7	1.803	1.721	1.603	1.509	1.692	1.554	1.593	1.450
8	1.808	1.723	1.602	1.509	1.691	1.554	1.593	1.450
9	1.810	1.724	1.603	1.509	1.691	1.551	1.592	1.449
10	1.811	1.724	1.603	1.510	1.691	1.551	1.592	1.449
11	1.816	1.724	1.599	1.508	1.691	1.551	1.591	1.448
12	1.816	1.724	1.599	1.510	1.691	1.551	1.591	1.447
13	1.816	1.724	1.603	1.510	1.691	1.551	1.591	1.447

The sorption curve (Figure 4.29) indicated that moist *Entandrophragma angolense* seeds were placed in an environment with temperature at 20°C and 3% RH, the moisture content dropped to an equilibrium moisture content of 1.6%. On the other hand, seeds at 20°C and 60% RH chamber had their moisture content settled at an equilibrium moisture content of 6.3% and at 20°C with 93% RH, the moisture content of the seed sample at equilibrated at 15.3%. The curve shows three zones with water content increasing rapidly between 3 and 25 % RH, followed by a more gradual, linear increase in water content between 25 and about 60% and a final, rapid region of hydration above 80%.

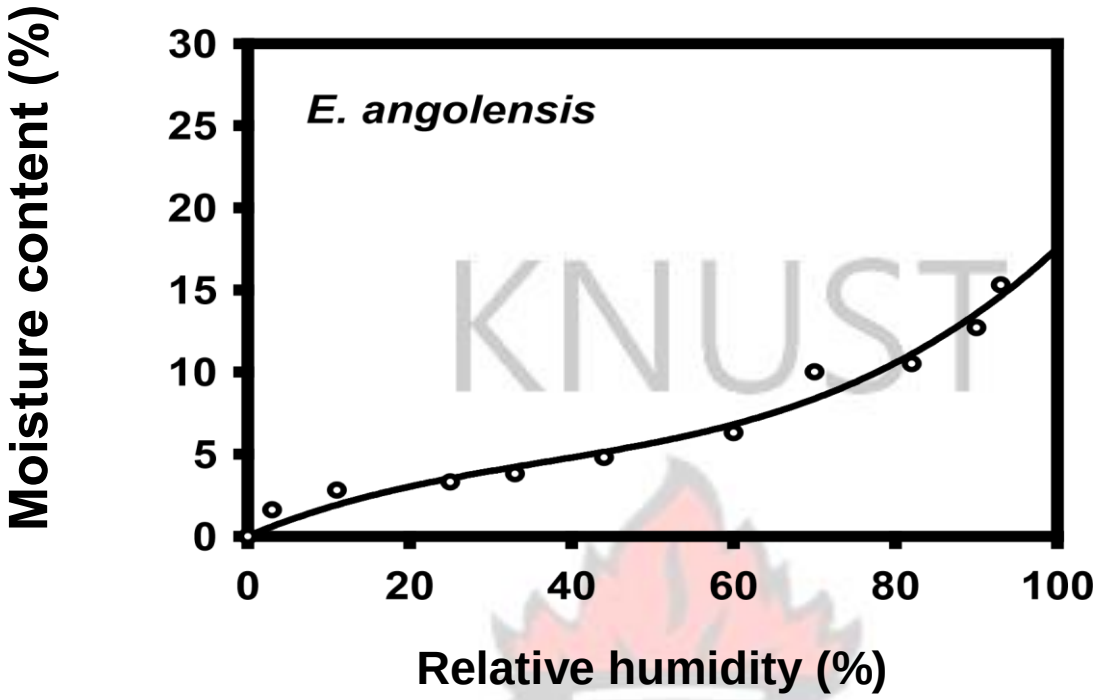

FIGURE 4.29: Water sorption isotherm of *E. angolense* seeds showing the relationship between the equilibrium MC and RH at 20°C.

4.11.6 Water sorption attributes of *Mansonia altissima* seeds:

Seeds placed at all the RHs from 70 to 93% gained weight whilst samples placed between 3 and 60% RH lost weight (Table 4.21) during the experimental period which lasted for about 30 days for all the seed samples to equilibrate.

Table 4.21 Weight changes of *M. altissima* seed samples recorded during the first 15 days under various RH chambers at 20°C

				Relative Humidity (%)				
	93	90	82	70	60	18	11	3
Drying Period(Days)				**Seed Sample Weight(g)**				

0	3.136	3.224	3.062		3.249	3.077	3.156	3.103	3.068
1	3.203	3.268	3.082	3.281	3.045	3.112	3.016	2.974	
2	3.256	3.297	3.095	3.303	3.025	3.084	2.974	2.922	
3	3.303	3.320	3.101	3.315	3.009	3.064	2.946	2.890	
4	3.311	3.325	3.103	3.318	3.007	3.060	2.940	2.884	
5	3.347	3.343	3.110	3.329	2.997	3.047	2.922	2.865	
6	3.383	3.364	3.117	3.341	2.988	3.036	2.907	2.848	
7	3.421	3.383	3.123	3.353	2.980	3.025	2.893	2.838	
8	3.456	3.402	3.128	3.363	2.974	3.017	2.881	2.820	
9	3.485	3.416	3.132	3.372	2.968	3.010	2.872	2.801	
10	3.515	3.427	3.135	3.382	2.962	3.004	2.866	2.799	
11	3.569	3.438	3.139	3.390	2.959	2.999	2.856	2.793	
12	3.620	3.480	3.152	3.414	2.950	2.988	2.842	2.776	
13	3.638	3.490	3.152	3.418	2.948	2.986	2.838	2.772	
14	3.656	3.499	3.154	3.422	2.946	2.983	2.829	2.767	
15	3.674	3.510	3.156	3.426	2.943	2.981	2.825	2.763	

The sorption curve (Figure 4.30) indicated that when moist *Mansonia altissima* seeds were placed in an environment with temperature of 20°C and 3% RH, the moisture content dropped to an equilibrium moisture content of 5.3%. For seeds of the species placed in an environment of 20°C and 60% RH, the moisture content settled at equilibrium moisture content of 10.3%. On the other hand when seeds of the species were placed in an environment at 20°C with 93% RH, the moisture content of the seed sample equilibrated at 30.8%.

The sorption curve is clearly divided into three zones. Water content increased rapidly between 3 and 20 % RH, followed by a more gradual, linear increase in water content between 25 and about 60% and a final, rapid region of hydration above 70%.

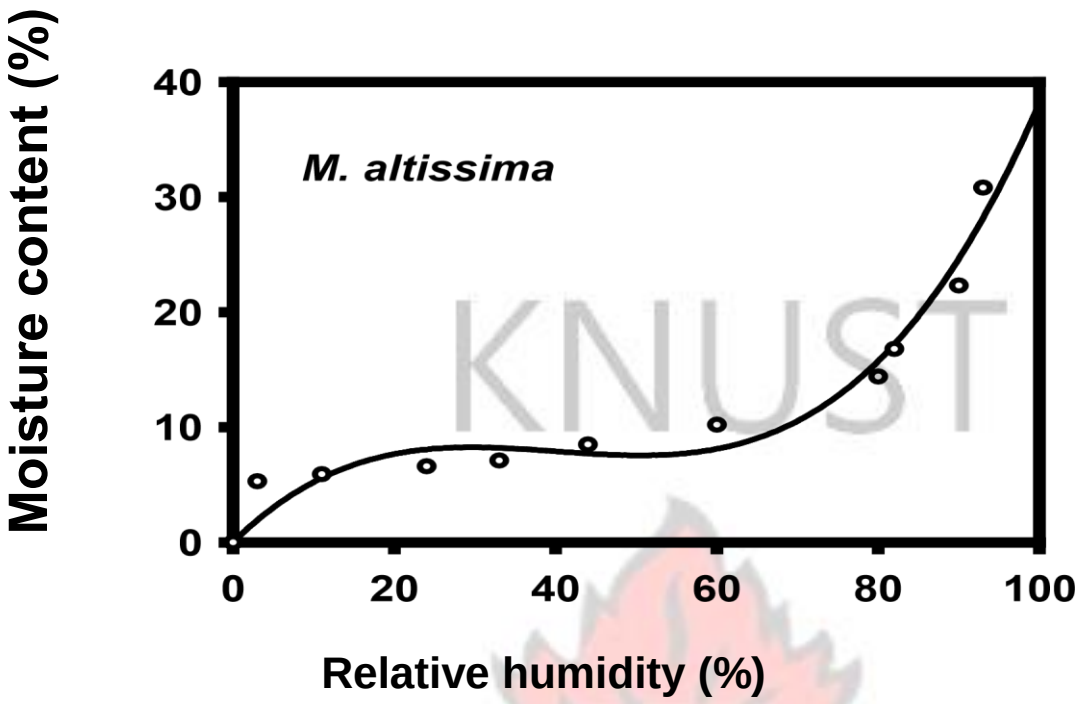

FIGURE 4.30: Water sorption isotherm of *Mansonia altissima* seeds showing the relationship between the equilibrium MC and RH at 20°C.

4.12 Electrical conductivity measurement of desiccated *Garcinia kola* seeds

The electrical conductivity measurement of *Garcinia kola* seeds desiccated from 50% to 27% moisture content are shown in Tables 4.22. There were significant differences between electrical conductivity measurement at different seed moisture contents and soaking times ($p < 0.001$). Electrical conductivity increased with the reduction in seed moisture content and incresead soaking time. Electrical conductivity was highest (285.54 µS) at the moisture content

of 27% after 8 hours of soaking seeds in deionized water. The lowest electrical conductivity (8.59 µS) was recorded at the moisture content of 50% after an hour of soaking seeds in deionized water.

Seed moisture content and seed weight effect on electrolyte leakage from seeds of *Garcinia kola* desiccated to various moisture contents is presented in Table 4.23. There were significant differences between electrical conductivity measurement at different seed moisture contents using various seed weight ($p < 0.001$). Electrical conductivity increased with the reduction in seed moisture content and incresead seed weight. Electrical conductivity measurement was highest (256.55 µS) at seed moisture content of 27% with seed weight of 0.66 g. The lowest electrical conductivity measurement was recorded at seed moisture content of 50% with seed weight of 0.16 g.

Table 4.22: Seed moisture content and soaking time effect on electrolyte leakage from seeds of *Garcinia kola* desiccated to various moisture contents

Soaking Time (Hours)

	1	2	3	4	5	6	7	8
Seed Moisture Content (%)	**Electrical Conductivity Measurement** (µS)							
27	131.77	158.31	181.61	202.98	230.02	252.22	256.85	285.54
30	99.73	115.07	123.89	133.84	154.03	167.98	177.41	185.95
35	66.15	72.45	75.97	78.56	83.43	86.89	90.81	93.48
38	58.15	62.98	64.97	66.92	69.18	71.49	73.76	75.57
41	17.13	22.98	29.85	35.62	38.94	43.14	46.23	49.57
45	13.94	21.24	25.88	32.45	37.38	42.84	45.57	47.92
50	8.59	13.33	18.68	25.51	29.92	34.15	38.84	42.68
Replications = 18						df = 672		
s. e.d = 0.08144						c.v. = 0.3%		

Table 4.23: Seed moisture content and seed weight effect on electrolyte leakages from seeds of *Garcinia kola* desiccated to various moisture content.

	Seed Weight (g)	**0.16**	**0.31**	**0.37**	**0.46**	**0.59**	**0.66**
Seed Moisture Content (%)		**Electrical conductivity measurement (µS)**					
27		182.20	193.65	197.61	213.82	230.67	256.55
30		117.50	125.59	137.24	148.80	160.05	179.10
35		71.99	73.43	76.33	80.42	86.11	97.54
38		60.35	62.41	65.31	68.07	72.18	78.98
41		24.47	26.71	29.65	33.83	45.34	50.86
45		22.16	23.80	24.84	26.96	50.66	54.48
50		10.89	17.22	18.46	19.99	43.91	48.38

Replications = 24 df = 672

s. e. d = 0.07053 cv = 0.3%

CONCLUSIONS

From the present study conducted on the seeds of these seven important forest tree species in Ghana namely: *Garcinia kola*, *Garcinia afzelii*, *Terminalia superba*, *Terminalia ivorensis*, *Khaya anthotheca*, *Entandrophragma angolense* and *Mansonia altissima* the following conclusions and recommendations could be made.

- *Garcinia kola* seeds are shed at very high moisture content at approximately 58.0% (fresh weight basis) and therefore fresh seeds of species recorded high equilibrium relative humidity (eRH). It can be said that the species do not go through the process of maturation drying.

- *Garcinia afzelli* seeds are also shed at high moisture content at approximately 32.0% (fresh weight basis) and therefore fresh seeds of the species recorded high eRH. It can be said that the species do not go through the process of maturation drying.

- *Garcinia kola* seeds gradually loose viability with decreasing seed moisture contents and when desiccated to moisture contents below 30% termed as the 'Critical Moisture Content' (CMC) or 'Lowest Safe Moisture Content' (LSMC), loose viability drastically and thus the species can be classified as desiccation intolerant/desiccation sensitive or recalcitrant.

- *Garcinia afzelii* seeds gradually loose viability with decreasing seed moisture contents and when desiccated to moisture contents below 25% termed as the 'Critical Moisture Content' (CMC) or 'Lowest Safe Moisture Content' (LSMC), loose viability drastically and thus the species can also be classified as desiccation intolerant/desiccation sensitive or recalcitrant.

- Fast drying of whole seeds and seed fragments of *Garcinia kola* in silica gel would not result in the reduction of the 'critical moisture content' of the species

- Fast drying of whole seeds of *G. afzelii* in silica gel would not result in the reduction of the 'critical moisture content' of the species.

- The pattern of germination described as 'garcinia-type' of seed germination by (de Vogel, 1980) in other *Garcinia species* like *Garcinia gummi-gutta* (Geeta *et al*., 2006) and in *Garcinia indica* (Malik *et al*., 2005) was also observed in *Garcinia kola* in the present study. In this type of germination, the primary root (PR) emerges from the distal end of the seed whilst the shoot emerges from the opposite end called the proximal end.

- *Garcinia kola* seedlings can be raised from the proximal sections (PS) and half proximal sections (HPS) and the complete/whole seed (WS) among others.

Distal sections (DS) and half distal sections (HDS) of the seed cannot be used to produce seedlings as they only develop primary roots (PR) which soon degenerate ending the life of the potential seedling. Middle section (MS) and half of the middle section (HMS) of *G. kola* seeds do not develop roots or shoots.

- Reduction in the speed of germination, seedling vigour and seed vigour index was associated with seed desiccation in *G. afzelii*.

- *Terminalia superba*, *Terminalia ivorensis*, *Khaya anthotheca*, *Entandrophragma angolense* and *Mansonia altissima* seeds are shed at moisture contents less than 20% and therefore they can be said to go through the process of maturation drying.

- *Terminalia superba*, *Terminalia ivorensis*, *Khaya anthotheca*, *Entandrophragma angolense* and *Mansonia altissima* seeds are orthodox in response to desiccation, tolerating desiccation down to 4-5% moisture content indicating that there is no adverse effect of drying on germination of the species.

- Seeds of *Terminalia superba*, *T. ivorensis* *Khaya anthotheca* and *Entandrophragma angolense* dry at an exponential rate until equilibrium moisture content are reached. In other words seed moisture content of the

species decline steeply during the initial stages of drying, and as seed moisture content reduce, the rate of seed moisture losses also reduce.

- The best media for germinating *Khaya anthotheca* seed was agar followed by soil and then by seed testing paper. The optimum temperature range for germinating the species was 20-35°C.

- The best constant temperatures for germinating *Entandrophragma angolense* were 25, 30 and 35°C. Agar, soil and seed testing paper were all suitable as germination media for the species

- The best constant temperature for germinating *Mansonia altissima* was 30°C. This was followed by 25 °C and then 20°C. Soil and agar were the best germination media for the species.

- The moisture sorption isotherms curves generated for the seven species showed that seed moisture content increases with increasing relative humidity.

- Moisture sorption isotherm curves developed for *T. superba*, *T. ivorensis*, *E. angolense*, *K. anthotheca* and *M. altissima* have been divided into three regions

indicative of three regions of water binding; they exhibited a reverse sigmoidal shape characteristic of all orthodox seeds.

- Contrarily to what was reported by Vertucci and Leopold (1987), water sorption isotherm of *Garcinia kola*, a desiccation intolerant/ desiccation sensitive/ recalcitrant species exhibited a reverse sigmoidal shape characteristics similar to that of desiccation tolerant/ desiccation insensitive/ orthodox seeds.

- Electrical conductivity measurements of leachates from *Garcinia kola* seeds increased as seeds were dried to lower moisture content. There was a close correlation between increased leakage of solutes from seeds and loss of seed viability.

RECOMMENDATIONS

- Using the proximal sections (PS) and the half proximal sections (HPS) of *G. kola* as materials for germination are recommended as these sections germinate early than the other seed fragments/sections and also in the case of the half proximal sections (HPS) multiple seedlings can be obtained from one seed.

- *Garcinia kola* and *G. afzelii* being desiccation sensitive species must be sown soon after collection without drying. If there is the need to keep the seeds/fruits for some few days /weeks they must be stored in a moistened medium such as sawdust or wood shavings with enough air circulation within the seeds/fruits.

- For a long term ex-situ conservation of *Garcinia kola* and *G. afzelii*, further research work on their cryo- conservation/ preservation (storage at ultralow temperature will be required.

- The recommended temperature for germinating *Garcinia kola* seed and seed fragments/sections on agar is 30°C.

- In selecting alternating temperatures for germinating *Terminalia superba* using agar as media, the recommended temperature combinations include:

15/35°C, 15/40°C, 20/35°C, 20/40°C, 25/35°C, 30/35°C, 35/15°C, and 35/25°C.

- In selecting alternating temperatures for germinating *Terminalia ivorensis* using seed testing paper as media, the recommended temperature combinations include: 15/35°C, 20/35°C, 35/40°C and 40/30°C.

- In selecting alternating temperatures for germinating *Khaya anthotheca* using agar as media, the recommended temperature combinations include: 15/35°C, 15/40°C, 20/35°C, 20/30°C, 20/35°C, 25/15°C, 25/20°C , 25/30°C , 25/35°C , 30/10°C , 30/10°C, 30/15°C , 30/20°C, 30/35°C, 35/10°C and 35/15°C.

- The recommended conditions for germinating *Mansonia altissima* using constant temperature is 30°C with agar or soil as media.