DORMANCY STUDIES AND PROXIMATE ANALYSIS IN *DIALIUM GUINEENSIS* AND *PROSOPIS AFRICANA*

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Abstract

Studies were made on the dormancy and proximate analysis of *Prosopis africana* (Guil & Perr) Taub and *Dialium guineensis* (wild).This is with aim of improving percentage germination in the seeds of these species. Some standard methods of releasing seed dormancy were used. Percentage germination range in fresh seeds of *P. africana* and *D. guineensis* was 0-10%. The seeds of the two tree species exhibit physical dormancy due to hard seed coat. Dormancy in *P. africana* and *D. guineensis* was terminated by soaking of seeds in 90% concentrated sulphuric acid for 10-15 minutes; and mechanical scarification using coarse sand, gravel and emery cloth abrasion. The percentage germination in pretreated seeds ranged between 70-100%. The emery cloth treatments gave the best result. The proximate analysis of the seeds including the moisture content, dry matter, fibre content, crude protein content, crude fibre content, ash content and carbohydrate content were estimated. The seeds of *P. africana* and *D. guineensis* contain moisture content (%) of 8.64 and 12.62, dry matter content (%) of 9.36 and 87.38; Iron (%) 4.57 and 8.85; crude protein (%) 78.00 and 21.61; crude fibre (%) of 2.43 and 2.33; Ash content (%) 2.19 and 4.71 and total carbohydrate (%) 54.19 and 51.88 respectively

Keywords: Dormancy, germination, seeds, seedlings, savanna, trees, scarification, proximate analysis

Introduction

The savanna vegetation in Nigeria occupies a land area between Latitude 7^{o}N and 10^{o}N (Agboola, 1995a). Three types of savanna are observable depending on the distance from the tropical rainforest and the river sources. The vegetation is well known for its combination of open woodland and grasses (Ebofin *et. al.*, 2002). Woody species

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of the woodland savanna are all fire tolerant with thick, corky and broken barks. Some of the tree species are the general utility types helping in nutrient recycling as well as providing food, fodder, charcoal and timber (Ola-Adams, and Onyeachusin 1993). The trees are also known to provide shade for man, livestock, timber for wood and fuel for domestic use (Okafor, 1993).

Hardness of seed coats, seed sizes, seed dormancy are some of the problems encountered in many tree species most especially the legumes of savanna which affect germination of seeds and growth of seedlings (Agboola, 1995a). The effects of these attributes on seedling procurement and nursery practices have been the focus in forestry and agroforestry practices (Ebofin *et. al*., 2002). The tree species have been categorized as lost, wild, lesser and endangered tree crops. This results from the fact that it is not cultivated, under-utilized, neglected and threatened by extinction (Okafor *et. al*., 1993; Odunfa, 1993).

This study aims at investigating the effect of seed sizes, gibberellic acid and hydration/dehydration presowing treatment on seed germination and seedling growth of *D. guineensis* and *P. africana*. The aim of this work is to study the germination of the seed of these multipurpose trees with the aim of breaking the dormancy. This is with aim of enhancing the availability of seedlings for afforestation.

Materials and methods

Seed procurement and processing: Seeds of *D. guineensis* were collected from the Federal Forestry Research Institute of Nigeria (FRIN) Ibadan, Nigeria. While that of *Prosopis africana* seeds were obtained from the tree stands at the main campus of University of Ilorin, Kwara State, Nigeria. viability tests were carried out on seeds using the floating and Triphenyl-tetrazohium chloride method of ISTA (1976). Seeds were surface-sterilized with 0.1% mercuric chloride solution for 30 seconds and rinsed severally with distilled water. About 20-50 seeds were broadcast on moistened filter paper in sterile 9cm petri dishes. All germination trials were carried out at room temperature of $30 \pm 2^{o}C$. Data collected in means of five replicates were subjected to analysis of variance at $P = 0.05$.

Hydration/Dehydration presowing treatments:

Seeds were immersed in 100ml of distilled water. The seeds were stirred for 24h as much as possible, after which they were air-dried for 2 days before preparing for germination in petri dishes. Germination percentages were recorded at alternate days.

Preparation and effect of Gibberellic Acid (GA_3):

The GA_3 of 100, 200, 300mg was dissolved into 50mls of 95% ethanol in 250mls volumentric flask. The solution was marked to 100mls by adding distilled water to give 1000mg/L, 2000mg/L and 3000mg/L respectively. Five percent (5%) ethanol solution with distilled water was used as control. The scarified seeds lots were moistened with1000-3000mg/L GA_3.

Effects of seed sizes on seed germination and seedling growth:

Seeds were sorted out into small and big size based on two sizes 1.1 x 0.6cm and 0.7 x 0.5cm respectively. The seeds were initially pretreated for dormancy by soaking in concentrated sulphuric acid for 10-15 minutes according to the methods of Agboola and Etejere (1991). Seeds were removed and washed several times with distilled water and then prepared for germination studies in 9cm petri dishes five replicates of the set up were made.

Effect of seed sizes on seedling growth:

Three pretreated seeds were planted in heat-sterilised loamy soil in black polythene planting bags in the nursery. Watering was done alternate day. Seedlings in each bag were thinned to one per bag. The leaf number, plant height, petiole length were measured for 3 months old seedling. Data were subjected to an analysis of variance at $P > 0.05$ and means compared by means of least significance difference test.

Proximate Analysis of the Seeds

Measurement of Moisture Content (MC) of the seeds

Seeds were put in an electric oven preset at 103^{o}C for 4 hours according to the method of Justice (1972). The % MC was calculated by subtracting final fresh weight of the seed from the initial weight of the seed divided by the original weight and then multiplied by 100.

The proximate analysis of the two seeds were carried out after the samples had been weighed with weighing balance and oven dried at 80^{o}C till they were well dried (when constant weights have been recorded). The dried samples were ground into powdery form in a blender and sieved through a sieve. The sieved powder were stored in a dessicator for the proximate analysis including moisture content, dry matter, fat and ash contents, crude fibre and total carbohydrate.

Dry matter of the seeds

Some quantity of the seed samples were weighed and dried in a glass vial in the oven and then cooled in a dessicator before it was transferred into a clean sample bottle, then labeled. The percentage dry matter were obtained for the sampled by deducting the moisture content from 100.

∴ Dry matter = 100 – moisture content

Ash content of the seeds

Twenty grams of finely ground, dried sample were weighed into a tared porcelain crucible. Dust contamination was avoided after weighing. The samples were charred on an electric heater or bunsen flame inside a fume cupboard after which samples in crucible were ashed in gallenkamp maffle furnace at 550^{o}C for 6 hours. The crucibles were then removed and cooled in a desicator after which the final weight was taken in order to know the weight of grey or white ash obtained. The ash contents were calculated as follows:

Ash content = weight of dried crucibles deducted from weight of crucible + ashed sample divided by sample weight.

Fat content of the seeds

Clean 250cm pyrex boiling flasks were dried in an electric oven at 105-110^{o}C for 30 minutes then cooled in a desiccator. Samples (2g) were accurately weighed into labeled thimbles. Corresponding labeled, cooled boiling flasks were filled with about 60ml of n-hexane (boiling point 4^{o}C). The extraction thimble was plugged lightly with cotton wool. Extraction was done in soxlet apparatus which was allowed to reflux for sic hours. Thimble was reviewed with care and n-hexane are collected in the top container of the set-up and drained into a container for re-use. After the flask might have been freed of n-hexane, residue were removed and dried at 105oC for one hour and transferred into a dessicator for cooling and then weighed. Fat content is calculated as weight of sample before extraction – weight of dry residue = Xg

% fat content is equal to weight of fat divided by weight of sample multiplied by100.

Determination of Crude Protein Content:

The crude protein in the seeds were determined by the Kjeldhal procedure involving digestion, distillation and titration.

Digestion: about 2g of sample are weighed into Kjeldhal flask. One tablet of Kjeldhal catalyst selenium was added, 5 glass beads were used to prevent bumping during

heating and 25ml of conc. H_2SO_4 were introduced into the mixture. Then the mixture was heated in the fume cupboard first gently then the heat was further increased with occasional shaking till the mixture assumed a green colour. The mixture was then allowed to cool after which the black particle showing at the mouth and neck of the flask were washed down with distilled water. It is then re-heated gently at first and then vigorously until the given colour disappeared and then cooled. The coloured digest was transferred with several washing into 100ml volumetric flask made up to the mark with distilled water after which it was distilled using Marham distillation apparatus.

The distillation was carried out with Marham distillation apparatus which allows volatile substance such as ammonia to be steam distilled with complete collection of the distillate. The apparatus was steamed out for about 15 minutes to allows the developing vacuum to remove condensed water. The steam generator is then placed on the heat source and coach component of the apparatus was fixed up appropriately. Ten milliliter of the digest was pipetted into the boiling apparatus via the small funnel aperture, washed down with distilled water followed by 10ml of 40% NaOH solution. The mixture was steam-distilled for 5-7 minutes into 100ml conical flask containing 100ml of boric acid indicator placed at the receiving tip of the condenser in order to collect enough ammonium sulphates. The receiving flask is then removed and washed down the tip of the condenser into the flask. The flame is then removed so that the resultant developed vacuum will remove the condensed water. The solution in the receiving flask was later titrated with 0.01N Hcl acid. The formular of crude protein is given below:
Crude protein is given by general factor (6.25) multiplied by Nitrogen content.

Crude Fibre Content:

One gram of each sample was introduced into 500ml conical flask and 100ml of digestion reagent (500ml of glacial acetic acid, 450ml water, 50ml nitric acid mixed together with 20g of trichlor acetic acid) were added to wash down the sides of the flask. This was then boiled and refluxed for 40 minutes, a water jacket condenser was used to prevent loss of liquid. The flask was then removed from the heater and cooled under cold tap. The digest was filtered through a 15cm of No. 4 Whatman filter paper and washed six times with hot water and once with methylated spirit. The filter paper was then opened and the residues were removed with a spatula. The fibre was transferred into a silica dish, dried overnight at 105^oC then cooled in a desicator. It is then ashed at 600^oC (overnight) in gallon camp muffle furnace and cooled in a desiccator and reweighed.

Determination of Carbohydrate Content:

This is also known as nitrogen free extract determination of each sample. It was obtained by deducting crude protein, crude fat, crude fibre and ash from 100.

Results

The result of hydration/dehydration presowing treatment are as in figures (1-2). It was observed that the highest percentage germination was recorded on the 8,10,12,14 and 16 days in seed scarified for 6 hours (50%). This was followed by 30% obtained from the seeds treated for 4 hours on the 10-16 days of germination. While about 20% germination was observed in seeds treated for 1 hour. The seeds treated for 2 hours gave as low as 10% germination percentage of 0% was observed with those seeds treated for 2-3 hours within 2-16 days (Fig. 1).

The seeds of *D. guineensis* subjected to hydration /dehydration presowing treatment for 5 hours gave up to 50% germination percentage withing 6-16 days. While the lowest germination percentage (0%) was recorded in seeds pre–treated for 3 hours gave up to 20% germination percentage within 8-16 days whereas, 10% germination was maintained with seeds treated for 2 hours.

Fig. 3. showed the direct effect of GA_3 on the on seed germination of *P. africana* seeds. It was observed that seeds treated with 3000mg/L GA_3 concentration gave the highest germination percentage of 70%. This was followed by 2000mg/L concentration of GA_3 which gave 50-60% germination while 1000mg/L GA_3 gave 10-20% germination of *P. africana* seeds.

Fig. 4. also showed the effect of GA_3 on D. guneensis seeds. The 300mg/L concentration GA_3 gave the highest percentage germination of 50-60% with 20-25 days. While the lowest germination percentage was recorded for 2000mg/L concentration of GA_3 giving 10% within 5-10 days of germination period. About 20% germination percentage was recorded for 1000mg/L GA_3 within 10-25 days.

Large sized seeds of *D. guineensis* gave 10% germination in 4-5 days, compared to 30% seed germination obtained in small-sized seeds. The germination percentage increased sharply to 60% within 5-10 days in large sized seeds. Up to 70% germination in small sized seeds of *D. guineensis* was obtained within the same days. The highest germination of 80% germination was obtained in large sized seeds when compared to 70% germination in small sized seeds within 10-15 days (Fig. 5).

Fig. 6 showed the *P. africana* seeds giving up to 80% and 70% germination in large sized and small sized seeds respectively within 10-20 days, followed by 60-70% germination within 5-10 days of period of germination. The lowest percentage germination was about 20-30% seed germination within 0-5 days for both small and large sized seed respectively.

Result showed that the plant height of seedlings of *P. africana* and *D. guineensis* raised from large-sized seeds were significantly higher ($P \leq 0.05$) in petiole length and leaf number than those from big sized seeds especially at 3 month old stage (Table 1).

Table 1. Effect of seed sizes on some aspect of seedling growth of two savanna tree species

Prosopis africana	Leaf number	Leaf number	Plant height		Petiole lengths	
	Ss	Bs	Ss	Bs	Ss	Bs
1 2 3	2.0 ± 1.0 1.1 ± 0.1 6.0 ± 3.0	3.0 ± 2.0 0.2 ± 0.1 4.0 ± 0.3	4.0 ± 3.0 0.2 ± 0.1 0.4 ± 0.3	3.0 ± 2.2* 6.1 ± 2.2 5.2 ± 1.1	1.0 ± 5.0 1.5 ± 0.2 1.4 ± 1.2	4.1 ± 6.2* 2.2 ± 3.1 1.1 ± 2.1*
Dialium guineensis						
1 2 3	3.1 ± 2.2* 4.4 ± 3.2 5.0 ± 2.0*	6.0 ± 3.2* 4.1 ± 4.0 3.3 ± 2.1*	4.2 ± 2.2* 6.1 ± 3.4 5.0 ± 2.2*	4.4 ± 2.1 2.2 ± 6.1 1.7 ± 6.6	3.2 ± 1.4 2.6 ± 7.8 1.9 ± 1.0	4.4 ± 2.6 1.1 ± 1.8 2.2 ± 2.8

Data are mean of five replicates
Ss = small sized seeds; Bs = Big sized seeds

Table 2. Proximate analysis of seeds of *P. africana* and *D. guineensis*

Species	MC (%)	DM (%)	Fe (%)	CP (%)	CF (%)	AC (%)	TCHO (%)
P. africana *D. guineensis*	8.64 12.62	9.36 87.38	4.57 8.85	78.00 21.61	2.43 2.33	2.19 4.71	554.15 51.88

MC = moisture, D.M = Dry matter, FC = fat content, CP = Crude protein,
CF = Crude fibre, AC =Ash content, TCHO = Total Carbohydrate content

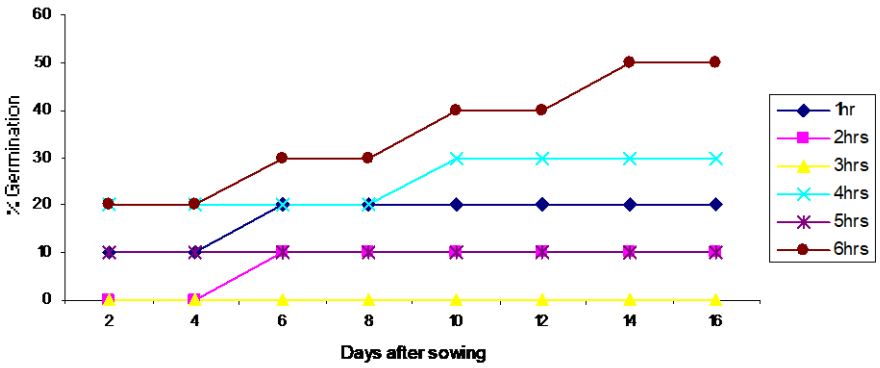

Fig 1 : Effect of hydration and dehydration treatments on germination of *D. guineensis* seeds.

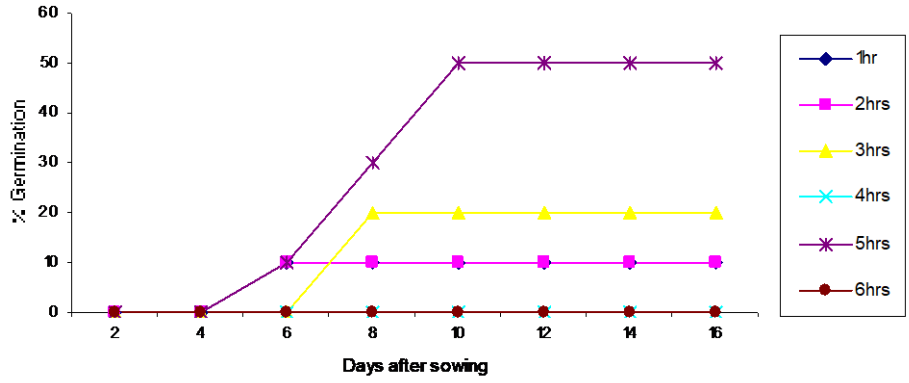

Fig 2: Effect of hydration and dehydration treatments on germination of *P. africana* Seeds.

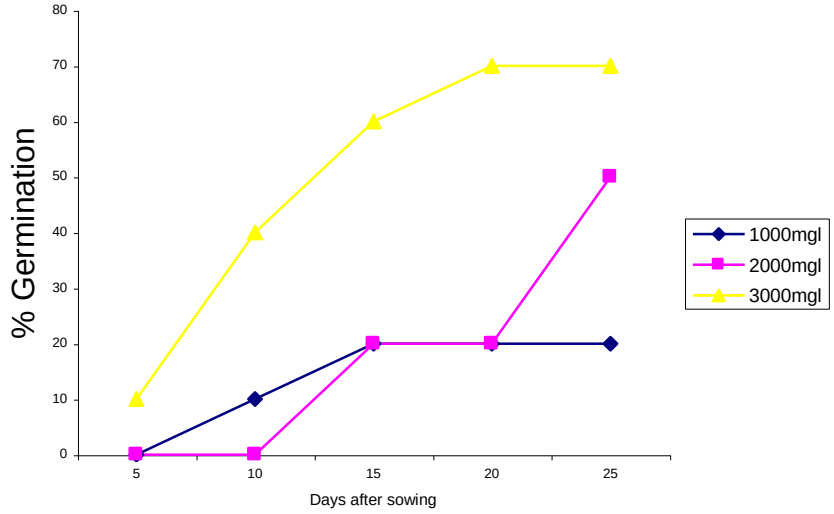

Fig 3: Effect of Gibberellic Acid (GA_3) on germination of *P. africana* Seeds.

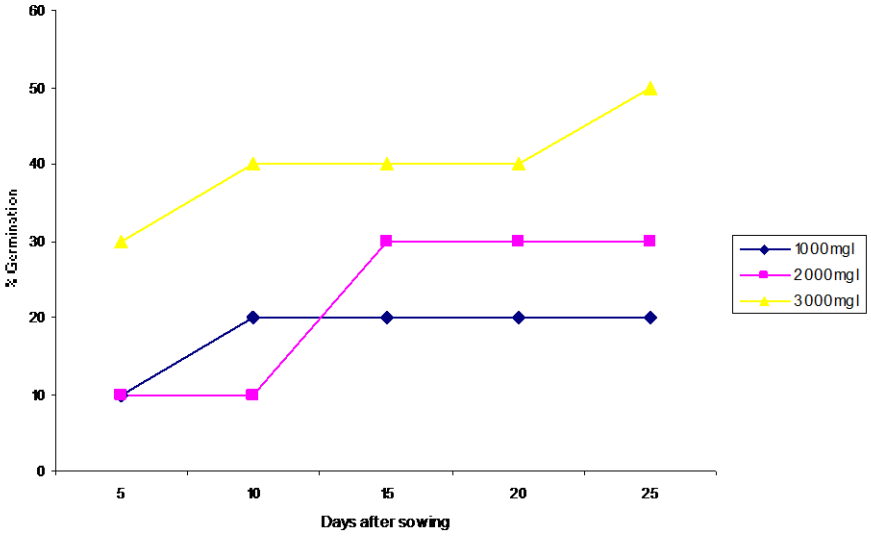

Fig 4 : Effects of GA3 on germination Of D. guineensis seeds

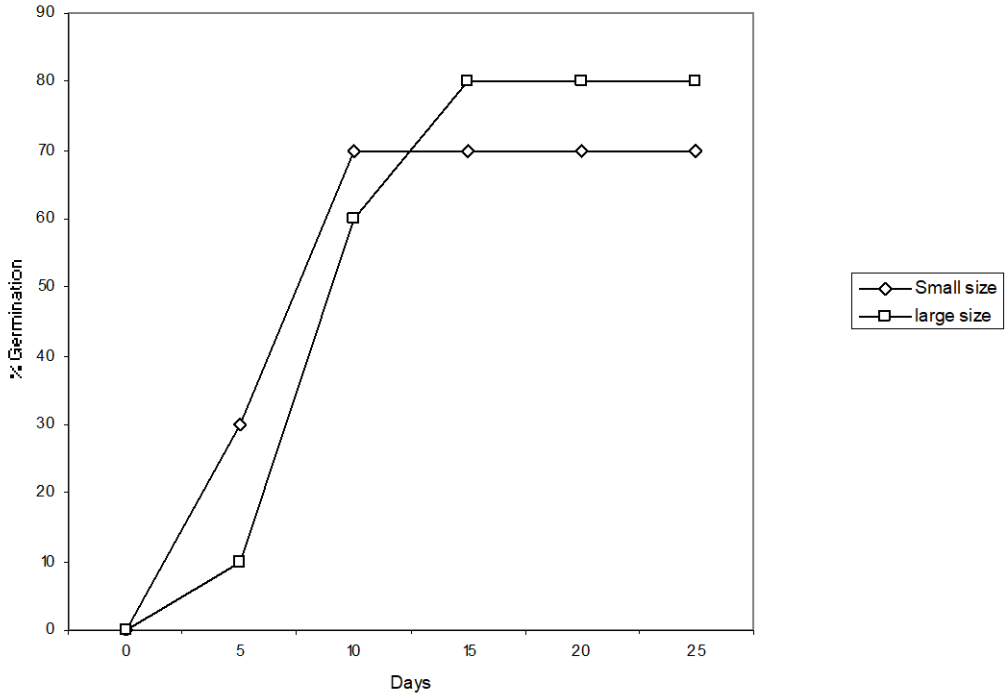

Fig:5: Effect of seed size on germination of Dialium guineensis seeds

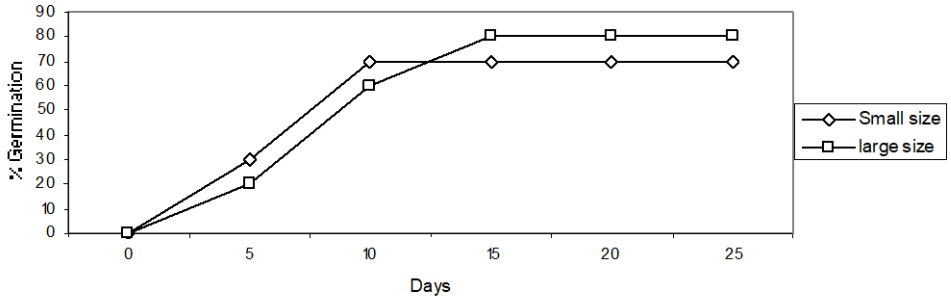

Fig.6: Effect of seed size on germination of Prosopis africana seeds

Discussion

P. africana and *D. guineensis* have two seeds types on the basis of colour that is LBS and DBS. They also have a varying sizes of seeds. Some of the colour types and seed sizes of some multipurpose tree such as *Parkia biglobosa, Uraria picta* etc. are well documented by (Etejere et al., 1982; Okusanya, et al., 1992). The colour differences are probably due to genetic attributes. The two distinct seed sizes observed were also common occurrence in trees such as *Gmelina arborea, Tectona grandis, Leucaena leucocephyla* (Agboola, 1992).

Seed size had significant effect on leaf number of plant height on *P. africana* and *D. guineensis*. Seedlings raised from big-sized seeds of these two tree species showed higher shoots and root dry weights (Ebofin, 2003). Dalianis (1980) found that larger seeds were superior to the medium and small in terms of seedling elongation rate and length. Agboola (1996) found that seed size had significant effect on growth performance as seedlings from larger fruit stones of *Gmelina arborea* were significantly better than those from smaller ones.

The seeds of *P. africana* and *D. guineensis* exhibits physiological dormancy due to hard seed coat. This was terminated using various scarification methods which include conc. H_2SO_4, hydration/dehydration treatments. These methods have been used to break seed coat dormancy in many seeds such as those of *Parkia biglobossa, Ceiba pentandra, Cercocarpus montamis, Centrosema pubescens* (Etejere et al., 1982;

Okusanya et al., 1992; Agboola, 1995a). Hard seed coverings have been found to be impervious to water and gases. The proper enzymatic actions and proper mobilization of food materials for the growth of the embryo are hampered due to the impervious nature of their seed coats. The degree of dormancy in seeds have been associated with many reasons including hard seededness (Agboola, 1995a).

Gibberellic acid is one of the major plant hormones involved in the control processes for mobilization of food reserves from the endosperm or cotyledons most especially enzyme production (Black, 1972). Hence acceleration of the rate of germination by 3000mg/L concentration of GA_3 in scarified seeds was due to the fact that there is an unhindered entry of GA_3, the seed coat barrier having been reduced and softened by acid scarification. Gibberellic acid has been known by plant physiologist to affect various processes in germination seeds, for example, metabolism of amino-acids and amides respiration and increase in amylase content. Paley (1960) has demonstrated increased amylse activity and increased formation of reducing sugars in barley seeds treated with gibberellins. This produces a possible explanation for the rapid swelling, splitting and peeling-off of testa observed in *D. guineensis* and *P. africana* seeds treated with gibberellic acid.

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