GERMINATION STUDIES ON SEEDS OF *TRICHILIA EMETICA* AND *VANGUERIA INFAUSTA*

By

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B.Sc. (For) (Hons) (Dar)

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF MASTER OF SCIENCE IN FORESTRY AT THE
SOKOINE UNIVERSITY OF AGRICULTURE

1988

DEDICATION

To my beloved parents, Petro and Niwaeli who laid the foundation for my education.

ACKNOWLEDGEMENTS

I am indebted to Professor J.A. Maghembe of Forest Biology Department, Faculty of Forestry, Sokoine University of Agriculture, who tirelessly supervised my work.

I express my sincere appreciation to Dr. S.A.O. Chamshama and Dr. L.L.L.Lulandala, both Senior Lecturers, of the Department of Forest Biology for their assistance during the planning phase of the work and during write up.

The Silviculturist, Mr. A.G. Kalaghe provided transport during seed collection. Mr. F. Malya, Officer Incharge, the National Official Seed Certification Laboratory, Morogoro, kindly allowed me to use the seed testing facilities.

The Norwegian Agency for International Development (NORAD) financed the whole programme.

Finally special thanks are due to E.R.Mkulasyai who typed this dissertation.

DECLARATION

I, HERIEL PETRO MSANGA, do hereby declare to the Senate of the Sokoine University of Agriculture that this dissertation is my own original work and has not been submitted for a degree in any other University.

Signature: H.P. Msanga .... Date : 15. Jul, 1987 ....

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ABSTRACT

The effect of various physical seed coat scarifications and chemical treatments were studied to determine presowing prescriptions that would ensure uniform, fast and high germination in seeds of Trichilia emetica and Vangueria infausta. The studies involved eight 3 x 5 factorial model experiments arranged in a randomized block design with with 4 blocks each. For each of the two species, two different experiments were conducted in the laboratory and repeated in the nursery.

The first experiment of Trichilia emetica involved 3 types of physical seed coat scarification and soaking the seeds in gibberellic acid (GA_3) at 5 concentrations; i.e. 0, 250, 500, 750, and 1000 ppm. A second experiment involved 3 types of physical seed coat scarification and soaking the seeds in potassium nitrate (KNO_3) at 5 concentrations; i.e. 0.0, 0.2, 0.4,0.6 and 0.8%.

The first study involving Vangueria infausta included 3 types of physical seed coat scarification and treatments of the seed with 5 concentration levels of hydrogen peroxide (H_2O_2); i.e. 0, 2, 4, 6 and 8%. In the second experiment, 3 types of physical seed coat scarification and soaking the seeds in 5 concentration levels of indole acetic acid (IAA); i.e. 0, 25, 50, 75 and 100 ppm were studied.

Unscarified seeds of Trichilia emetica (without chemical treatment) attained cumulative germination of 29% and 35%, while seeds with complete removal of both the aril and the seed coat attained cumulative germination of 94% and 93% in the laboratory and nursery experiments respectively.

The period from sowing to completion of germination was 18 and 34 days for unscarified seeds, while for seeds with complete removal of both the aril and seed coat the period was 13 and 23 days in the laboratory and nursery experiments respectively. Partial removal of the seed coat at the radicle end and removal of the aril alone produced satisfactory germination results. It is recommended that the aril alone in seed of Trichilia emetica be completely removed before sowing.

Application of gibberellic acid did not have significant effects on germination of seeds of Trichilia emetica. The data also shows that potassium nitrate (KNO_3) should not be used during germination in seeds of Trichilia emetica.

Unscarified seeds of Vangueria infausta (without chemical treatment) attained cumulative germination of 49% while treatments involving partial removal of the testa by abrasion attained cumulative germination of 55% for laboratory and nursery experiments in both cases. The period from sowing to completion of germination was 38 and 45 days for unscarified seeds, while treatments involving partial removal of the testa by abrasion the period was 30 and 42 days in the laboratory and nursery experiments respectively.

Application of 6% hydrogen peroxide (H_20_2) stimulated germination in seeds of Vangueria infausta, but concentrations above this level produced detrimental effects. The data also shows that indole acetic acid (IAA) should not be used in pretreating seeds of Vangueria infausta.

CHAPTER 1

INTRODUCTION

Trees are propagated from seed, with a few exceptions, such as *Euphorbia*, *Ficus*, *Populus* and *Salix* species which are propagated by stem cuttings. Tree planting through seeds is therefore the principal means of meeting the increasing demand for forest products. Operationally, tree planting may involve establishment of industrial plantations, shelter belts, enrichment planting, or scattered planting for soil stabilization, urban and rural amenity and agroforestry. With such a variety of planting purposes, the scale of tree planting and the variety of species to be planted have continued to grow in the tropics. This has introduced new problems in seed processing and germination.

A lot of research has been done on seed germination of temperate tree species. In the tropics however, little or no information is available on the germination of many indigenous species. Exploitation of valuable indigenous species has continued for years without due considerations for planting the exploited species. As a result, many valuable indigenous species have been over exploited in their natural habitats to the extent that they can no longer recover through natural regeneration. Two such species are *Trichilia emetica* and *Vangueria infausta*.

based on endogenous or exogenous factors. On the other hand, seeds of V. infausta show sporadic germination over a long time and at the end, the germination is very low.

There is no published information on how to germinate seed of the species, although some scanty information is available in publications dealing with other silvicultural aspects of the species (Watkins, 1960 ; Mugasha et al., 1980 ; FAO, 1983; Forest Division, 1984). The work reported here focused on solving seed germination problems in the two species. More specifically, the work examined:-

- The effects of various physical seed coat scarifications on the germination parameters (cumulative germination, germination energy, germination value, dormancy periods and initial seedling growth);
- The effects of various chemicals at various concentrations on germination parameters as above;
- The effects of various physical seed coat scarifications in combination with various chemicals at various levels on germination parameters as above;
- Comparisons between laboratory and nursery germination parameters.

CHAPTER 2

LITERATURE REVIEW

2.1 Description and distribution of the trees

2.1.1 Trichilia emetica

Trichilia emetica Vahl. Syn. T. roka (Forsk.) Chiov. is commonly known as Woodland Mahogany in English and Mgolimasi or Mafura in Kiswahili. It belongs to the family Meliaceae. The name Trichilia has been coined from the Greek "tricho" meaning "in 3 parts", and it refers to the often 3 celled fruits. The specific name emetica is based on the emetic properties of the infusion of the bark (Palmer and Pitman, 1972).

T. emetica is a large, much branched evergreen tree reaching 8 - 25 m high with a dense rounded crown. The bark is grey and flaking. The leaves are imparipinnate, pubescent beneath or subglobrous, varying in length from 10 to 40 cm. The number of leaflets varies from 3 to 13. They are oblong elliptic, 4 to 23 cm long and 2 to 10 cm wide. Flowers are yellowish - green, or white cream, produced on congested axillary panicles. Each fruit is a globose capsule, about 2.5 cm in diameter. At maturity the capsule wall splits slowly, over a long period - into 3 valves exposing about 6 black seeds almost completely concealed by a scarlet aril.

Each seed is about 2.0 cm long and 0.8 cm wide and slightly flattened (Exell et al., 1963).

T. emetica grows naturally from Senegal to the Red Sea, through East and Central Africa to the Congo, Natal, and the Transval and also in Yemen (Palmer and Pitman, 1972). The tree is wide spread in Tanzania, absent only in the miombo woodlands and dry thicket belts. It is most abundant on heavy soils in riverine and alluvial forests, usually in company with Acacia campylacantha, A. albida and Ficus sycomorus (Brenan and Greenway, 1949). The tree species occurs widely in Kyela, Ifakara,Muheza, Korogwe and Morogoro districts.

2.1.2 Vangueria infausta

Vangueria infausta Burch. Subsp. rotundata (Robyns) Verdc. Syn. V. tomentosa Hochst . is commonly known as Wild Medlar in English and Muviru in Kiswahili. It belongs to the family Rubiaceae. The specific name "infausta" means "unlucky" due to its supposed magic properties (Palmer and Pitman, 1972). Vangueria infausta is a small deciduous tree about 5 m but occasionally reaching 8 m, with a grey trunk. Leaves and flower buds have densely matted hairs. Leaves are simple opposite, broadely ovate to lanceolate, medium to large, usually from 5 to 30 cm long and 3 to 18 cm wide and tomentose on both surfaces. Cymes are borne in axils of fallen leaves or second year shoots. They are about 2 to 3 cm long. The whole inflorescence is pilose (Troupin, 1982). The fruits are fleshy drupes, subglobose, 3 to 4.5 cm long and 3.5 to 6 cm wide, crowned with a shallow ring (the veins of a calyx) covered with a greenish leathery skin which turns light brown when ripe (Palmer and Pitman, 1972). Within the fruits there are 5 pyrenes (or few by abortion) which are areolate. Seeds in each pyrene are solitary, hard - coated, 2.0 to 2.5 cm long and 1 to 1.5 cm wide, embeded in a yellow - brown pulp.

Lushoto herbarium records show that V. infausta is widely distributed in Tanzania particularly on the margins of disturbed woodlands. It has also been reported to occur in South Africa (Palmer and Pitman, 1972) and Rwanda (Troupin, 1982).

2.2 Seed germination

Germination consists of three overlapping processes (Ching, 1963; 1966):

- Absorbtion of water mainly by imbibition, causing a swelling of the seed with eventual breaking or splitting of the coat;
- Concurrent enzymatic activity, increased respiration and assimilation rates and translocation of metabolites to growing regions;
- Cell division and enlargement resulting in emergence of the radicle and plumule.

Detailed accounts of the biochemical processes occuring during seed germination have been made in many reviews (Toole et al., 1956; Amen, 1968; Rolston, 1978). Figure 1 summarises these processes for a dormant seed.

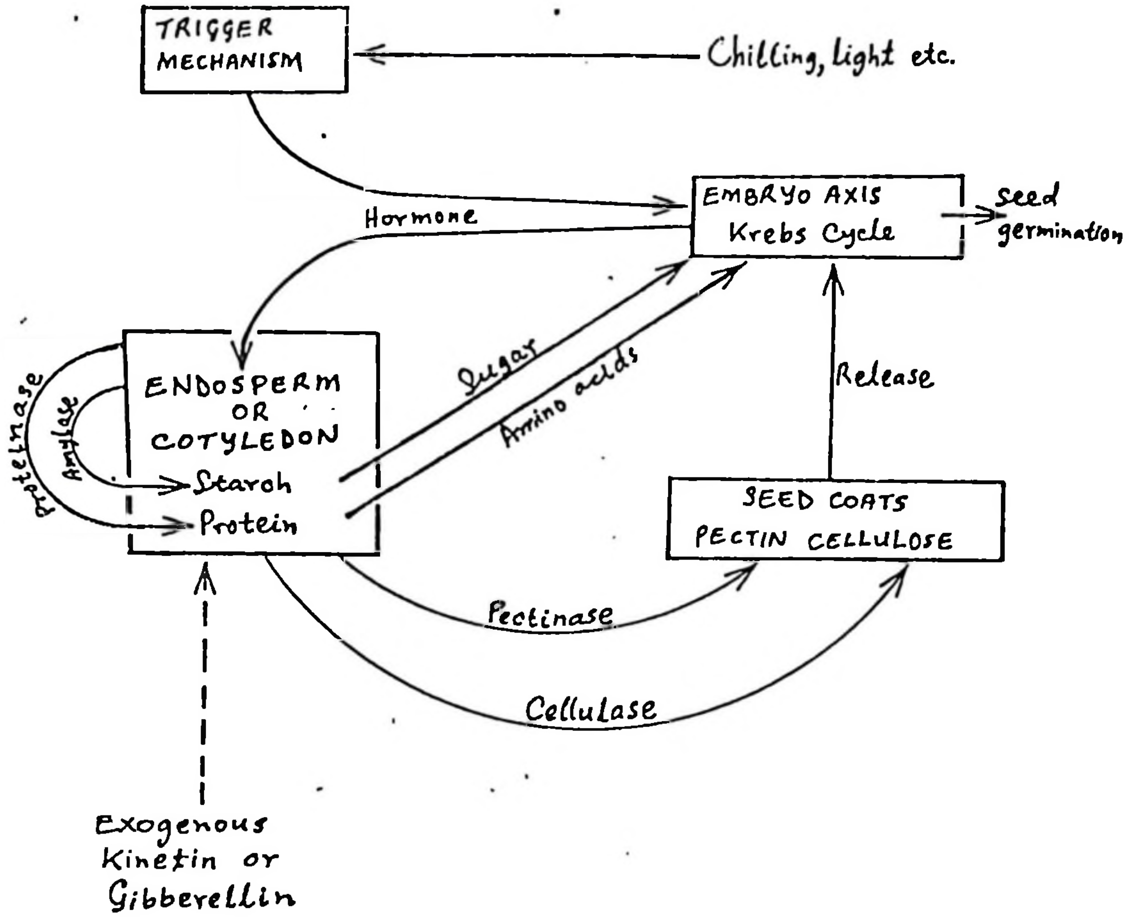

Fig. 1 : Processes during the germination of a dormant seed. (Adopted from Amen, 1968).

2.3 Factors affecting seed germination

Seed germination is initiated when environmental conditions are just right. These conditions include adequate supply of water; optimal temperature; sufficient oxygen for normal aerobic metabolism; biocids and in certain instances light. The quantitative and qualitative requirements for these factors vary according to species and seem to represent plant adaptation to their environment (Berrie, 1984). Following is an examination of these factors in detail.

2.3.1 Water

Water is a basic requirement for seed germination. It is essentjal for enzyme activation and for the breakdown, translocation and use of food reserves. In their resting stage, seeds are characteristically low in moisture content (water potential as low as - 10 bars). Germination is not possible in most plant seeds until imbibitión has brought up the seed water potential to values greater than - 0.15 bars (Kaufman and Ross, 1970). At this water potential most seeds will have acquired the 30 - 60% moisture content which is required for germination to occur (Heydecker et al., 1969).

Ordinarily, when dry seeds are exposed to liquid water, there is a general increase in moisture content until about 40% of initial fresh weight. The rate at which this plateau is attained varies with species, but once reached, it is succeeded by another increase in water uptake. This pattern

Seeds which respond to diurnal variations are probably responding to internal senses of their position on the upper soil horizon. Generally the limits of the fluctuations are separated by about 15°C in the miombo. For most temperate ecosystems however, seasonal temperature fluctuations of 20 to 35°C are common (Black and Wareing, 1955 ; Attims and Come, 1978; Totterdel and Roberts, 1980).

Detailed studies on the effect of alternating temperatures on germination have shown that a temperature range of 25°C for 24 hours and 5°C for 10 hours strongly prompted germination in seeds of *Hyscyamus muticus*. Kinetin 60 ppm substituted for the periodic temperature changes. Both kinetin and diurnal temperature fluctuations increased the acetic acid level in seed prior to germination (Elkinawy and Hemberg, 1974). It is considered that alternating temperatures create a balance of the intermediate products of respiration at the high temperature part of the cycle, which though unfavourable for germination at that temperature, may promote germination at a lower one (Toole *et al.*,1956).

2.3.2 In other plant species, an optimum temperature exist at which germination occurs best. The optimum temperature for germination of *Erythrina caffra* seed was found to be 30°C. The relatively high optimum temperature (30°C) for germination of seed of this species correlates with the fact that the species range is within the warm subtropical regions of the world (Small *et al.*, 1977). The optimum temperature for most provenances of *Eucalyptus microtheca* in Australia is 35°C and this is believed to be an adaptation to the climatic conditions prevailing in the period of general seed fall (Doran and

Boland,1984). For *Lotus carniculatus* and *Dystaenia takesimana* the optimum temperature for germination is 20°C (Hur and Nelson, 1985).

2.3.3 *Oxygen*

Respiration during seed germination increases rapidly initially, then gradually diminishes as germination is completed (Opic and Simon, 1963). An adequate supply of oxygen must therefore be available. Oxygen plays a primary role as the electron acceptor in catabolism. In some species it may also be involved in activation of an inhibitor (Black and Wareing, 1959).

For some tree species the seed coat appears to be a barrier to oxygen uptake. For example, the removal of the seed coats in seeds of *Pinus strobus* markedly accelerates oxygen uptake. Exposure of intact seeds to high oxygen concentration also accelerates their rate of oxygen uptake. Removal of seed coats, followed by exposures of seeds to high oxygen concentration often results in higher rates of seed respiration and improved germination (Kozlowski and Gentle, 1959). In other studies, removal of the testa in seeds of *Erythrina caffra* or increase of oxygen partial pressure increased the rate of germination. This is attributed to an increased oxygen permiability (Small *et al*., 1977).

The intact seed coat of *Betula pubescens* and *B. verrucosa* prevented germination in the dark by reducing oxygen supply below a critical level. Nevertheless, isolated embryos germinated in low concentrations of oxygen. Hence, the embryo appeared to be reached by very limited supplies of oxygen when the seed coat was present. The data suggested that barrier presented by the seed coat increased the oxygen requirement for germination and that oxygen above a threshold level was required to supress the action of the seed coat (Black and Wareing, 1959).

Most seeds will not germinate when the germination medium is too wet, when the seeds are planted too deep, or when other conditions limit the supply of oxygen. Even then the rate of oxygen absorbtion during seed germination is highly variable among species. Populus deltoides and Salix nigra seem to be triggered to germinate by anaerobic conditions as they normally begin to germinate while submerged in water. Interestingly, Ulnus americana and Planatus occidentalis would not be induced under similar treatment (Hosner, 1957). Seeds of Typha latifolia will normally germinate under water if given a light stimulus. This treatment is important since anaerobic conditions seem to be preferred during part of the germination process (Sifton, 1959).

2.3.4 Light

Another important environmental factor which is sometimes critical to seed germination is light. Seeds which respond to light are termed photoblastic. Non -responding seeds are non-photoblastic. Light may induce or promote germination, these seeds being positively photoblastic, or light may prevent or retard germination, in which case the seeds are negatively photoblastic. Germination of the tropical and subtropical grasses Chloris gayana and Cynodon dectyton is promoted by light while a few species like Phacelia tanacetifolia will only germinate in darkness, as light may be inhibitory (ISTA, 1985). Seeds of *Vitis venifera* do not germinate in darkness, but will germinate promptly, just after harvest, in the presence of appropriate light exposure at alternating temperatures of 35 and 15°C (Pereira and Maeda, 1986).

Seed germination in some temperate plant species is better under continuous light as in the case of *Pseudotsuga menziesii* and *Pinus banksiana* (Jones, 1961). In some situations, the continuous light requirement can be replaced by one or several exposures to red light. Studies in seeds of several forage species, have demonstrated initiation of germination following eight minutes of red light treatment (Schultz and Kinch, 1976). In certain cases, there seems to be an interaction between light and some environmental factors such as soil temperature and nutrients (Vincent and Roberts, 1977).

Light affects seeds germination in many tree species. For most species, the maximum total germination and speed of germination occur in daily light periods of 8 to 12 hours. Interrupting the dark period with a short light flash or increasing temperature usually give the same effect as lengthening the duration of exposure to light. However there are some variation in photoperiodic requirements among species; *Eucalyptus* seeds germinate well in 8 hour days and those of *Betula* in 20 hour days, but not in 8 - hour days (Jones, 1961). At low temperatures there is a requirement for several cycles of long day treatment for germination of *Betula* seeds. At 20°C, however, the seed germination occurs following a single light exposure and at 25°C to 30°C germination occurs in the dark (Black and Wareing, 1955).

Light intensity has relatively minor effects on seeds of forest species while photoperiod and wavelength often have pronounced effects. Many seeds usually germinate at low light intensities. For example seeds of Betula generally require only 1 Lux (Black and Wareing, 1955), Pinus 5 Lux, some conifer and hardwood species require up to 100 Lux for germination (Jones, 1961).

Germination in some species of forest tree is sensitive to wavelength, with red light (about 650 mu) stimulating germination and for red light (750 m u) inhibiting it. Therefore, the occurance of failure of germination depends on which irradiation was given first (Toole et al., 1961).

Light has considerable influence on the regeneration of trees in the forest. Seeds of Gmelina arborea, Sapium baccatum and Vitex pinnata complete germination in the open, in less than two months (Mohamed and Ng, 1982). Under dense forest canopy, they are completely inhibited but are capable of germination after 6 months if moved to the open. Under moderate shade of palm roofing, the seeds differentiate into two physiolocically distinct portions. One portion germinates within 2 months, thereby behaving as if the seeds were in the open. The remainder lie dormant until moved to the open. For species that have evolved two staged behaviour, the obvious advantage is that seeds will remain domant under heavy shade when conditions are unsuitable for growth, produce a partial germination response when the canopy is thinned or partially opened, and full response when the canopy is open.

2.4 Seed dormancy

Seed dormancy is a physiological state in which viable seeds fail to germinate when exposed to suitable conditions for germination (Taylorson and Hendricks, 1977). The causes of seed dormancy have been studied in detail and can be separated into 5 broad categories.

- Impermeable seed coats;
- Mechanically resistant seed coats;
- Rudimentary embryo;
- Physiologically immature embryos;
- Presence of germination inhibitor

In some reviews of literature on the subject, these factors have been grouped into three types of seed dormancy namely (Wareing and Saunders, 1971):

- Exogenous or seed coat dormancy;
- Endogenous or embryo dormancy;
- Combined dormancy.

2.4.1 Exogenous or seed coat dormancy

This type of dormancy may include one or a combination of three conditions:

- Physical impermeability of seed coat to fluids;
- Chemical inhibitors present in the seed coat or
- Mechanical resistance of seed coat to embryo growth (Gordon and Rowe, 1982).

Impermeable seeds are common in many legumes and are also found in the Lamiaceae and Malvaceae (Amen 1963; Quincivan, 1968; Mathur et al., 1984). The common factor affecting seed impermeability in species from different families is a layer of macrosclerid cells that form a palisade layer in the testa (McNaughton and Robertson, 1977).

An example of seeds with chemical inhibitor in the seed coat is Strelitzia species (Van de Venter and Small, 1975). The common inhibiting substances in seed germination are phenols (Mayer and Poljakoff - Mayber, 1975), Coumarin (Lerner et al., 1959) and abscisic acid (ABA) (Aspinall et al., 1967; Sankhla and Sankhla, 1968).

Seeds with mechanically resistant seed coats are found in many plant genera including Juniperus, Corylus, and Cocos (Amen, 1963).

2.4.2 Endogenous or embryo dormancy

Three types of embryo dormancy have been identified (Wareing, 1971; Webb and Wareing, 1972) including:

- Cases of embryo dormancy resuting from morphologically underdeveloped embryos at the time the seed is separated from the parent plant;
- Cases of embryos which are morphologically mature but physiologically immature at the time of seed dispersal or collection;
- Cases in which dormancy is apparently due to growth inhibiting chemicals present in the embryo. The most commonly mentioned examples of seeds with embryo dormancy include Nicotiana tabacum (Ogawara and Ono, 1961); Eucalyptus delegatensis, E. regnans (Bachelard, 1967); Protea compacta (Brown and Van Staden, 1971).

2.4.3 Combined dormancy

Seed coat dormancy and embryo dormancy may occur at the same time in some plant species. Examples are found in *Cercis canadensis*, *Carpinus caroliniana* and *Viburnum opulus* (Bonner *et al*., 1974).

2.4.4 Significance of seed dormancy

Seed dormancy is considered to be a biologically advantageous characteristic. It enables a species to regulate its growth cycles to seasonal or accidental changes in environmental conditions. Seed dormancy enables germination to take place at a time during the growth season when seedling establishment is likely (Berrie and Brennan, 1971). In the case of seed coat dormancy, impermeable seed coats permit extension of life to the seeds and permits extensive dispersal by birds and other animals (Burton and Andrews, 1948; Williams and Elliott, 1960).

In silviculture, seed dormancy has other advantages as well as disadvantages. One of the advantages in establishing perenial crops is that the dormant seeds may be considered as a potential reserve of the species to make up for empty spots in the stand or to help in establishing the stand following unfavourable conditions (Rolston, 1978). The spontaneous establishment of pioneer species in clearings in Malaysia has been attributed mainly to the presence of a reservoir of dormant viable seeds in previously undisturbed and shaded forest soil (Liew, 1973). Under tropical conditions delayed germination is also advantageous in providing a large period for disposal as the case for *Styrax benzoin* in Malaysia (Kiew, 1982).

Seed dormancy causes difficult problems in seed testing, as dormant seeds often require complicated treatments and long periods of testing (Rolston, 1978). In nurseries, seed dormancy causes erratic germination, reduces confidence in the final result and tends to increase the cost of nursery stock by lengthening the total nursery period. In addition, dormancy produces initially uneven stock and often leads to a waste of seed (Nagaveni and Srimathi, 1981).

2.5 Methods of overcoming seed coat dormancy

2.5.1 Physical seed coat scarification

Scarification is any treatment which eliminates or reduces the impermeability of the seed coat to fluids (Bonner, 1984). Seeds of many leguminous species respond well to physical scarification as examplified by *Erithrina* (Small *et al*., 1977), *Dialium*, *Sindora* (Ahmad, 1978), and *Intsia* species (Sasaki and Ng, 1981).

In this method seeds are treated by rubbing on rough stones, filing, sand papering or by splitting the seed coat as long as damage to the endosperm and embryo is avoided. The best site for scarification is that part of the seed coat immediately above the tips of the cotyledons (ISTA, 1985). For large quantities of seed, tumbling in concrete mixer with sharp gravel, crushed glass or sand is effective. This method has been used successfully with seeds of *Acacia farnesiana*. Seeds of this species bottled with crushed glass for five hours gave a germination of 20.7% in 43 days, compared with 0.7% for untreated seed. There was also a positive relationship between the time used for scarification, and cumulative germination (Kisou *et al.*, 1983). In similar studies complete removal of the seed coat of *Podocarpus usambarensis* resulted in 81% germination after 6 weeks compared to no germination for untreated seed (Chamshama and Downs, 1982).

Germination in seeds of *Erythrina caffra* is staggered out over a long period and at the end, the germination percentages are very low. Studies have shown this to be due to impermeability of the seed coat to water and oxygen. In a detailed study, mechanical abrasion and incubation for 7 days at 30°C achieved 92% germintion compared to 4% for unscarified seeds (Small *et al.*, 1977). Similarly, seeds of *Dialium mainganji* and *Sindora coriaceae* (Leguminosae) normally take many months to achieve a good level of germination. Mechanical scarification by cutting away part of the seed coat has been found to give the best germination results i.e. 68 - 74% within one month (Ahmad, 1978).

Some of the most extensive studies on seed scarification in India have been made with Sandal (*Santalum album*). In this species seeds have been found to germinate fast when the seed coat is completely removed. The decoated seeds attain a maximum germination of 90% whereas untreated seeds attain unsatisfactory level of germination. Following removal of the seed coat, germination starts in about 9 days and completes germination in 30 days. It is considered that in addition to the thin but hard seed coat being substaintially impermeable to water, the seed coat posseses some inhibitory principle making the seed liable to delayed germination (Nagaveni and Srimathi, 1981).

Manually applied physical seed scarification is one of the simplest and most direct method of seed scarification. Manual treatment of individual seeds is safe and effective. It is most suited to larger and more refractory seeds (Wunder, 1966), although it can be slow in large nurseries. This technique is especially suitable for seeds with abundant resin or pulp which tend to clog machines in mechanical scarification. In most cases care must be taken to avoid overtreating which may cause damage to the seeds (Bonner et al., 1974) or make the the treated seeds more susceptible to attack by pests and pathogens (Kemp, 1975).

2.5.2 Soaking in water

The testa and its structures, the hilum, micropyla, strophiole and chalaza have been implicated as barriers to imbibition in leguminous seeds (Ballard et al., 1976). The hilum fissure, by virtue of its counter palisade cells, opens when the moisture in the air is less than that of the seed resulting in moisture loss from the seed, when the relative humidity increases the hilum fissure closes (Hyde, 1954). Hot water soaking has given successful results with many dormant leguminous seeds. In Leucaena leucocephala the hard seed coat can be softened to permit water uptake by immersing the seed in boiled water and allowing them to soak as the water cools to room temperature (Lulandala, 1978).

Treatment with boiling water improves germination of *Acacia sieberiana* seed from 2 - 10%, whereas continuous boiling in water for 60 minutes achieved 60% germination (Larcen, 1964). Treating *Acacia nilotica* seeds with hot water (80°c) for ten minutes and then soaking them in ordinary water for 24 hours gives better germination results than untreated seeds. These studies also indicated variations between provenances in response to hot water soaking (Mathur *et al*., 1984). In our own studies, seeds of *Albizia schimperana* germinated better after soaking in hot water at an initial temperature of 75°C than in boiling water (99°C) (Msanga and Maghembe, 1986). On the other hand, seeds of many *Acacia* species germinate more readily after soaking for up to 48 hours in water at ambient temperature. The longer the duration of soaking the greater the germination (Bebawi and Mohamed, 1985).

Some seeds respond well to a regime of alternate soaking and drying. In Tanzania *Tectona grandis* seeds (strictly fruits) are packed in sacks and soaked in running streams for 72 hours. They are then dried in the sun for two days before sowing (Wood, 1967). Prolonged soaking in stagnant water may serve to soften the seed coats but unless the water is changed daily, there is a risk of injury on the seed. Soaking seeds of *Cercocarpus montanus* and *Atriplex canescens* in cold water for 48 hours has been found to depress germination (Riffle and Springfield, 1968). As the time of soaking increased from 1 to 15 days, germination capacity of *Santalum album* decreased from 45 - 11%, respectively in sunken seeds (Nagaveni and Srimathi, 1985).

In studies involving soaking seeds of Cassia fistula in water, it was found that soaking increased microbial activities resulting in substantial increases in rotting seeds and abnormal seedlings (Randhawa et al., 1986). To avoid this drawback, frequent changes of water are recommended during prolonged soaking (ISTA, 1985).

2.5.3 Chemical scarification

In this method seeds are soaked in a chemicals for a period of time depending on species and the type and concentration of the chemical. Concentrated (95 - 98%) sulphuric acid (H_2SO_4) has been used effectively to break dormancy for many species with hard seed coats. Seeds of Gledistia tricanthos are soaked for 1 hour (Kisou et al., 1983); Prosopis chilensis for 2 minutes (Winer, 1983) and those of Acacia tortilis for 1 minute (Chamshama and Downs, 1984). Sulphuric acid treatment for 10 minutes was highly effective for Albizia richardiana and Samanea saman. Other species like Acacia farnesiana require up to 40 minutes treatment (Kumar and Purkayastha, 1972). The extremely hard seed of Erythrina caffra attained 30% and 95% germination after soaking in sulphuric acid for 30 minutes and 120 minutes, respectively compared to untreated controls which attained only 4% germination (Small et al., 1977). Seed dormancy in Cassia fistula is easily broken when seeds are treated with sulphuric acid for 5 to 11 minutes. This treatment increases seed germination from 4% (of check) to more than 67% (Randhawa et al., 1986).

The mode of action of sulphuric acid in scarification has been studied in a number of species. Sulphuric acid dissolves the counter palisade layer which obstructs the hilar fissure in *Lupinus angustifolius* (Burns, 1959). Scanning electron micrographs of *Coronila varia* seed scarified by acid indicated that lumens of macrosclerid cells were exposed, permitting imbibition of water (Brant *et al*., 1971). In *Erythrina caffra* it was found that the hilar region was affected most, as judged by the swelling pattern of the seeds (Small *et al*., 1977). In *Sinapis arvensis* seeds it was the rapid dessication provided by the sulphuric acid and not its strong oxidising ability or its high hydrolic capacity which seems to cause the fragmentation of teguments allowing the passage of oxygen to the embryo (Duran and Tortosa, 1985).

Chemicals other than sulphuric acid have also been found to overcome seed coat dormancy. A concentration of 67% potassium hydroxide (KOH) has been found to overcome dormancy in seeds of *Sinapis arvensis* by removing barriers based on the seed coat (Duran and Tortosa, 1985). Acetone and petroleum ether significantly reduced seed coat hardness and increased germination in seeds of *Coronila varia* (Brant *et al*., 1971).

2.5.4 Biological methods

In nature, animals and micro-organisms are an important factor in the breakdown of seed coat impermeability. Under arid conditions in Northern Sudan, *Prosopis chilensis* seeds that have passed through the digestive tracts of goats show a higher germination value compared to soaking in concentrated sulphuric acid, hot water or cold water (Winer, 1983).

Insects play a big role in breaking seed dormancy for seeds with hard seed coats. Indeed, one of the methods used to increase seed germination in Pterocarpus angolensis is to let termites eat up the tough wings and bristles of the fruit (Groome et al., 1957). Puncturing of the seed coat by a small beetle (Bruchieus sp.) can contribute to dormancy breaking in seeds of Erythrina caffra and where sufficient moisture is available, seeds can germinate before being killed by the developing larvae (Small et al., 1977).

Many microorganisms known to be associated with tree seeds have been linked to seed germination. For example, the fungus Rhizopus has been found to corrode the seed coat of Gymnocladus dioica and positively increasing its germination value (Raleigh, 1930).

2.5.5 Fire

In the savanna, fire is a powerful factor in the removal of seed dormancy. Hot enough fires, will reduce seed coat impermeability and stimulate germination. Light fires have been used in this way to stimulate germination in seeds of Pterocarpus angolensis (Groome et al., 1957) while the impermeable seed of Acacia melanoxylon will germinate following fire, allowing rapid colonization of the burnt area (Villiers, 1972). At Arusha National Park in Tanzania, regeneration of Juniperus procera is usually very dense after the seeds have been stimulated by light fires (Hall and Ndosi, 1982).

2.6 Methods of overcoming embryo dormancy

2.6.1 Stratification

Stratification is the practice of placing seeds in alternate layers of moist medium to hasten afteripening and even overcome dormancy. The term is generally used to describe any technique which keeps seeds in strata of cold and moist environments (Bonner, 1984).

The period of stratification vary considerably from species to species, from seed lot to seed lot, and even from tree to tree within the same species (Milby, 1986). For most tree species, seeds are kept at a temperature between 3°C and 5°C for a period, varying with species from seven days to 12 days (ISTA, 1985).

The optimal period for stratification in *Fraxinus pennysylvana* is 30 days at 3°C (Tinus, 1982), 21 days in *Pinus taeda* (Biswas *et al*., 1972) , and 40 to 60 days in *Acer sacchurum* (Shin *et al*., 1985). As expected for tropical species low temperature stratification produces very low germination values when compared to unstratified treatments due to "prechill damage" (Gordon *et al*., 1972). This finding seems to contradict with results observed in *Pinus caribaea* var *hondurensis* where stratification of seeds at temperatures slightly above 0°C for one week improves germination and shortens the period of completion of germination (Madhawaraja, 1982).

2.6.2 Chemical treatments

2.6.2.1 Gibberellins

There is a conclusive data to show that gibberellins (GAs) at high concentrations can break the dormancy of positively photoblastic and/or temperature sensitive seeds (Beevers et al., 1970 ; Vidaver and Hsiao, 1974), negatively photoblastic seed (Chen and Thiamann, 1964; Macleod and Reid, 1985) and non - photoblastic seed requiring stratification (Black and Naylor, 1959). Gibberellic acids are also effective in breaking stress imposed dormancy (Kahn, 1960).

Over 60 individual GAs are known today and there is ample evidence that their effectiveness in promoting seed germination varies widely. In many experiments, GA_4 is the most effective and able to break dormancy at concentrations as low as 35 ppm (Thomson, 1968). Gibberellic acid (GA_3) is effective in stimulating germination in a number of species but where this is active GA_4 and GA_7 are more active even at lower concentrations (Ikuma and Thiamann, 1963). Because it is needed in large amounts, GA_3 might appear to be a universal dormancy breaking agent. This assertion has led to the erroneous hypothesis that other dormancy breaking treatments like light, promotes the synthesis of gibberellic acid which in turn stimulates germination (Berrie and Taylor, 1981). In any case; the International Seed Testing Association recommends that in seed testing the germination substratum should be moistened with 500 ppm solution of GA_3, when the dormancy is weaker, 200 ppm may be enough; when it is stronger up to 1000 ppm may be used (ISTA, 1985).

GA_3 has been recommended for breaking dormancy in many seeds, including Eucalyptus delegatensis, E. nitens and E. regnans (Bachelard, 1967); Datura ferox (Sánchez et al., 1967); Avena sativa, Hordeum vulgare, Secale cereale, Triticum aestivum (ISTA, 1985).

2.6.2.2 Potassium nitrate

The effect of potassium nitrate (KNO_3) on seed germination was realized when it was noted that Knop's solutions promoted the germination of some seeds. Further experiments showed that potassium nitrate was responsible for this stimulation (Mayer and Poljakoff - Mayber, 1975). Today potassium nitrate is the most widely used chemical for promoting seed germination. A solution of 0.2% potassium nitrate is commonly used in routine seed germination tests (ISTA, 1985). Potassium nitrate increases light sensitivity to positively photoblastic seed and may interact with temperature to increase germination in some plant species (Ferreira and Small, 1974). For example, a significant germination response was observed when positively photoblastic weed seeds were subject to 0.2% potassium nitrate alone (e.g. Chenopodium album), or to a combination of potassium nitrate and alternating temperatures of 25°/15°C (Vincent and Roberts, 1977). These effects alternating temperature and potassium nitrate in the absence of light are often more marked after chilling. However, in all cases, the highest germination is obtained when all three factors are combined. In other studies potassium nitrate has also been shown to act cooperatively in combination with gibberellic acid and kinetin to induce germination of Nicotiana tabacum seed in light (Ogawara and Ono, 1961).

The stimulation obtained by potassium nitrate is dependant on its concentration. For example, the germination of Agrostis tenuis seed was increased progressively from 75 - 85% when the potassium nitrate concentration was increased from 0.1 - 0.5% (Gadd, 1955). The germination of Epilobium montanum seed in the dark at 16° - 20°C increased from 8% (untreated controls) to a maximum of 90% at 0.01% potassium nitrate and then declined to 30% when the concentration of potassium nitrate was increased to 1% (Hesse, 1924; cited by Mayer and Poljakoff - Mayber, 1975). The germination of Albizia schimperana seed in light at ambient temperature (20° - 31°C) was not improved by application of 0.2% potassium nitrate in seeds previously pretreated by soaking in water at 50°C. However, it depressed germination in seeds which were soaked in water at 75°C (Msanga and Maghembe, 1986).

Nearly 30 years ago, data was presented to show that potassium nitrate may be detrimental to the germination of seeds in some plant species. In some studies, the germination of Lactuca species seed was inhibited by potassium nitrate despite the fact that the seed required light and low temperatures to germinate (AOSA, 1959). The literature shows that potassium nitrate shows only slight improvements on seed germination and is seldom stimulating on its own. Its influence on seed germination is strongly depending on other factors such as light, temperature and may show profound effects under interactions with other chemicals.

2.6.2.3 Hydrogen peroxide

The stimulating effect of hydrogen peroxide (H_2O_2) on seed germination has been studied in detail in many species. The germination in seeds of *Pinus penderosa*, *P. edulis*, *Juniperus monosperma*, *Cercocarpus montanus* and *Cowania mexicana* is usually increased after washing in water for 48 hours; followed by a half hour soak in 30% hydrogen peroxide. The hydrogen peroxide treatment also effectively reduced seed bone microflora (Riffle and Springfield, 1968).

The stimulating effect of hydrogen peroxide on seed germination and subsequent seedling vigor has been observed in many other species (Ching, 1959; Trappe, 1961). It has been suggested by Ching (1959) that the stimulation by hydrogen peroxide is mediated through one or a combination of the following process :

- A direct destruction of the peroxidase by catalase adds molecular oxygen which in turn increases the rate of respiration and so facilitates the oxidation of fatty substances;
- A stimulation of lipoxidase through increase of the oxidation rate of unsaturated fatty acids;
- A peroxidase function with the hydrogen to oxidise growth inhibitors possibly present in tissue;
- The chemical acts directly as a reducing agent, thus the rate of respiration rises.

A half - hour soaking of seed in 5% solution of hydrogen peroxide of smooth coated seeds, such as those in the genera Picea, Larix, Pseudotsuga and Tsuga provides effective surface sterilization without injury. Seeds of recinous coats or attached with wings, such as those of Abies, require up to an hours' soaking (Ching and Parker, 1958; Trappe, 1961). The use of weak solutions requires either extended soaking or opening of the seed coat to be effective. When solutions as strong as 35% are used, relatively brief soaking stimulates germination without individual handling of the seeds (Trappe, 1961). In other experiments, soaking seeds of Drocera aliciae in a hydrogen peroxide solution (5 - 30%) for 24, 48 and 72 hours gave in consistent germination results. At low hydrogen peroxide concentrations (i.e. 5%), seed germination was improved relative to other treatments. It was apparent that concentrations above 5% inhibited germination (Ferreira and Small, 1974). This can be attributed to embryo damage by prolonged soaking in high concentrations of hydrogen peroxide. Thus the stimulating effect of hydrogen peroxide on seed germination depends on the species concentration, period of soaking, and other pre - sowing treatments. Because treatment of seeds with hydrogen peroxide disinfects their seed coats and the germination media in addition to stimulation of germination, hydrogen peroxide has been used widely in the germination of seed for asceptic studies (Piche and Fortin, 1982).

2.6.2.4 Indole - 3 - acetic acid

Amongst the agents known to control cell elongation and structural compounds in plant tissues is indole acetic acid (IAA). In some plant species therefore, this growth substance has a regulatory role in seed germination (Robertson et al., 1976; Jann and Amen, 1977). The effect of indole acetic acid on germination depends upon its contact in the embryo, the rate of embryo differentiation, the site of indole acetic acid localization and its interactions with other hormones (Nikolaeva *et al*., 1974; cited by Khan, 1977).

Detailed studies show that the auxin indole acetic acid is involved in dormancy regulation. In stratified and dormant seeds of *Acer tartaricum* and *Eucomymums europaea* there is an interaction between indole acetic acid and other hormones (Nikolaeva *et al*., 1974; Cited by Khan, 1977). In seeds of *Lactuca sativa* where germination in the dark was of the order 4 - 10% indole acetic acid at a concentration of 0.02 ppm raised the germination in the dark to 20 - 30%. For seeds germinating in the range of 60 - 80% in the dark, indole acetic acid had no effect. In other experiments the responses of the seeds to indole acetic acid was found to be temperature dependant. At 20°C 0.02 ppm indole acetic acid raised germination from 27 - 47%, while at 27°C the germination was 8% both in water and indole acetic acid (Poljakoff- Mayber, 1958; Cited by Mayer and Poljakoff - Mayber, 1975). In another study the effect of a range of indole acetic acid concentration supplied to *Lactuca sativa* seed depended on the source of the seed. Seed which was imported into Israel and germinated in darkness was not promoted by indole acetic acid and high concentrations proved inhibitory. This response pattern was not modified by irradiation with red or far - red light. With locally grown (i.e. Israeli) seed, on the other hand, low concentrations promoted germination but high concentrations again proved inhibitory (Poljakoff - Mayber, 1958; Cited by Robertson *et al*., 1976).

The application of indole acetic acid did not bring about germinations of excised embryos in seeds of Protea compacta. The number of embryos in which chlorophyll appeared, however, increased five - fold by the application of 1 ppm indole acetic acid (Van Staden et al., 1972). It would appear therefore, that indole acetic acid is unable to overcome the inhibition of radicle growth, but can overcome the inhibition of chlorophyll synthesis. However, a conflicting result was obtained in the carnivorous plant (Drocera aliciae) in which germination was stimulated slightly by 0.05 ppm indole acetic acid. This effect was greater with one year old seed than with fresh seed (Ferreira and Small, 1974). In other studies soaking of Capsicum annuum seeds in 20 or 40 ppm indole acetic acid increased germination but at 80 ppm indole acetic acid, germination was depressed (Patil and Ballal, 1979). Another study carried out at 20°C and 30°C in darkness, showed that concentration of indole acetic acid greater than 0.02 ppm could inhibit the germination of Lactuca sativa seeds. In the same study it was also observed that the gibberellins A_4 and A_7 benzylamine and red light were capable of reversing the effects of indole acetic acid (Robertson et al., 1976).

The review shows that clear effects of hormones of the indole acetic acid type on germination are still unknown. Numerous workers have investigated the effects of indole acetic acid on the germination of a variety of seed, and have obtained conflicting results. In some studies clear stimulation of seeds of particular plant species have been obtained following demonstrations of inhibition in another study. It is apparent that the concentrations of the indole acetic acid used and the type of seed germinated differ considerably making generalizations difficult. In general, therefore, it can be concluded that indole acetic acid can, under special conditions, stimulate germination although most seeds will germinate without artificial application of the hormone.

2.6.2.5 Cytokinins

Like gibberellic acids, different cytokinins vary in their effectiveness as germination promoters and synthetic cytokinins are more effective than natural ones. This phenomenon has been ascribed to a possible higher absorbtion rate for the synthesis or resistance from degradation by the enzymes involved in cytokinin metabolism (Bidington and Thomas, 1976).

The naturally occuring cytokinins are found in tRNAs located near the anti - codon (Kende, 1971). It is possible to consider therefore that a low or different cytokinin would result in a deficiency of tRNA concentration in tissue, and that protein synthesis would be depressed. The cytokinins are very effective promoters of germination, and since early protein synthesis is a necessity, it can be concluded that exogenously applied cytokinin could enhance protein synthesis allowing initial cell division and elongation.

In experiments involving kinetin, a concentration of 1 to 10 ppm was effective in promoting Nicotiana tabacum seed germination when the seeds were irradiated. The combination of kinetin and GA_3 was synergistic under light but not in darkness (Ogawara and Ono, 1961).

Application of 1 ppm kinetin to embryos of Protea compacta seeds, which have been found to have innate embryo dormancy (Brown and Van Staden, 1971), a considerable elongation of the radicle occured, but no new roots were formed. This was probably due to excessively concentrations of kinetin in the substratum for root formation to occur (Van Staden et al., 1972). In another experiment, it was found that application of 10 ppm kinetin increased germination of Protea compacta seed from 57% (control) to 78%. The same concentration increased germination of Leucadondra daphroides from 20% (control) to 33% (Brown and Van Staden, 1973). Kinetin has also been shown to induce germination in positively photoblastic Lactuca sativa seeds, and like in the case of GA_3, a high concentration was needed (Thomas, 1977). Cytokinins can have also been used to increase the germination potential of Apium graveolensis in place of GA_3 (Bidington et al., 1980).

2.6.2.6 Ethylene

Ethylene (C_2H_4) is produced by germinating seeds of various species (Maherink and Spencer, 1964; Spencer and Olson, 1965). Its production has also been shown to stimulate the germination of certain seeds (Toole et al., 1964). Experimental results show that the embryonic axis of non dormant varieties of Arachis hypogaea actively produce ethylene during germination, whereas those of very dormant varieties produce only low levels of ethylene when imbibed (Ketring and Morgan, 1969; 1970). Similar results have been obtained with Trifolium subterranean seed in which more ethylene is produced by non - dormant seeds than dormant ones; and that the production of ethylene proceeds the first emergency of the radicle (Wareing and Saunders, 1971).

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An evaluation of the published literature shows that ethylene enhances the germination rate of aged as well as immature seed (Wareing, 1971; Ketring, 1977). The specific response vary with species and concentration of ethylene. For example, a satisfactory germination response in seeds of Strelitzia regnae were obtained only at ethylene concentration of 0.2% (Van de Venter, 1978). In the laboratory tests, the germination of Amaranthus retrofleuxus was enhanced by ethylene at 1 to 10 ppm. In further studies, germination increased substantially with increasing concentration, and was greater in light than in darkness. Ethylene promoted germination to nearly 100% in dormant seeds incubated at 35°C and in non - dormant seeds incubated at 30°C. These results suggest that naturally occuring ethylene may exert a significant effect on the germination of.soil - borne Amaranthus retrofleuxus seeds (Schonbeck and and Egley, 1979).

It can be inferred therefore that ethylene is involved in the regulation of seed dormancy and germination although its effect is not limited to dormant seed. Its effect is more pronounced in light than in the darkness. Recently, it has been shown that ethylene can substitute for far - red light inhibiting red promoted germination of Pobentilla norvegica. In these studies, sequential treatment of red light and ethylene were the same as red and far - red light (Suzuki and Taylorson, 1981).

CHAPTER 3

MATERIALS AND METHODS

3.1 Seed collection and processing

3.1.1 Trichilia emetica

Ripe fruits of Trichilia emetica were picked in April, 1986 from 15 trees growing naturally at Korogwe (38° 17'E longitude, 4° 53'S. latitude, at 400 m a.s.l.)in Tanga region Tanzania. The fruits were packed in open weave gunny bags and immediately transported to the laboratory for processing. In the laboratory fruits were spread on hessian cloth under shade for two days. After their carpels had opened, the seeds were removed by hand. The extracted seeds were immersed in water to discard floating seed and other chaff materials.

3.1.2 Vangueria infausta

Ripe fruits of Vangueria infausta were picked in March 1986 from 20 trees growing naturally at Lushoto (38° 17' E longitude, 4° 47'S latitude, at 1300 m a.s.l.)in Tanga region Tanzania. The fruits were packed in open weave gunny bags and transported to the laboratory for processing.

The fruit skin and pulp around the seeds were removed by a knife and the seeds washed in a few changes of water. The empty seeds were separated from filled ones by flotation in water as described for Trichilia emetica. After drying in the sun for four days, the seeds were packed in cotton cloth bags and stored at room temperature until used for experimentation in May, 1986.

3.2 Seed weight and moisture content determination

Seed weight, moisture content and other laboratory experiments were conducted at the Tanzania Official Seed Certification Laboratory, Morogoro. The number of seeds per kilogram and moisture content (low constant temperature oven method) were determined according to ISTA (1985).

3.3 Test for seed viability

The procedures for testing viability by tetrazolium method in seeds of Trichilia emetica and Vangueria infausta have not been recorded in the International Seed Testing Association (ISTA) rules. So the general principles described for Prunus spp. seeds which are morphologically closest to T. emetica and V. infausta were adopted (ISTA, 1985).

Four replicates of 100 seeds each were drawn at random from seedlots of each species. In seeds of T. emetica the seed coat was removed from the seeds by means of a scalpel. In case of V. infausta, the hard seed coat was cracked with a small hammer and removed by means of a scalpel. The scarified seeds of both species were then soaked in water for 20 hours. Then they were cut longitudinally through the middle of the distal half of the cotyledons, leaving the embryo axis uncut. The cotyledons which carried a radicle and plumule were immersed in 1% 2, 3, 5-triphenyl tetrazolium chloride solution prepared according to ISTA (1985) rules.

The immersed embryos were maintained constantly at 30°C in complete darkness for a period of 24 hours. At the end of this period, the solution was decanted. After rinsing with water, the treated embryos were spread on a plate and kept wet for examination.

The following seeds were considered viable :

1. Completely stained embryo;
2. Embryo showing an unstained spot at the radicle tip;
3. Embryo showing unstained spots on the cotyledons, allowing superficial necrosis affecting up to 1/2 of the cotyledon and pervading necrosis affecting up to 1/3 of the cotyledon;
4. Cases (2) and (3) combined.

3.4 Experimental design

A 3 x 5 factorial model arranged in a randomized block design with 4 blocks was used. For each species, two different experiments of this model were conducted in the laboratory and repeated in the nersery. Altogether, there were eight experiments. In each experiment there were 15 plots per block or a total of 60 plots per experiment. Each plot had 25 seeds.

Each experiment involved two factors. The first factor involved 3 levels of physical seed coat scarification and a second factor involved soaking the seeds in a chemical at 5 concentration levels. The treatments were as follows :

i) Experiment No. 1 - *Trichilia emetica* in the laboratory

Factor 1 = Physical scarification (S):

S_o = Unscarified

S_1 = Partial removal of seed coat at the radicle end

S_2 = Complete removal of seed coat

Factor 2 = Chemical treatment (C) :

C_o = Soaking in 0 ppm GA_3 (distilled water)

C_1 = Soaking in 250 ppm GA_3

C_2 = Soaking in 500 ppm GA_3

C_3 = Soaking in 750 ppm GA_3

C_4 = Soaking in 1000 ppm GA_3

ii) Experiment No. 2 - *Trichilia emetica* in the laboratory

Factor 1 = Physical scarification (S) :

S_o = Unscarified

S_1 = Complete removal of aril alone

S_2 = Complete removal of aril + partial removal the seed coat at the radicle end.

Factor 2 = Chemical treatment (C)

C_o = Soaking in 0.0% KNO_3 (distilled water)

C_1 = Soaking in 0.2% KNO_3

C_2 = Soaking in 0.4% KNO_3

C_3 = Soaking in 0.6% KNO_3

C_4 = Soaking in 0.8% KNO_3

iii) Experiment No. 3 - *Trichilia emetica* in the nursery

Same as Experiment No. 1 but conducted in the nursery.

iv) Experiment No. 4 - *Trichilia emetica* in the nursery

Same as Experiment No. 2 but conducted in the nursery.

v) Experiment No. 5 - *Vangueria infausta* in the laboratory

Factor 1 = Physical scarification (S) :

S_o = Unscarified

S_1 = Partial removal of testa by abrasion at the radicle end

S_2= Complete removal of testa

Factor 2 = Chemical treatment (C) :

C_o = Soaking in 0% H_2O_2 (distilled water)

C_1 = Soaking in 2% H_2O_2

C_2 = Soaking in 4% H_2O_2

C_3 = Soaking in 6% H_2O_2

C_4 = Soaking in 8% H_2O_2

vi) Experiment No. 6 - *Vangueria infausta* in the laboratory

Factor 1 = Physical scarification (S) :

S_o = Unscarified

S_1 = Splitting part of testa (i.e. cracking part of testa) at the radicle end

S_2 = Partial removal of testa by chipping(i.e. cutting off a portion of testa) at the radicle end.

Factor 2 = Chemical treatment

C_0 = Soaking in 0 ppm IAA(distilled water)

C_1 = Soaking in 25 ppm IAA

C_2 = Soaking in 50 ppm IAA

C_3 = Soaking in 75 ppm IAA

C_4 = Soaking in 100 ppm IAA

vii) <u>Experiment NO. 7 - *Vangueria infausta* in the nursery</u>

Same as Experiment No. 5 but conducted in the nursery.

viii) <u>Experiment No. 8 - *Vangueria infausta* in the nursery</u>

Same as Experiment No. 6 but conducted in the nursery.

3.5 Techniques for physical seed scarification

The various degrees of physical seed coat scarification are demonstrated in Figures 2 and 3. The aril and seed coat of *Trichilia emetica* were peeled manually by using a scalpel. In *Vangueria infausta* the outer seed coat (testa) was cracked by slight hammering. Then where required, it was separated from the kernel by a scalpel. In another treatment, seed scarification was made by holding individual seeds with finger tips and abrading the testa at the radicle end against the rough surface of a wood-rasp until a faint brown colour of the inner coat (tegmen) appeared. Every time care was taken to avoid overtreatment which could cause damage to the internal structures of the seed. Where damage occured, the seed was discarded.

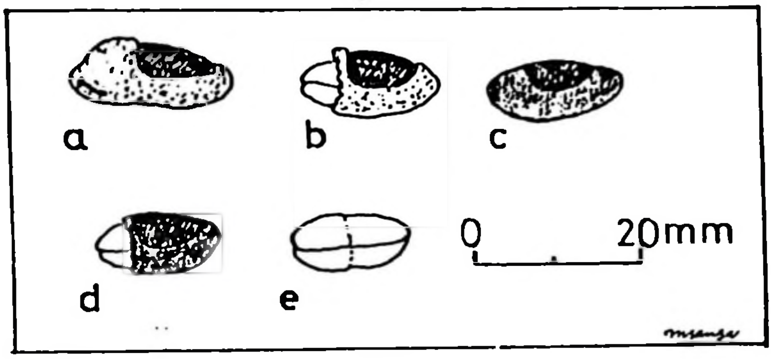

Fig. 2 : Various types of physical seed coat scarification in *Trichilia emetica* seed,

(a) unscarified seed,
(b) partial removal of seed coat at the radicle end,
(c) complete removal of aril,
(d) complete removal of aril + partial removal of seed coat at the radicle end, and
(e) complete removal of seed coat.

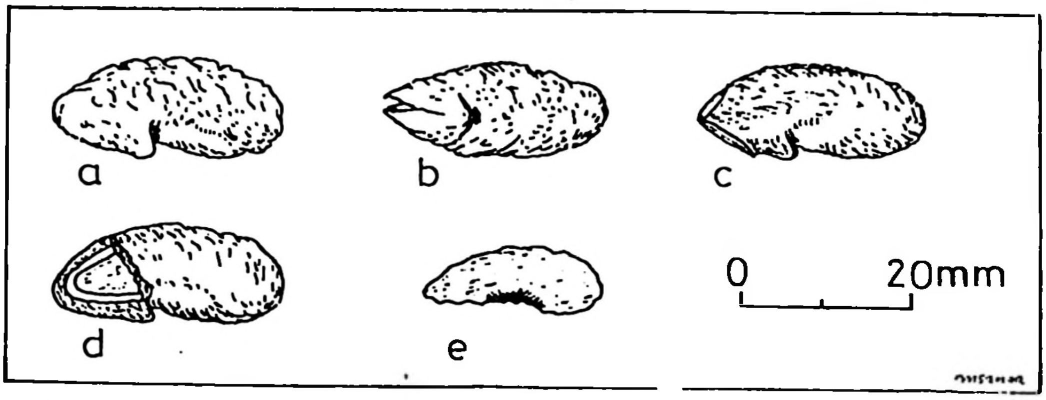

Fig. 3 : Various types of physical seed coat scarification in *Vangueria infausta* seed,
(a) unscarified seed,
(b) splitting the testa at the radicle end,
(c) partial removal of testa by abrasion at the radicle end,
(d) partial removal of testa by chipping at the radicle end, and
(e) complete removal of testa.

3.6 Preparation and application of chemicals

3.6.1 Preparation of gibberellic acid (GA_3) - $C_{19}H_{22}0_6$ and Indole 3 - acetic acid (IAA) - $C_8H_6NCH_2COOH$

Solutions of GA_3 (250, 500, 750 and 1000 ppm) and 1AA (25, 50, 75, and 100 ppm) were made up in distilled water. When concentrations greater than 800 ppm were required a 0.01M phosphate buffer, prepared according to International Rules (ISTA, 1985) was used to increase solubility of the chemicals.

3.6.2 Preparation of potassium nitrate (KNO_3)

The required concentrations (0.2, 0.4, 0.6 and 0.8%) were prepared by dissolving KNO_3 crystals in distilled water.

3.6.3 Preparation of hydrogen peroxide (H_20_2)

The required concentrations (2, 4, 6 and 8%) were prepared by diluting in distilled water a stock solution of H_20_2 (30% by volume).

3.6.4 Application of chemical

The seeds were immersed into their respective chemicals for a period of 12 hours. As controls, some seeds were immersed in distilled water (0 concentration) for the same period of time. At the end of this period, seeds were removed and immediately sown in the laboratory and nursery as described in 3.7 and 3.8, respectively.

3.7 Seed germination in the laboratory

Filter paper substratum (three layers of Whatman No. 4) was laid inside transparent plastic dishes (size 18 x 12 x 6 cm). Then 25 seeds were evenly distributed on top of the substratum, without touching each other to reduce the chance of cross contamination by microflora.

The substratum in each dish was moistened with 10 ml of the respective chemical at the beginning of the experiment. Thereafter the substratum was kept moist with distilled water at all times to supply the necessary moisture, but not so wet that a film of water forms around the seeds.

The dishes were covered and placed in a germination incubator. Each dish represented an observation plot and the shelves inside the incubator represented blocks. Constant temperature (25 ± 1°C) and relative humidity approximately 90% were maintained inside the incubator. Seeds were illuminated all the times by four 20W cool white fluorescent tubes, and the photon flux density at seed level was about 20 u mol. m^{-2} sec^{-1} (400 - 700 mm).

3.8 Seed germination in the nursery

Nursery experiments were conducted at the Forestry Nursery at Sokoine University of Agriculture, Morogoro. Timber frames of internal dimensions 20 x 20 cm and depth of 15 cm were used as observation plots. The frames were laid on the ground and filled with sand which had been washed of organic matter and sieved through a mesh No. 5 (size 5/64" or 2 mm). The sand particles were however large enough to be retained on mesh No. 1 (size 1/64" or 0.4 mm).

The seeds were distributed on top of the surface of the sand without touching on each other, then covered with sand of the same particle size to a uniform depth of 10 mm. Watering with a fine rose was applied manually in such a way that the medium was kept moist all the times without becoming water logged.

Shelter was provided by a roof of corrugated iron sheets, supported by timber frames and tall poles, in order to prevent rainsplash and direct heating from the sun.

3.9 Data collection and analysis

3.9.1 Germination

In the laboratory experiments, seeds were scored as having germinated when the protruding epicotyl had reached at least 10 mm and exhibited negative geotropism and all essential seedling structures were normal (ISTA, 1985). Seeds which were obviously dead and decayed were removed at every assessment to avoid cross contamination of healthy ones by microflora. In the nursery the criteria for germination was a visible protrusion of the shoot apex or epicotyl on the surface of the sand.

In both laboratory and nursery experiments, all germinants were recorded every day until no new germination occured. At this stage, the height (epicotyl + hypocotyl) of the seedlings at the nursery, were measured to the nearest mm. In addition, ungerminated seeds were tested for viability by the tetrazolium method (ISTA, 1985).

3.9.2 Dormancy periods

The complete dormancy period (i.e. the number of days from sowing day to commencement of germination) and the total dormancy period (i.e. the number of days from sowing day to completion ot germination) and the differential dormancy period (i.e. the number of days from commencement to completion of germination OR the difference between total and complete dormancy periods) were determined.

3.9.3 Data analysis

At each assessment the number of germinated seeds was expressed as percent of all seeds sown per plot. The germination energy or the germination percentage when the mean daily germination (cumulative germination percentage divided by the time elapsed since sowing date) reached its peak, was also calculated. In addition the germination value (GV) or the composite value which combines both germination speed and total germination was determined. It was calculated by using a formula for objectively evaluating results of germination tests (Djavanshir and Pourbeik, 1976):

$$GV = \frac{DGS}{N} \times (gp \times 10)$$

Where GV = Germination value

DGS = Daily germination speed which was computed by dividing cumulative germination percent by the number of days since beginning of the test

N = Number of days since beginning of germination

GP = Germination percent of the tests conclusion. In this formula it is used as the number of germinated seeds over 100.

10 = Constant

Prior to statistical analyses, data from all experiments were transformed into their appropriate values as follows : Cumulative germination percentage and germination energy were transformed into Arcsin angle values ; Germination values was computed according to Djavanshir and Pourbeik (1976); Complete, total and differential dormancy periods were transformed into square root values;Height of seedlings was transformed into common logarithmic values.

Analysis of variance (ANOVA) was performed using appropriate statistical routines for factorial experiments. Means and standard deviations were calculated for each treatment. Duncan's Multiple Range test (Alder and Roesller,1972)was used to separate significant differences among means. Linear regression analysis for the relationship between germination and various chemical concentrations; and correlation analysis for the relationship between laboratory germination and nursery emergence were performed using the regression analysis routine available for the Hewlett Packard Computer (HP 85).

CHAPTER 4

RESULTS

4.1 Seed morphology and structure

Trichilia emetica seed is slightly flattened, about 2.0 cm long and 0.8 cm broad, black and almost completely covered by a soft scarlet aril (Figure 4). A longitudinal section through the seed shows a minute radicle and plumule attached to the two cotyledons near the hilum. For Vangueria infausta, the seed is almost oval, with a low ridge above the hilum. Yellowish brown, about 2.5 cm long and 1.5 cm broad (Figure 5). The seed coat consists of an outer shell-like covering (testa) with an underlaying tegmen. The testa is rough and woody, while the tegmen is a thin delicate papery covering. The embryo is spathulate and enclosed in a fleshy endosperm. It consists of two cotyledons positioned parallel to the hilum, and an elongated superior radicle.

4.2 Seed weight, moisture content and viability

The number of seeds per kilogram was 1340 for T. emetica and 500 for V. infausta. The moisture content (wet weights) of the seed at use was 34.6% for T. emetica and 17.1% for V. infausta. Based on the tetrazolium biochemical test, the seed viability was estimated to be 98% for T. emetica and 87% for V. infausta.

4.3 Germination characteristics in Trichilia emetica

The germination of Trichilia emetica seed is epigeal. The radicle emerges from the hilum side and develops into a tap root

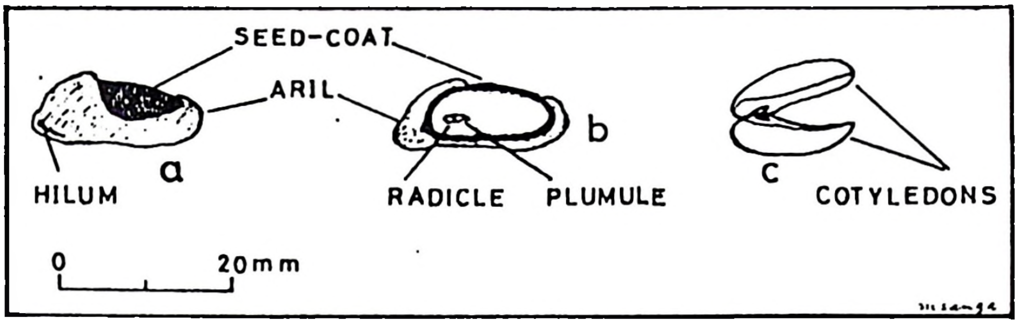

Fig. 4 : Morphology and structure of Trichilia emetica seed,
(a) external characteristics,
(b) longitudinal section, and
(c) embryo.

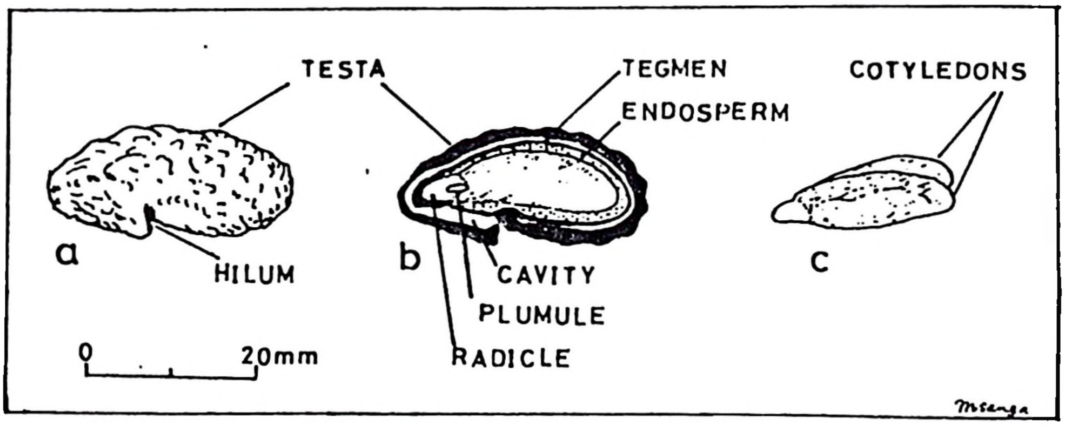

Fig. 5 : Morphology and structure of Vangueria infausta seed,
(a) external characteristics,
(b) longitudinal section, and
(c) embryo.

(Figure 6). Then the plumule extricates itself and develops into a young shoot. The aril usually rots while the seed coat is carried above the germination medium by the hypocotyl on the cotyledons. Later the cotyledons open and the seed coat falls off. The cotyledons change from white to green and remain attached to the seedling.

4.4 Effect of physical seed coat scarification and GA_3 treatments on the germination of *Trichilia emetica* seed

4.4.1 Germination in the laboratory

4.4.1.1 Cumulative germination and germination energy

The treatments studied had profound effects on the germination of *Trichilia emetica* seed (Figure 7). Overall, seed scarification treatments produced the highest germination, and were superior to the application of gibberellic acid (Tables 1 and 2). Unscarified seeds (S_0) started germination as late as 2 weeks after sowing and attained only 29% overall germination at termination of the experiment. Partial removal of the seed coat (S_1) and complete removal of the seed coat (S_2) significantly hastened the germination process and highly increased the germination percentages (Figure 7). At the end of the experiment, germination in these two scarification treatments ranged from 88 - 99%.

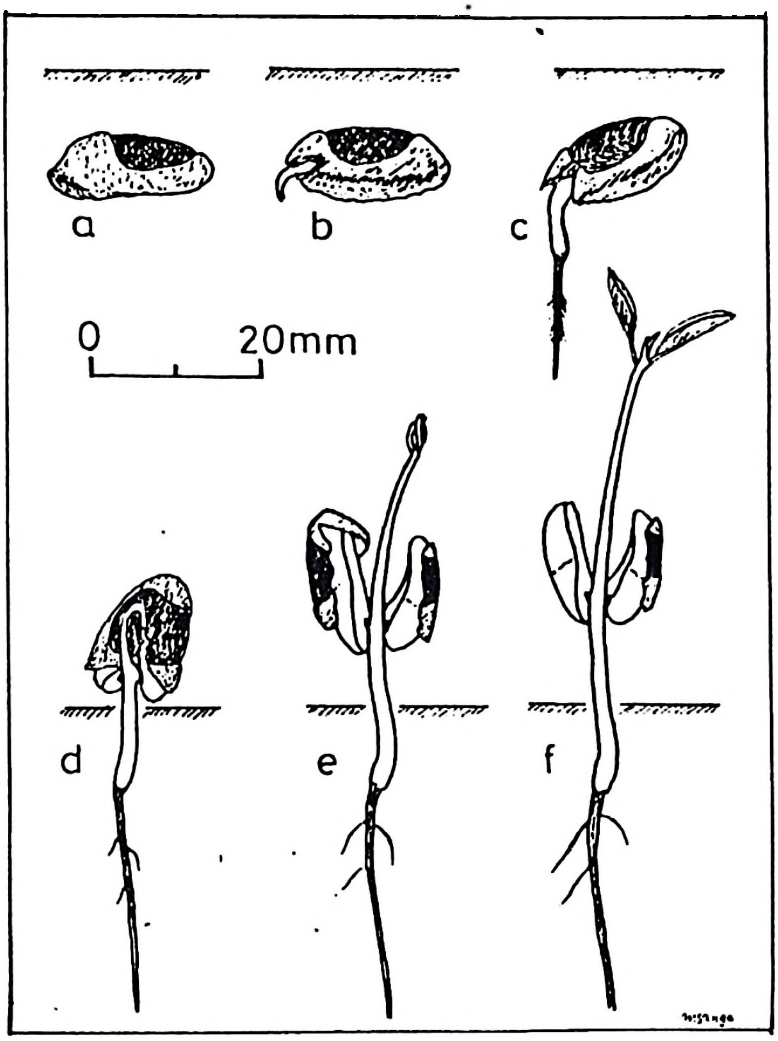

Fig. 6 : Stages in the germination and early development of a *Trichilia emetica* seedling,
(a) at sowing,
(b) at 12 days,
(c) at 17 days,
(d) at 22 days (germinated),
(e) at 25 days,
(f) at 35 days after sowing.

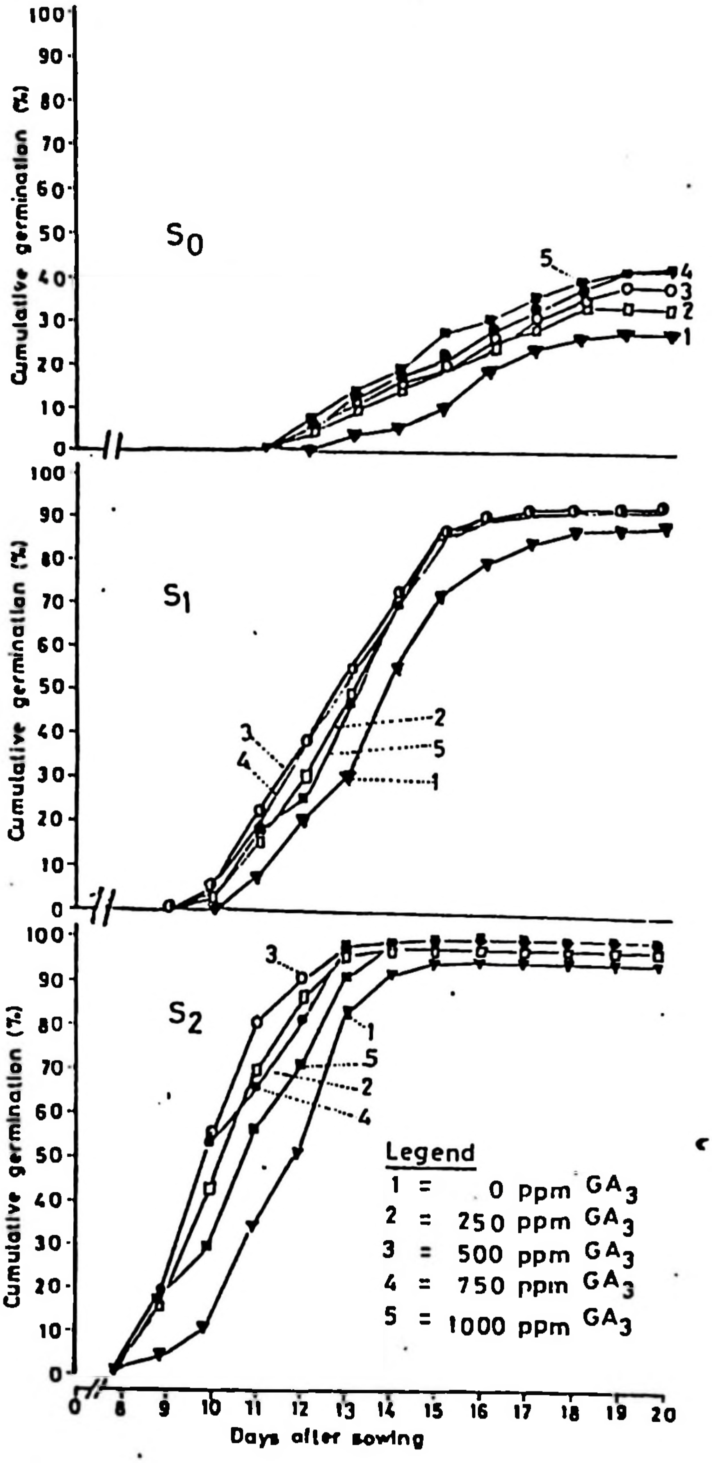

Fig. 7 : Effect of physical seed coat scarification (S_0 - unscarified; S_1 - partial removal of seed coat; S_2 - complete removal of seed coat) and concentrations of GA_3 on cumulative germination of Trichilia emetica seed in the laboratory.

Table 1 : F - values from different ANOVA tables for the effect of physical seed coat scarification and GA_3 concentrations on the germination of *Trichilia emetica* seed in the laboratory

Source of variation	Cumulative germination	Germination energy	Germination value	Dormancy periods		
				Complete	Total	Differential
Replicates	1.289 NS	0.448 NS	1.731 NS	1.428 NS	0.321 NS	1.745 NS
Physical scarification	317.033**	207.554**	392.030**	202.143**	68.82**	2.157 NS
Gibberellic acid (GA_3)	3.560*	3.806**	5.323**	2.428 NS	1.357 NS	1.037 NS
Physical scar. X GA_3	0.466 NS	0.705 NS	2.147 NS	1.286 NS	1.714 NS	2.167 NS

* = Significant at $P < 0.05$

** = Significant at $P < 0.01$

NS = Not significant

Table 2 : Effect of physical seed coat scarification and GA_3 concentrations on the germination of *Trichilia emetica* seed 20 days after sowing in the laboratory

Treatments					Dormancy periods (Days)		
Physical scarification	Chemical GA_3 (ppm)	Cumulative germination %	Germination energy %	Germination value (GV)	Complete (C)	Total (T)	Differential (T-C)
Unscarified (S_o)	0	29 ± 6.8a*	29 ± 6.8a	2.9 ± 0.9a	13 ± 1.0c	18 ± 0.5c	5 ± 1.0c
	250	35 ± 7.6ab	35 ± 7.6ab	5.0 ± 2.3b	12 ± 1.5c	18 ± 1.5c	6 ± 1.7c
	500	40 ± 7.3b	40 ± 7.3b	5.9 ± 1.7b	11 ± 0.5c	19 ± 0.8c	8 ± 1.3c
	750	44 ± 3.3b	44 ± 3.8b	7.0 ± 2.3b	11 ± 0.5c	19 ± 1.0c	8 ± 1.0c
	1000	45 ± 6.8b	45 ± 6.8b	7.7 ± 2.1b	11 ± 0.0c	19 ± 1.0c	8 ± 0.6c
Partial removal of seed coat (S_1)	0	88 ± 3.3c	82 ± 5.2c	31.9 ± 7.8c	11 ± 1.0b	18 ± 0.5b	7 ± 1.0b
	250	92 ± 7.3d	88 ± 8.6d	34.2 ± 7.8c	10 ± 0.5b	16 ± 1.2b	6 ± 1.3b
	500	92 ± 5.7d	88 ± 11.8d	35.3 ± 4.4c	10 ± 0.6b	16 ± 0.5b	6 ± 0.0b
	750	93 ± 3.8d	89 ± 3.8d	30.2 ± 7.8d	10 ± 0.5b	16 ± 1.0b	6 ± 1-0b
	1000	93 ± 5.0d	88 ± 5.7d	38.7 ± 8.1d	10 ± 0.8b	16 ± 1.0b	6 ± 0.6b
Complete removal of seed coat (S_2)	0	94 ± 6.9e	90 ± 7.7e	38.2 ± 6.4e	9 ± 0.5a	15 ± 1.3a	6 ± 0.0a
	250	97 ± 3.8f	97 ± 3.8ef	48.5 ± 7.0f	8 ± 0.0a	13 ± 0.5a	5 ± 0.8a
	500	99 ± 2.0f	98 ± 5.2f	59.3 ± 2.9g	8 ± 0.0a	13 ± 0.5a	5 ± 0.5a
	750	99 ± 2.0f	98 ± 4.0f	53.3 ± 5.0fg	8 ± 0.0a	13 ± 0.5a	5 ± 0.5a
	1000	99 ± 2.0f	95 ± 3.8ef	49.8 ± 6.3fg	8 ± 0.0a	13 ± 0.5a	4 ± 0.5a

Data represents a mean of four replicates ± standard deviation.

* = Values in the same column and within a scarification treatment followed by the same letter do not differ significantly based on Duncan's Multiple Range test ($P < 0.05$)

Where the seed coat was completely removed (S_2) virtually all the viable seeds germinated, showing statistical superiority over the other two scarification treatments. When tested for viability by the tetrazolium biochemical method, all ungerminated seed at 20 days were found to be non - viable. Unscarified seed (S_0), and seed whose seed coat was partially removed (S_1) were attacked by a fungus later identified as Fusarium sp. The severity of the attack was highest in unscarified seed.

Within scarification treatments, the application of gibberellic acid (GA_3) produced marginal but at times significant improvements in germination (Table 2). Unscarified seeds not treated with GA_3 showed significantly lower cumulative germination when compared to GA_3 treated ones. Treatment of seeds with GA_3 under complete removal of the seed coat produced nearly 100% germination. Linear regression analysis of cumulative germination on the concentration of GA_3 is presented in Table 3. Trends in germination energy were similar to cumulative germination (Table 2). Seed scarification had profound effects on germination energy compared to only a marginal effect produced by the application of GA_3. Complete removal of the seed coat induced the highest germination energy, while the lowest germination energy was obtained under unscarified seeds; partial removal of the seed coat being intermediate.

4.4.1.2 Germination value

Data for germination value (GV) are presented in Figure 8. Germination value increased progressively from unscarified seeds, seeds with partial removal of seed coat to seeds with the seed

Table 3 : Relationships between laboratory germination and GA_3 concentrations for *Trichilia emetica* seeds given different physical seed coat scarification treatments

Physical seed coat scarification	Cumulative germination (Arcsin $\sqrt{\%}$)	Germination energy (Arcsin $\sqrt{\%}$)	Germination value
Unscarified (S_o)	$Y = 33.375 + 0.009x$	$Y = 33.375 + 0.009x$	$Y = 3.424 + 0.004x$
	$R^2 = 0.492$	$R^2 = 0.492$	$R^2 = 0.485$
	$F = 17.4^{**}$	$F = 17.4^{**}$	$F = 17.0^{**}$
Partial removal of seed coat (S_1)	$Y = 72.175 + 0.005x$	$Y = 67.205 + 0.007x$	$Y = 32.230 + 0.007x$
	$R^2 = 0.060$	$R^2 = 0.121$	$R^2 = 0.157$
	F = 1.2 NS	F = 2.5 NS	F = 3.3 NS
Complete removal of seed coat (S_2)	$Y = 80.220 + 0.008x$	$Y = 76.530 + 0.006$	$Y = 44.264 + 0.011x$
	$R^2 = 0.183$	$R^2 = 0.070$	$R^2 = 0.197$
	F = 4.0 NS	F = 1.4 NS	F = 4.4 NS

Each regression is based on 20 data points

Y = Parameter indicated

x = GA_3 concentration (ppm)

** = Significant at $P < 0.01$

NS = Not significant

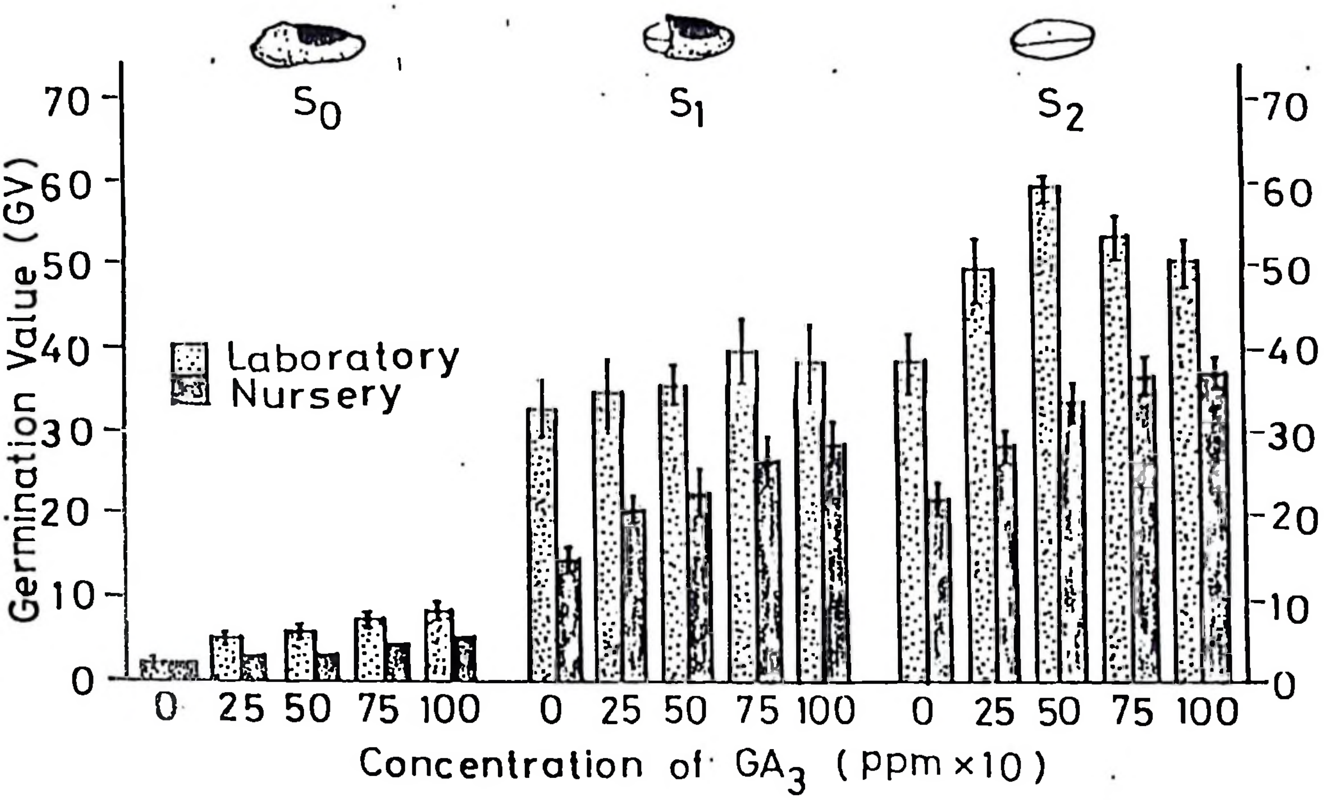

Fig. 8 : Influence of physical seed coat scarification and GA_3 concentrations on the germination of *Trichilia emetica* seed in laboratory and nursery experiments, (S_0) unscarified, (S_1) partial removal of seed coat, (S_2) complete removal of seed coat. Each bar is a mean of four replicates. Vertical lines indicate ± standard deviations. A bar without a line denotes that the standard deviation is nearly zero.

coat completely removed. Application of GA_3 improved GV for both unscarified and scarified seeds (Table 2). Linear regression analysis of GV on the concentrations of GA_3 is presented in Figure 9a.

4.4.1.3 Dormancy

Data on complete dormancy, total dormancy and differential dormancy periods are presented in Table 2. The significance of these parameters when subject to analysis of variance is shown in Table 1. Complete dormancy period was significantly reduced when seed scarification involved complete removal of the seed coat. The longest complete dormancy period occured under unscarified treatments, partial removal of the seed coat being intermediate. For each seed scarification treatment, GA_3 application did not significantly influence the dormancy periods (Table 2).

4.4.2 Germination in the nursery

4.4.2.1 Cumulative germination and germination energy

Cumulative germination percentages for all treatments are presented in Figure 10. Seed scarificatio produced highly significant differences in cumulative germination (Table 4). In the final analysis, cumulative germination was highest in treatments with complete removal of seed coat and lowest in unscarified seeds. In general cumulative germination in scarified seeds was improved by increasing the concentration of GA_3 (Table 5). Detailed description of the relationships between GA_3

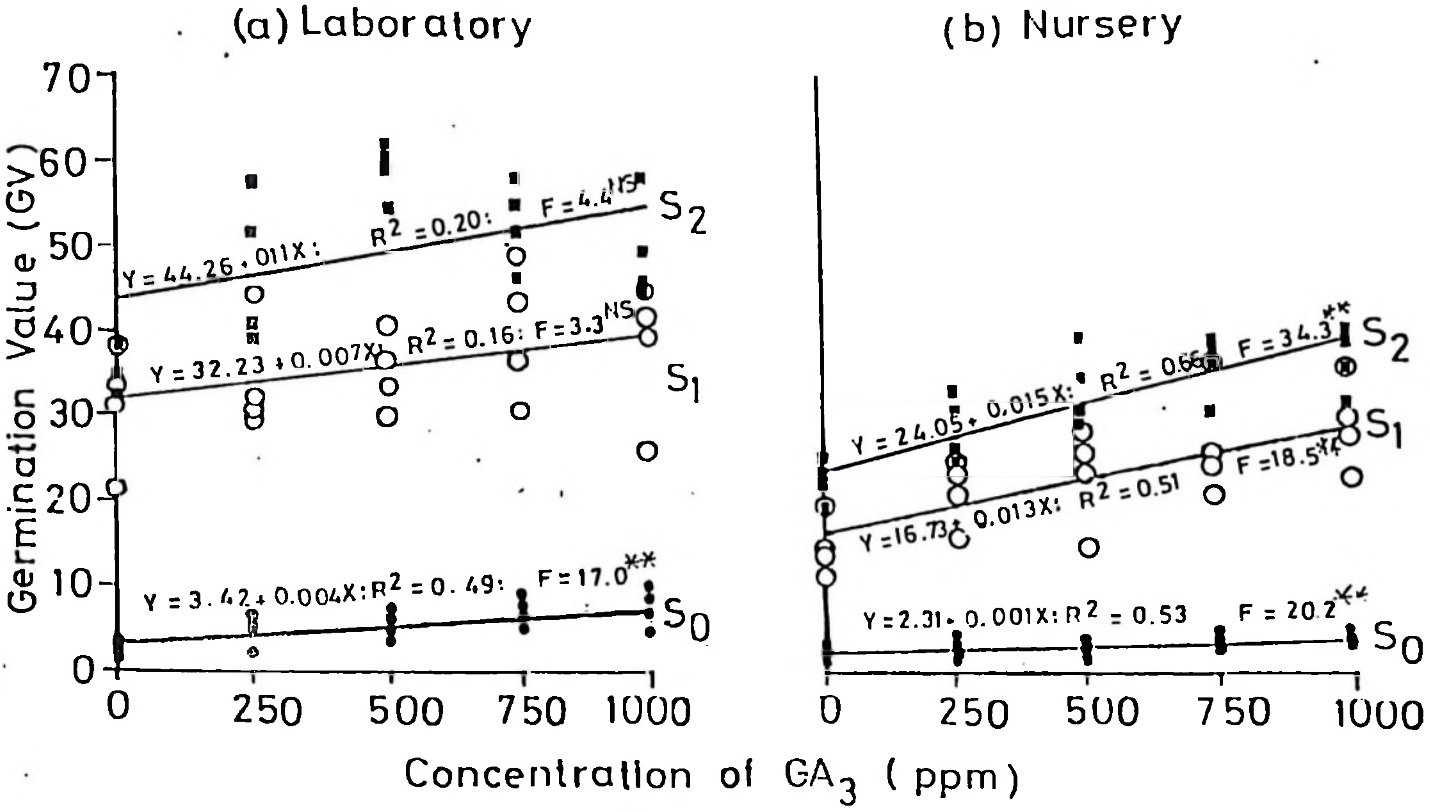

Fig. 9 : Relationships between germination and GA$_3$concentrations for different types of physical feed coat scarification in *Trichilia emetica* seed. (S$_0$) unscarified (S$_1$) partial removal of seed coat, (S$_2$) complete removal of seed coat.

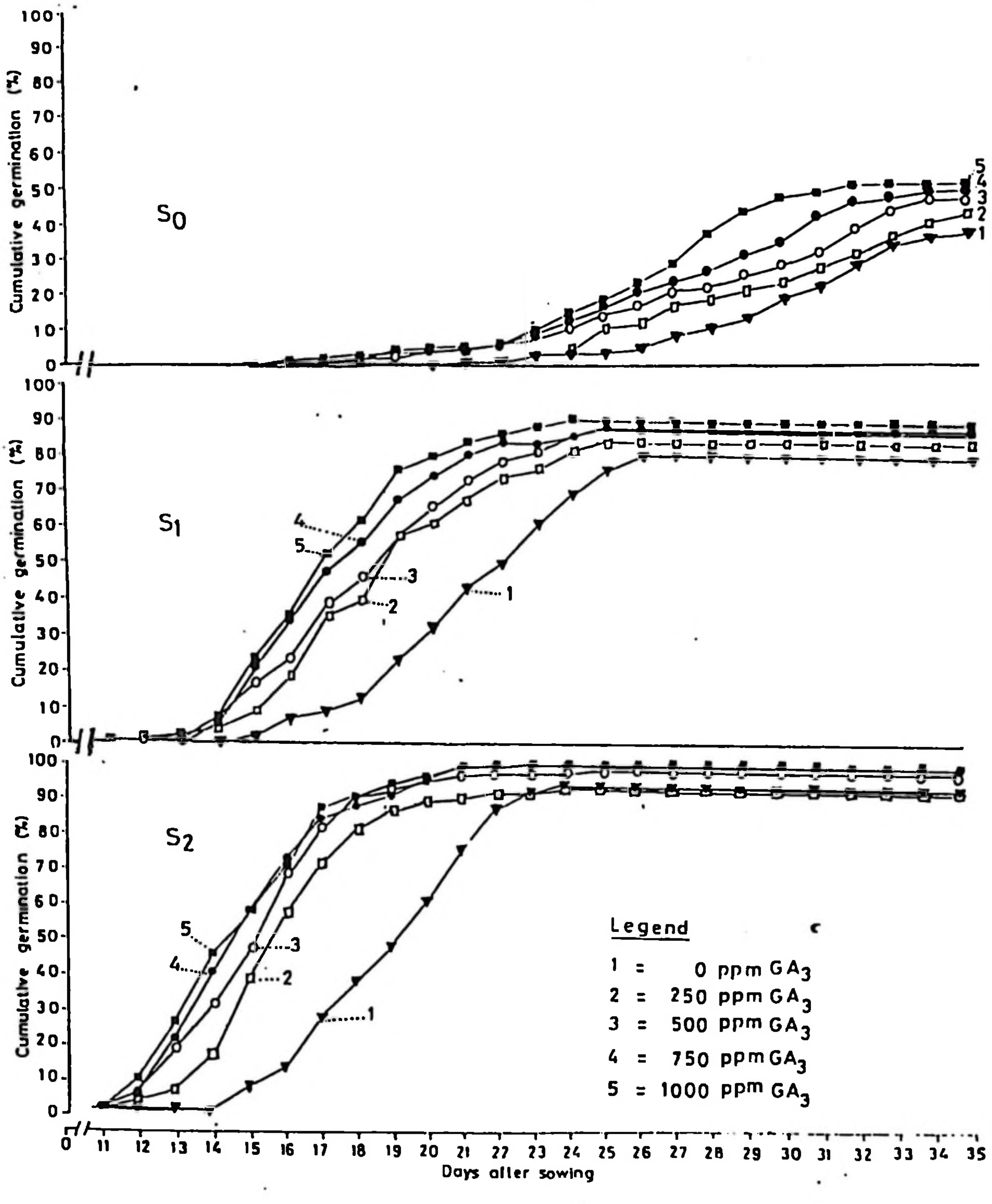

Fig. 10 : Effect of physical seed coat scarification (S_0-unscarified; S_1-partial removal of seed coat; S_2-complete removal of seed coat) and concentrations of GA_3 on cumulative germination of Trichilia emetica seed in the nursery.

Table 4 : F - values from different ANOVA tables for the effect of physical seed coat scarification and GA_3 concentrations on the germination of Trichilia emetica seed in the nursery

Source of variation	Cumulative germination	Germination energy	Germination value	Seedling height	Dormancy periods		
					Complete	Total	Differential
Replicates	0.470 NS	1.552 NS	1.783 NS	2.589 NS	0.467 NS	0.843 NS	0.360 NS
Physical scarification	418.217^{**}	499.289^{**}	311.593^{**}	1116.095^{**}	140.644^{**}	325.579^{**}	6.750^{**}
Gibberellic acid(GA_3)	11.370^{**}	4.977^{**}	15.038^{**}	30.077^{**}	2.378 NS	2.632 NS	0.920 NS
Physical scar. x GA_3	1.487 NS	1.501 NS	2.665 NS	1.000 NS	1.222 NS	0.579 NS	1.280 NS

* = Significant at $P < 0.05$

** = Significant at $P < 0.01$

NS = Not significant

Table 5 : Effect of physical seed coat scarification and GA_3 concentrations on the germination of *Trichilia emetica* seed 35 days after sowing in the nursery

Treatments						Dormancy periods (Days)		
Physical scarification	Chemical GA_3 (ppm)	Cumulative germination (%)	Germination energy (%)	Germination value (GV)	Seedling height (mm)	Complete (C)	Total (T)	Differential (T - C)
Unscarified (S_0)	0	35 ± 3.3a*	35 ± 3.3a	1.9 ± 0.4a	14 ± 3.1a	23 ± 3.4c	34 ± 1.0c	11 ± 3.0c
	250	40 ± 4.6b	40 ± 4.6b	3.3 ± 0.7b	16 ± 2.1b	22 ± 2.2c	35 ± 0.6c	13 ± 1.7c
	500	44 ± 5.0b	44 ± 5b	3.5 ± 0.9b	19 ± 2.6c	20 ± 2.6c	34 ± 0.6c	14 ± 3.2c
	750	47 ± 3.8bc	47 ± 3.8bc	3.8 ± 0.8b	19 ± 2.6c	19 ± 3.8c	33 ± 1.0c	14 ± 3.0c
	1000	49 ± 2.0c	49 ± 2.0c	4.7 ± 0.4b	22 ± 4.8d	19 ± 1.4c	33 ± 1.0c	14 ± 2.1c
Partial removal of seed coat (S_1)	0	82 ± 2.3d	82 ± 2.3d	15.4 ± 3.2c	50 ± 4.1e	15 ± 1.0b	26 ± 0.0b	11 ± 1.0b
	250	86 ± 2.6de	86 ± 7.3de	21.4 ± 3.8d	71 ± 8.3f	14 ± 1.9b	25 ± 0.5b	11 ± 2.1b
	500	89 ± 4.0ef	88 ± 3.3e	23.4 ± 5.7de	80 ± 9.5fg	13 ± 0.5b	25 ± 0.5b	12 ± 0.5b
	750	90 ± 5.0ef	87 ± 3.8de	27.2 ± 6.8e	83 ± 7.1g	14 ± 0.6b	24 ± 0.7b	10 ± 1.7b
	1000	92 ± 3.3f	87 ± 5.0de	28.8 ± 5.2e	86 ± 8.9g	13 ± 1.4b	24 ± 0.6b	11 ± 0.8b
Complete removal of seed coat (S_2)	0	93 ± 6.0g	89 ± 6.8g	21.7 ± 4.3f	71 ± 8.6h	13 ± 1.5a	23 ± 0.6a	10 ± 1.3a
	250	92 ± 3.2g	84 ± 5.7f	29 ± 3.9g	93 ± 7.4i	12 ± 0.6a	23 ± 2.6a	11 ± 2.6a
	500	98 ± 2.3h	87 ± 3.8fg	33.6 ± 4.6gh	100 ± 10.3ij	11 ± 0.5a	22 ± 2.2a	11 ± 2.5a
	750	99 ± 2.3h	91 ± 3.8g	36.7 ± 3.7h	107 ± 8.4j	11 ± 0.5a	21 ± 1.3a	10 ± 0.8a
	1000	99 ± 2.3h	90 ± 4.0g	37.4 ± 3.6h	110 ± 6.2j	11 ± 0.5a	21 ± 2.5a	10 ± 0.5a

Data represents a mean of four replicates ± standard deviation.

* = Values in the same column and within a scarification treatment followed by the same letter do not differ significantly based on Duncan's Multiple Range test ($P < 0.05$).

concentration and cumulative germination are presented in Table 6. For all seed scarification treatments, the relationships were highly significant.

Germination energy results followed the same trends for cumulative germination (Table 5). In general, however, the germination energy percentages were lower than their corresponding cumulative germination percentages.

4.4.2.2 Germination value

Data for germination value (GV) are presented in Figure 8. The effect of seed scarification on GV was similar to cumulative germination and germination energy. For each physical seed scarification treatment, GV was progressively increased by the application of GA_3 (Table 5). In addition, a regression analysis of GV on GA_3 concentrations showed a highly significant relationship for all physical seed scarification treatments (Figure 9b).

4.4.2.3 Height of seedlings

The initial height attained by the seedlings was also influenced significantly by presowing treatment, especially seed scarification (Tables 4 and 5). Complete removal of the seed coat induced the highest initial growth and unscarified seed the lowest. For each scarification treatment, increases in initial height growth were significantly associated with increase in GA_3 concentrations (Table 6).

Table 6 : Relationships between nursery germination and GA_3 concentrations for Trichilia emetica seeds given different physical seed coat scarification treatments

Physical seed coat scarification	Cumulative germination (Arcsin $\sqrt{\%}$)	Germination energy (Arcsin $\sqrt{\%}$)	Germination value	Seedling height ($\log_{10}$ mm)
Unscarified (S_o)	$Y = 37.590\ 0\ 0.007x$ $R^2 = 0.495$ $F = 17.6^{**}$	$Y = 37.970 + 0.007x$ $R^2 = 0.661$ $F = 35.1^{**}$	$Y = 2.305 + 0.001x$ $R^2 = 0.529$ $F = 20.2^{**}$	$Y = 1.150 + 0.001$ $R^2 = 0.487$ $F = 17.1^{**}$
Partial removal of seed coat(S_1)	$Y = 65.750 + 0.008x$ $R^2 = 0.496$ $F = 17.7^{**}$	$Y = 64.580 + 0.005x$ $R^2 = 0.225$ $F = 5.3^{*}$	$Y = 16.730 + 0.013x$ $R^2 = 0.507$ $F = 18.5^{**}$	$Y = 1.745 + 0.001$ $R^2 = 0.647$ $F = 33.0^{**}$
Complete removal of seed coat (S_2)	$Y = 74.250 + 0.014x$ $R^2 = 0.469$ $F = 15.9^{**}$	$Y = 68.875 + 0.002x$ $R^2 = 0.587$ $F = 9.5^{*}$	$Y = 24.049 + 0.015x$ $R^2 = 0.656$ $F = 34.3^{**}$	$Y = 1.887 + 0.001$ $R^2 = 0.681$ $F = 38.3^{**}$

Each regression is based on 20 data points

Y = Parameter indicated
x = GA_3 concentration (ppm)
* = Significant at $P < 0.05$
** = Significant at $P < 0.01$

4.4.2.4 Dormancy

Seed scarification treatments had highly signfiicant effects on all types of dormancy periods. Treatments with GA_3 were insignificant (Table 4). Like the results in the laboratory experiments, complete removal of the seed coat produced the best results, shortening the complete dormancy period to within 11 - 13 days compared to 19 - 23 days in unscarified treatments. Seeds with partial removal of the seed coat were intermediate.

4.4.3 Comparisons between laboratory and nursery germination results

Unscarified seeds which were sown in the laboratory had lower cumulative germination and germination energy percentages than those sown in nursery. With scarified seeds, cumulative germination and germination energy percentages were higher in the laboratory than in the nursery (Tables 2 and 5). Seeds sown in the laboratory attained higher germination values and shorter dormancy periods than those sown in the nursery (Figure 8). In the laboratory the most dormant seeds completed their germination after 19 days compared to 35 days in the nursery. Unscarified seeds which were sown in the laboratory were infected by a rotting fungus (Fusarium sp.) when those in the nursery were not.

Significant correlation coefficients between laboratory and nursery tests for unscarified seeds were found in all parameters except complete dormancy period. For seeds whose seed coat was partly removed, there was a highly significant correlation coefficient only for total and differential dormancy periods. The correlation was not significant for other parameters. For seeds whose seed coat was completely removed there were significant correlation in all parameters except total and differential dormancy periods (Table 7). Figure 11 illustrates the correlation between laboratory and nursery germination values for *T. emetica* seed.

4.5 Effect of physical seed coat scarification and KNO_3 treatments on the germination of *Trichilia emetica* seed

4.5.1 Germination in the laboratory

4.5.1.1 Cumulative germination and germination energy

Cumulative germination for all treatments is shown in Figure 12. At termination of the experiment (20 days after sowing), cumulative germination was highest in treatments involving the removal of the aril and part of the seed coat (S_2) and lowest in unscarified seeds (S_o) (Tables 8 and 9).

In treatments where only the aril was removed (S_1) cumulative germination varied significantly from 71% in seeds which were soaked in 0.8% KNO_3 to 90% in seeds which were soaked in 0.2% KNO_3. In unscarified seeds (S_o), treatments with KNO_3 reduced cumulative germination from 27% for untreated controls to 5% for seeds treated with 0.8% KNO_3.

Table 7 : Correlation coefficients for the relationship between laboratory and nursery germination and GA_3 concentrations when *Trichilia emetica* seeds are given different physical seed coat scarification treatments

Physical seed coat scarification	Cumulative germination (Arcsin $\sqrt{\%}$)	Germination energy (Arcsin $\sqrt{\%}$)	Germination value
Unscarified (S_o)	$R = 0.4546^*$ $F = 4.7^*$	$R = 0.5270^*$ $F = 6.9^*$	$R = 0.4545^*$ $F = 4.6^*$
Partial removal of seed coat (S_1)	R = 0.1553 NS F = 0.4 NS	R = 0.2765 NS F = 1.5 NS	$R = 0.6164^{**}$ $F = 11.0^{**}$
Complete removal of seed coat (S_2)	$R = 0.5136^*$ $F = 6.5^*$	$R = 0.475^*$ $F = 5.3^*$	$R = 0.6505^{**}$ $F = 13.2^{**}$

Each correlation is based on 20 data points

* = Significant at $P < 0.05$
** = Significant at $P < 0.01$
NS = Not significant

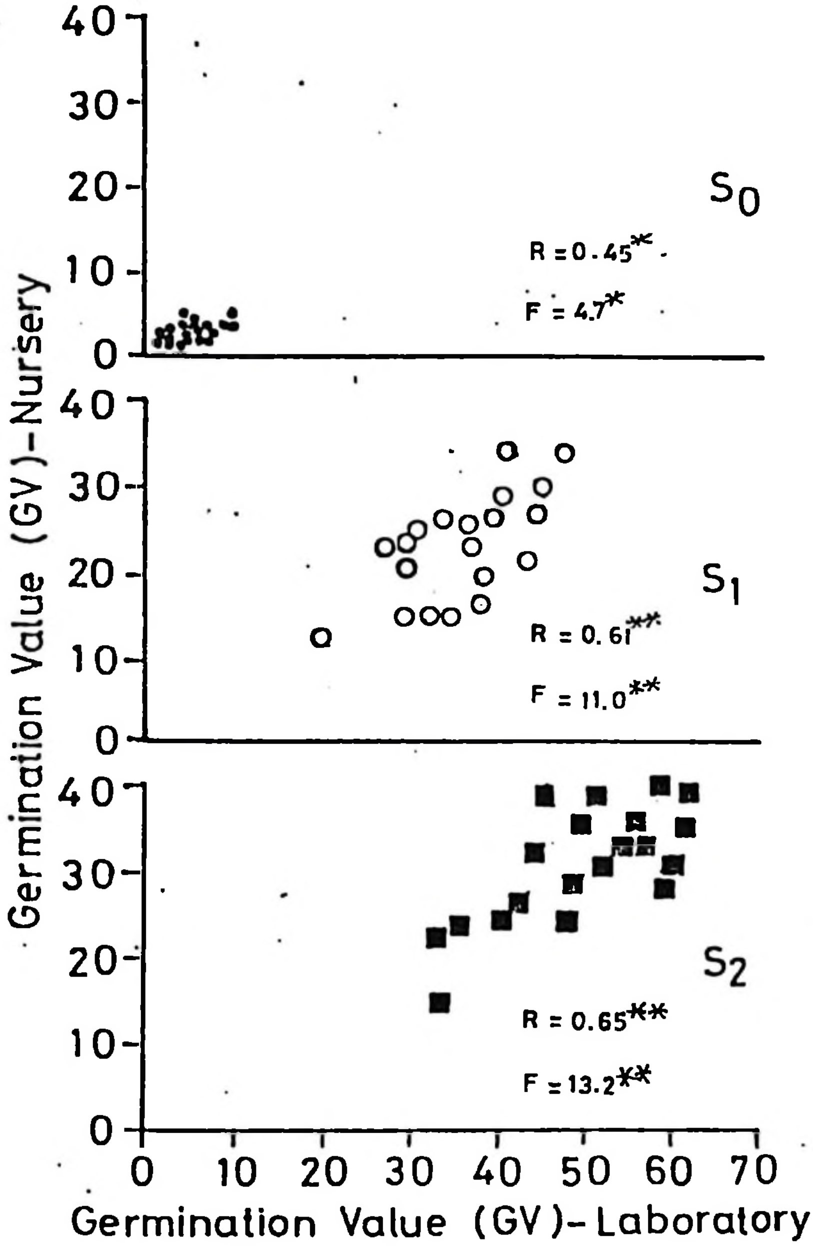

Fig. 11 : Scatter diagrams for the correlation between laboratory and nursery germination of Trichilia emetica seed after different types of physical seed coat scarification and GA_3 treatments. (S_0) unscarified, (S_1) partial removal of seed coat, (S_2) complete removal of seed coat. (*) significant at $p < 0.05$, (**) significant at $p < 0.01$.

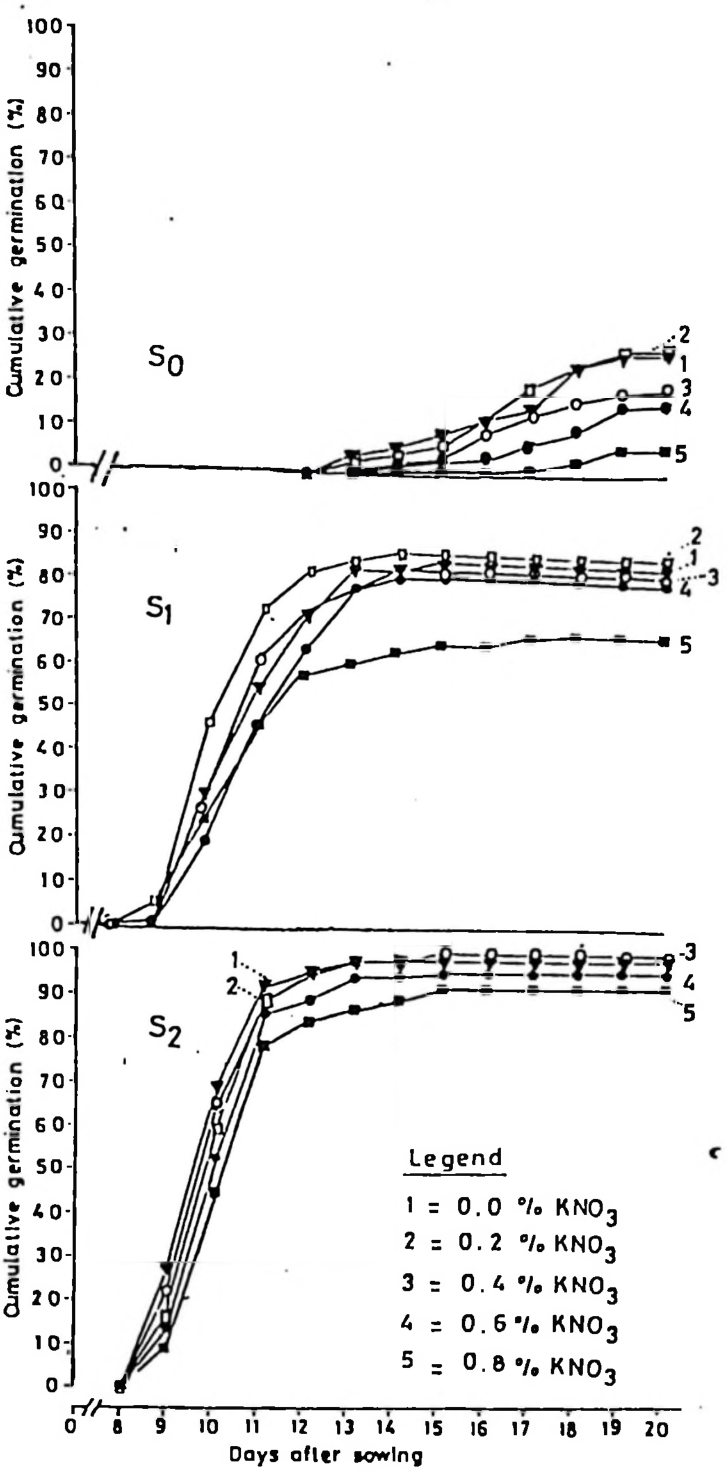

Fig. 12 : Effect of physical seed coat scarification (S_0-unscarified; S_1 - complete removal of aril; S_2 - complete removal of aril + partial removal of seed coat) and concentrations of KNO_3 on cumulative germination of <u>Trichilia emetica</u> seed in the laboratory.

Table 8 : F - values from differenti ANOVA tables for the effect of physical seed coat scarification and KNO_3 concentrations on the germination of Trichilia emetica seed in the laboratory

Source of variation	Cumulative germination	Germination energy	Germination value	Dormancy periods		
				Complete	Total	Differential
Replicates	2.655 NS	0.649 NS	0.298 NS	1.200 NS	0.900 NS	0.122 NS
Physical scarification	71.009**	269.623**	277.653**	80.677**	85.375**	0.429 NS
Potassium nitrate (KNO_3)	6.822**	3.419*	5.687**	0.908 NS	0.725 NS	0.429 NS
Physical scar. x KNO_3	0.686 NS	1.449 NS	1.691 NS	1.246 NS	0.550 NS	0.674 NS

* = Significant at $P < 0.05$

** = Significant at $P < 0.01$

NS = Not significant

Table 9 : Effect of physical seed coat scarification and KNO_3 concentrations on the germination of <u>Trichilia</u> <u>emetica</u> seed 20 days after sowing in the laboratory

Treatments					Dormancy periods (Days)		
Physical scarification	Chemical KNO_3 (%)	Cumulative germination (%)	Germination energy (%)	Germination value (GV)	Complete (C)	Total (T)	Differential (T-C)
Unscarified (S_o)	0.0	28 ± 6.5b*	27 ± 8.2b	2.7 ± 1.2a	13 ± 1.5c	19 ± 0.5c	6 ± 1.4c
	0.2	29 ± 7.6b	29 ± 7.6b	2.9 ± 1.8a	14 ± 1.7c	19 ± 0.5c	5 ± 1.5c
	0.4	19 ± 3.8ab	19 ± 3.8ab	1.3 ± 0.6a	14 ± 1.2c	19 ± 0.8c	5 ± 1.8c
	0.6	16 ± 3.3a	16 ± 3.3a	0.8 ± 0.1a	14 ± 1.7c	19 ± 0.5c	5 ± 2.2c
	0.8	5 ± 2.0a	5 ± 2.0a	0.2 ± 0.1a	15 ± 0.5c	19 ± 0.5c	4 ± 0.0c
Complete removal of aril (S_1)	0.0	88 ± 11.3d	84 ± 13.9d	46.1± 14.7cd	9 ± 0.0b	14 ± 1.2b	5 ± 1.3b
	0.2	90 ± 6.9d	86 ± 6-9d	52.6 ± 11.2d	9 ± 0.5b	14 ± 1.3b	5 ± 1.3b
	0.4	86 ± 13.7cd	83 ± 15.4d	45.5 ± 13.2cd	9 ± 0.5b	14 ± 0.5b	5 ± 0.8b
	0.6	84 ± 7.3cd	84 ± 7.3d	36.9 ± 7.0c	9 ± 0.5b	14 ± 0.6b	5 ± 0.5b
	0.8	71 ± 5.0c	71 ± 5.0c	26.3 ± 4.5b	9 ± 1.5b	14 ± 3.1b	5 ± 1.7b
Complete removal of aril + partial removal of seed coat (S_2)	0.0	99 ± 2.0e	94 ± 2.3e	62.8 ± 6.5f	8 ± 0.0a	13 ± 0.6a	5 ± 0.6a
	0.2	99 ± 2.0e	94 ± 2.3e	62.0 ± 5.3f	8 ± 0.0a	13 ± 0.5a	5 ± 0.8a
	0.4	100 ± 0.0e	93 ± 3.8e	65.0 ± 2.2f	8 ± 0.0a	14 ± 1.0a	6 ± 0.5a
	0.6	96 ± 5.7e	91 ± 8.2e	61.4 ± 7.0f	8 ± 0.0a	13 ± 0.5a	5 ± 0.5a
	0.8	92 ± 5.7f	86 ± 4.0f	51.5 ± 3.8g	8 ± 0.5a	14 ± 1.5a	6 ± 1.4a

Data represents a mean of four replicates ± Standard Deviation.

* Values in the same column and within a scarification treatment followed by the same letter do not differ significantly based on Duncan's Multiple Range test ($P < 0.05$).

Treatment with 0.8% KNO_3 clearly suppressed cumulative germination in all scarification treatments. All unscarified seeds were severely infected by Fusarium sp. Based on the tetrazolium test ungerminated seeds at the end of the experiment were found to be non - viable.

There was a significant negative linear relationship between cumulative germination and KNO_3 concentrations for both scarified and unscarified seeds (Table 10).

Germination energy results followed the same trends as for cumulative germination. In general, the germination energy percentages were lower than their corresponding cumulative germination percentages (Table 9).

4.5.1.2 Germination value

Germination values (GV) are illustrated in Figure 13 Germination value was highest in seeds with treatments involving complete removal of aril and part of the seed coat (S_2), intermediate in seeds with aril removed alone (S_1) and lowest in unscarified seeds (S_0) (Table 9).

There were negative linear relationships between GV and KNO_3 concentrations for scarification treatments (Table 10).

4.5.1.3 Dormancy

Data on complete dormancy, total dormancy and differential dormancy periods are shown in Table 9. Scarified seeds attained shorter complete dormancy and total dormancy periods than unscarified seeds. Scarification had no effect on differential

Table 10 : Relationships between laboratory germination and KNO_3 concentrations for *Trichilia emetica* seeds given different physical seed coat scarification treatments

Physical seed coat scarification	Cumulative germination (Arcsin $\sqrt{\%}$)	Germination energy (Arcsin $\sqrt{\%}$)	Germination value
Unscarified (S_o)	$Y = 34.715 - 23.613x$ $R^2 = 0.742$ $F = 51.7^{**}$	$Y = 34.265 - 22.863x$ $R^2 = 0.695$ $F = 41.0^{**}$	-
Complete removal of aril (S_1)	$Y = 74.480 - 16.500x$ $R^2 = 0.242$ $F = 5.8^{*}$	$Y = 70.045 - 10.613x$ $R^2 = 0.224$ $F = 5.3^{*}$	$Y = 52.591 - 27.704x$ $R^2 = 0.358$ $F = 14.0^{**}$
Complete removal of aril + partial removal of seed coat	$Y = 89.975 - 13.913x$ $R^2 = 0.243$ $F = 5.8^{*}$	$Y = 77.440 - 8.375x$ $R^2 = 0.4545$ $F = 4.7^{*}$	$Y = 65.222 - 11.620x$ $R^2 = 0.251$ $F = 6.0^{*}$

Each regression is based on 20 data points

Y = Parameter indicated

x = KNO_3 concentration (%)

* = Significant at $P < 0.05$

** = Significant at $P < 0.01$

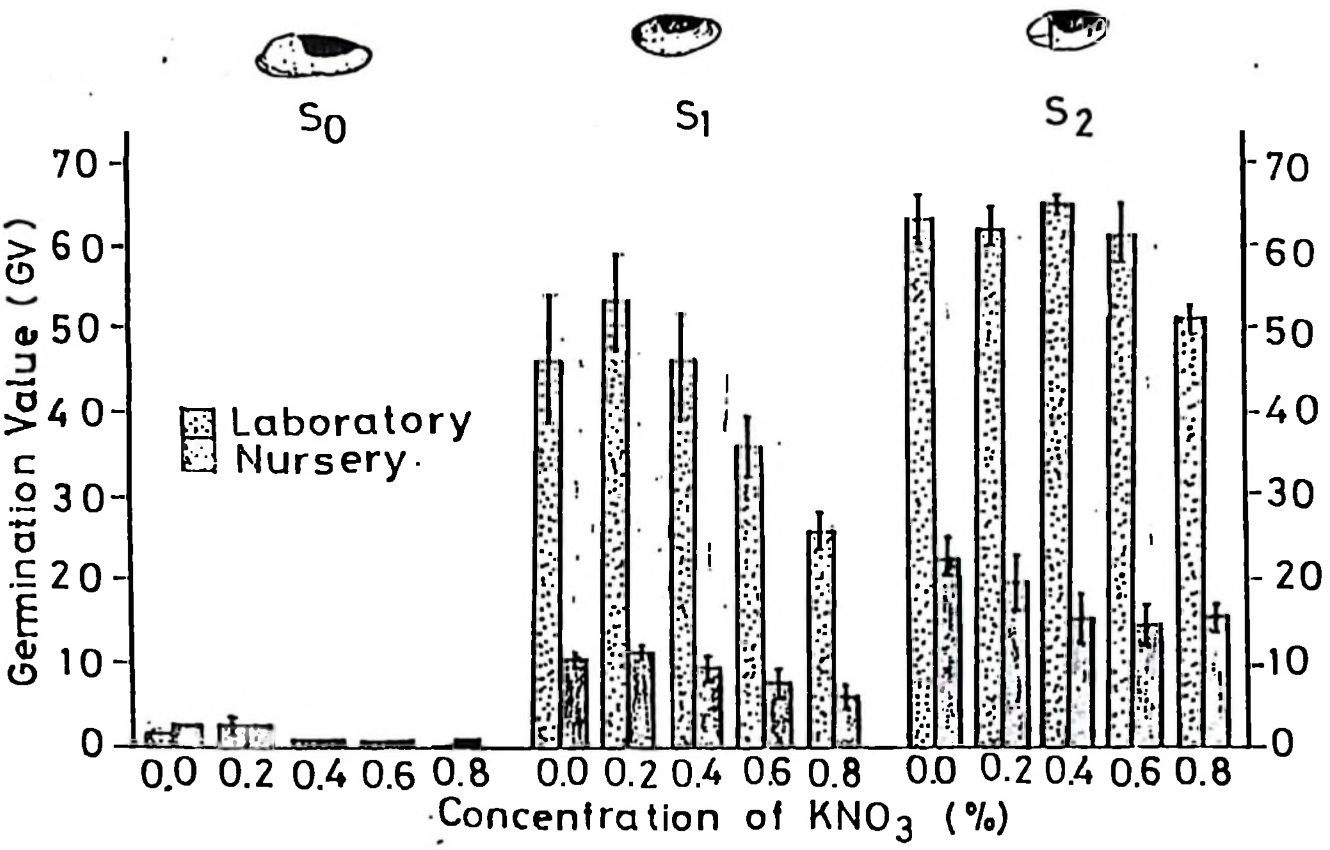

Fig. 13 : Influence of physical seed coat scarification and KNO_3 concentrations on the germination of *Trichilia emetica* seed in laboratory and nursery experiments. (S_0) unscarified, (S_1) complete removal of aril, (S_2) complete removal of aril + partial removal of seed coat. Each bar is a mean of four replicates. Vertical lines indicate ± standard deviations. A bar without a line denotes that the standard deviation is nearly zero.

dormancy period (Table 8). Application of KNO_3 did not significantly influence dormancy periods (Table 9).

4.5.2 Germination in the nursery

4.5.2.1 Cumulative germination and germination energy

Cumulative germination percentage for all treatments are presented in Figure 14. Analysis of variance showed significant effects of both physical seed coat scarification and KNO_3 treatments (Table 11).

Cumulative germination percent was highest in treatments involving the removal of the aril and part of the seed coat, intermediate under aril removal alone and lowest in unscarified seeds (Table 12). As in the case of laboratory studies the application off KNO_3 reduced cumulative germination; although this reduction was significant only when the concentration of KNO_3 was raised up to 0.8% (See Table 13).

Germination energy results followed the same trend as for cumulative germination. For most treatments, the germination energy percentages were lower than their corresponding cumulative germination percentages (Table 12). Based on the tetrazolium test, ungerminated seeds were non-viable at the end of the experiment.

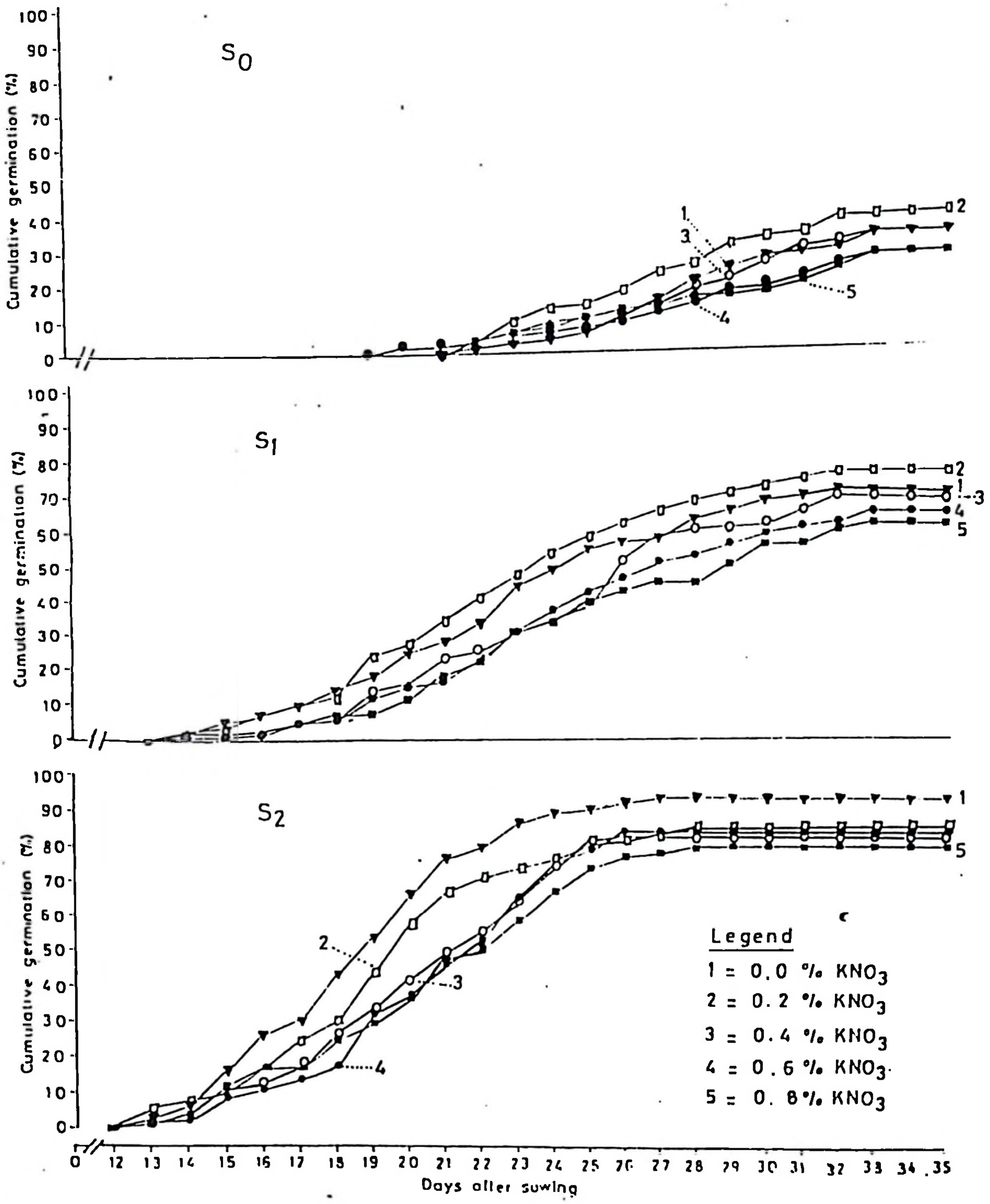

Fig. 14 : Effect of physical seed coat scarification (S_0-unscarified; S_1-complete removal of aril; S_2-complete removal of aril + partial removal of seed coat) and concentrations of KNO_3 on cumulative germination of Trichilia emetica seed in the nursery.

Table 11 : F - values from different ANOVA tables for the effect of physical seed coat scarification and KNO_3 concentrations on the germination of *Trichilia emetica* seed in the nursery

Source of variation	Cumulative germination	Germination energy	Germination value	Seedling height	Dormancy periods		
					Complete	Total	Differential
Replicates	1.292 NS	2.344 NS	0.665 NS	1.445 NS	1.060 NS	1.035 NS	0.939 NS
Physical scarification	154.648**	154.323**	108.566**	16.070**	11.304**	2.667*	26.900**
Potassium nitrate (KNO_3)	3.689*	3.064*	3.357*	1.633 NS	1.345 NS	1.012 NS	1.006 NS
Physical scar. x KNO_3	0.819 NS	1.288 NS	1.131 NS	0.921 NS	0.976 NS	0.983 NS	1.322 NS

* = Significant at $P < 0.05$

** = Significant at $P < 0.01$

NS = Not significant

Table 12 : Effect of physical seed coat scarification and KNO_3 concentrations on the germination of <u>Trichilia</u> <u>emetica</u> seed 35 days after sowing in the nursery

Treatments						Dormancy periods (Days)		
Physical scarification	Chemical KNO_3 (%)	Cumulative germination (%)	Germination energy (%)	Germination value (GV)	Seedling height (mm)	Complete (C)	Total (T)	Differential (T - C)
Unscarified (S_o)	0.0	33 ± 3.8b*	31 ± 3.8ab	2.1 ± 0.3a	15 ± 0.5a	24 ± 2.2c	31 ± 2.2c	7 ± 1.0a
	0.2	39 ± 7.6b	37 ± 9.5b	2.6 ± 0.8a	21 ± 2.6a	20 ± 1.0c	31 ± 1.5c	11 ± 2.1a
	0.4	33 ± 8.9ab	33 ± 8.9b	1.9 ± 0.9a	17 ± 3.7a	21 ± 1.9c	31 ± 2.1c	10 ± 2.5a
	0.6	27 ± 3.8a	27 ± 3.8a	1.4 ± 0.6a	16 ± 3.9a	22 ± 3.1c	31 ± 4.4c	9 ± 2.7a
	0.8	27 ± 11.0a	25 ± 10.5a	1.3 ± 1.1a	16 ± 3.7a	23 ± 2.6c	31 ± 2.7c	8 ± 3.0a
Complete removal of aril (S_1)	0.0	71 ± 3.8d	71 ± 3.8d	10 ± 1.1c	47 ± 3.2b	14 ± 0.5b	31 ± 1.3b	17 ± 1.7c
	0.2	76 ± 6.5d	73 ± 9.8d	11.9 ± 0.9c	59 ± 4.2b	14 ± 0.5b	31 ± 1.3b	17 ± 1.4c
	0.4	69 ± 6.8cd	64 ± 5.7c	10.2 ± 3.1c	42 ± 8.4b	16 ± 2.5b	31 ± 0.0b	15 ± 2.6c
	0.6	65 ± 3.8cd	62 ± 2.3c	8.2 ± 2.9bc	40 ± 7.1b	15 ± 1.4b	31 ± 1.7b	16 ± 2.6c
	0.8	62 ± 10.6c	60 ± 9.8c	6.4 ± 3.0b	39 ± 3.3b	15 ± 1.7b	29 ± 4.6b	14 ± 5.2c
Complete removal of aril + partial removal of seed coat (S_2)	0.0	93 ± 6.8f	92 ± 5.7g	23.5 ± 5.0e	68 ± 6.2c	13 ± 0.6a	25 ± 1.9a	12 ± 2.4b
	0.2	85 ± 14.0f	80 ± 13.9ef	19.9 ± 6.5e	62 ± 10.5c	14 ± 1.8a	27 ± 1.7a	13 ± 0.6b
	0.4	83 ± 10.5f	80 ± 7.3ef	16.2 ± 7.4d	60 ± 5.9c	13 ± 1.2a	25 ± 1.0a	12 ± 0.5b
	0.6	84 ± 12.6f	84 ± 12.6fg	15.4 ± 4.7d	58 ± 14.3c	13 ± 1.3a	26 ± 1.0a	13 ± 1.9b
	0.8	81 ± 7.6e	76 ± 8.6e	16.5 ± 2.6d	54 ± 6.3c	14 ± 1.3a	28 ± 1.3a	14 ± 2.2b

Data represents a mean of four replicates ± Standard Deviation.

* Values in the same column and within a scarification treatment followed by the same letter do not differ significantly based on Duncan's Multiple Range test ($P < 0.05$).

Table 13 : Relationships between nursery germination and KNO_3 concentrations for <u>Trichilia emetica</u> seeds given different physical seed coat scarification treatments

Physical seed coat scarification	Cumulative germination (Arcsin $\sqrt{\%}$)	Germination energy (Arcsin $\sqrt{\%}$)	Germination value
Unscarified (S_o)	$Y = 37.280 - 7.763x$ $R^2 = 0.194$ F = 4.3 NS	$Y = 36.305 - 8.575x$ $R^2 = 0.197$ F = 4.4 NS	-
Complete removal of aril (S_1)	$Y = 59.680 - 9.013x$ $R^2 = 0.305$ $F = 7.9^{*}$	$Y = 58.290 - 9.788x$ $R^2 = 0.332$ $F = 8.9^{**}$	$Y = 11.838 - 5.898x$ $R^2 = 0.344$ $F = 9.4^{**}$
Complete removal of aril + partial removal seed coat (S_2)	$Y = 74.660 - 8.350x$ $R^2 = 0.062$ F = 1.2 NS	$Y = 70.833 - 10.688x$ $R^2 = 0.111$ F = 2.3 NS	$Y = 22.042 - 9.251x$ $R^2 = 0.214$ $F = 4.9^{**}$

Each regression is based on 20 data points

Y = Parameter indicated

x = KNO_3 concentration (%)

* = Significant at $P < 0.05$

** = Significant at $P < 0.01$

NS = Not significant

4.5.2.2 Germination value

Germination value (GV) for all treatments are illustrated in Figure 13. Germination value was highest in treatments where aril removal was associated with partial removal of the seed coat (S_2). Unscarified seeds (S_0) showed significantly GV while seeds with aril removal alone (S_1) were intermediate. The results demonstrated negative effects of applying KNO_3 at concentrations greater than 0.4% for scarified seeds. Potassium nitrate had no effect on unscarified seeds (Table 12).

4.5.2.3 Height of seedlings

The heights of seedlings were influenced by scarification treatments as for cumulative germination (Table 12). Application of KNO_3 did not affect the initial height of seedlings.

4.5.2.4 Dormancy

Scarification of seeds had profound effect on complete dormancy period. Complete dormancy period was shortest in seeds where aril removal was associated with partial removal of the seed coat, intermediate in seeds with aril removal alone and longest in unscarified seeds (Table 12).

Total dormancy period followed nearly the same trends as for complete dormancy period.

Differential dormancy period was shortest in unscarified seeds, intermediate in seeds where aril removal was associated with partial removal of the seed coat, and longest in seeds with aril removal alone. Application of KNO_3 seemed to have little effect on dormancy periods (Tables 11 and 12).

4.5.3 Comparisons between laboratory and nursery germination results

Unscarified seeds which were sown in the laboratory had lower cumulative germination and germination energy percentages compared to those sown in the nursery. With scarified seeds, the cumulative germination and germination energy percentages were higher in the laboratory than in the nursery (Tables 9 and 12). Seeds sown in the laboratory attained higher germination values and shorter dormancy periods than those sown in the nursery (Figure 13). In the laboratory the most dormant seeds completed their germination after 19 days while those in the nursery completed after 31 days. Unscarified seeds which were sown in the laboratory were infected by *Fusarium* sp. when those in the nursery were not.

Correlation analysis between laboratory and nursery tests for all scarification treatments did not show significant relationships between laboratory and nursery germination results.

4.6 Germination characteristics in *Vangueria infausta*

The germination of *Vangueria infausta* seed is epigeal. The endosperm and the embryo expand and cause the testa to burst at the radicle end (Figure 15). The radicle and the cotyledons elongate greatly and both emerge through the radicle end of the seed and not through the hilum as the embryo is oriented transversely. The radicle develops into a stout vigorous taproot while the first leaves and the young shoot extricate themselves from the sides of the cotyledons. The hypocotyl is very short and the cotyledons are partly submerged in the germination medium. They become green and remain attached to the seedlings. The testa

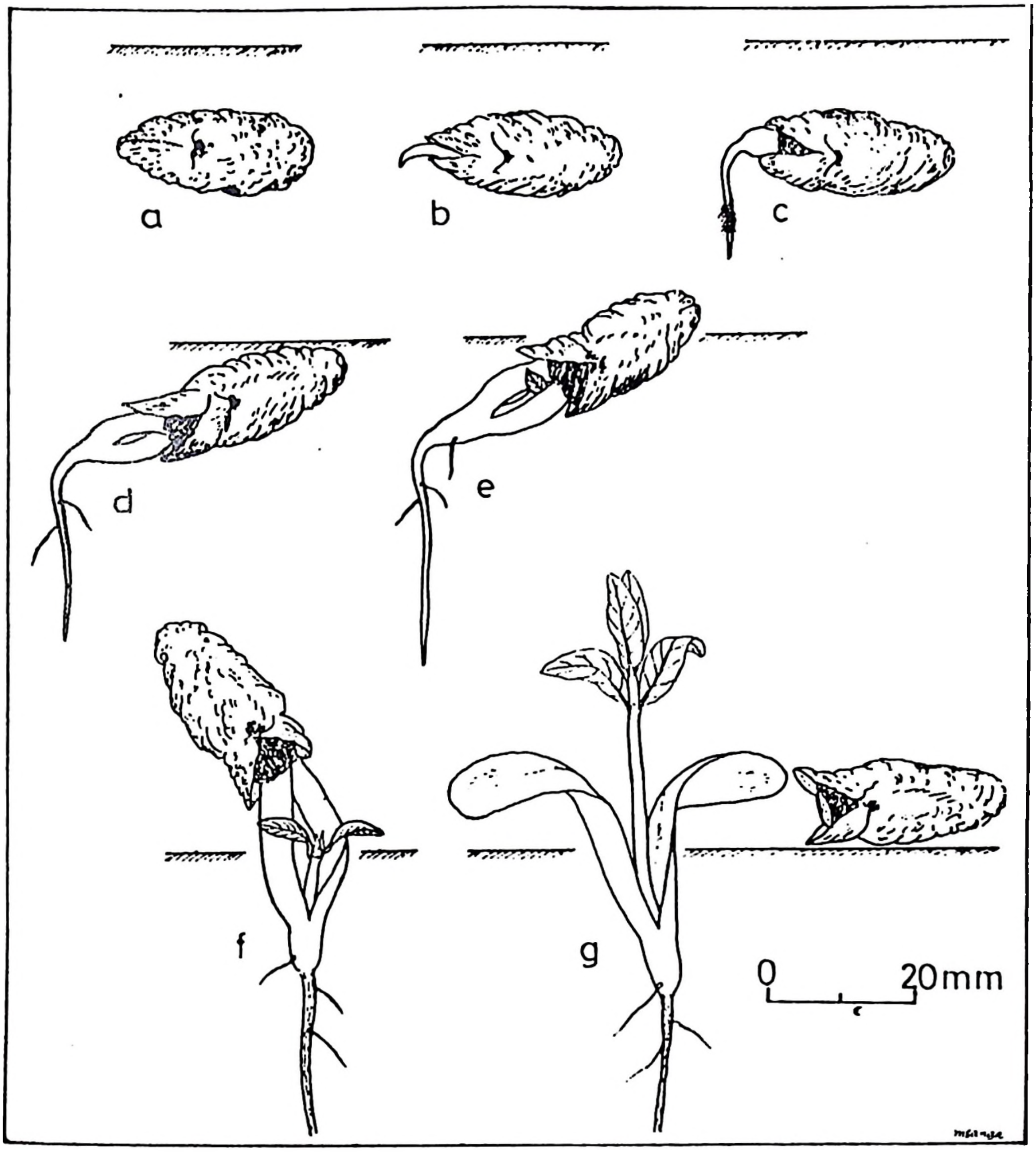

Fig. 15 : Stages in the germination and early development of a *Vangueria infausta* seedling,
(a) at sowing,
(b) at 15 days,
(c) at 20 days,
(d) at 25 days,
(e) at 30 days,
(f) at 35 days (germinated),
(g) at 45 days after sowing.

may remain partly enclosing the cotyledons together with the shrivelled endosperm or may fall off.

4.7 Effect of physical seed coat scarification and H_2O_2 treatments on the germination of *Vangueria infausta* seed

4.7.1 Germination in the laboratory

4.7.1.1 Cumulative germination and germination energy

Both seed scarification treatments and application of hydrogen peroxide (H_2O_2) produced significant effects on the germination of *Vangueria infausta* seed (Table 14). Data for cumulative germination percentages at final assessment are presented in Table 15. At final assessment cumulative germination in unscarified seeds (S_0) was nearly the same as for treatments involving partial removal of testa by abrasion (S_1). Cumulative germination was lowest in seeds with complete removal of testa (S_2) (Figure 16).

In unscarified seeds and seeds whose testa was partly removed by abrasion, application of 2% - 6% H_2O_2 increased cumulative germination percent. Application of H_2O_2 at the concentration of 8% on the other hand was harmful to germination (Table 15).

Linear regression analysis between cumulative germination percent and H_2O_2 concentrations did not

Table 14 : F - values from different ANOVA tables for the effect of physical seed coat scarification and H_2O_2 concentrations on the germination of *Vangueria infausta* seed in the laboratory

Source of variation	Cumulative germination	Germination energy	Germination value	Dormancy periods		
				Complete	Total	Differential
Replicates	1.737 NS	1.313 NS	1.525 NS	0.429 NS	1.119 NS	2.013 NS
Physical scarification	215.981^{**}	186.560^{**}	62.030^{**}	4.607^{**}	27.436^{**}	44.819^{**}
Hydrogen peroxide (H_2O_2)	4.776^{**}	4.393^{**}	6.022^{**}	3.571^{*}	3.487^{*}	3.434^{*}
Physical scar. x H_2O_2	1.677 NS	1.462 NS	2.141 NS	1.607 NS	0.744 NS	2.130 NS

* = Significant at $P < 0.05$

** = Significant at $P < 0.01$

NS = Not significant

Table 15 : Effect of physical seed coat scarification and H_2O_2 concentrations on the germination of *Vangueria infausta* seed 40 days after sowing in the laboratory

Treatments					Dormancy periods (Days)		
Physical scarification	Chemical H_2O_2 (%)	Cumulative germination (%)	Germination energy (%)	Germination value (GV)	Complete (C)	Total (T)	Differential (T-C)
Unscarified (S_0)	0	49 ± 6.8f*	44 ± 8.6f	4.2 ± 1.1b	22 ± 0.6d	38 ± 1.7c	16 ± 1.4h
	2	55 ± 11.5fg	53 ± 15.1fg	6.6 ± 2.1c	22 ± 1.3d	36 ± 1.9c	14 ± 2.9gh
	4	63 ± 21.3g	62 ± 20.3g	8.2 ± 5.5c	23 ± 1.5d	37 ± 2.9c	14 ± 6.8gh
	6	64 ± 6.5g	62 ± 4.0g	7.7 ± 4.1c	22 ± 0.5d	37 ± 1.0c	15 ± 1.0gh
	8	46 ± 6.9f	46 ± 6.9f	3.8 ± 1.2b	26 ± 0.5e	37 ± 1.9c	11 ± 1.7g
Partial removal of testa by abrasion (S_1)	0	55 ± 6.8cd	54 ± 7.7cd	6.0 ± 2.3d	21 ± 0.0a	30 ± 2.2d	9 ± 2.2ef
	2	57 ± 5.0cd	55 ± 6.8cd	7.3 ± 0.9e	21 ± 0.0a	31 ± 4.2d	10 ± 4.0f
	4	62 ± 2.3de	62 ± 2.3de	8.2 ± 2.6e	21 ± 0.0a	28 ± 2.1d	7 ± 2.1e
	6	71 ± 9.5e	68 ± 10.8e	10.9 ± 2.9f	21 ± 0.0a	30 ± 1.9d	9 ± 1.9ef
	8	48 ± 8.6c	48 ± 8.6c	4.6 ± 1.6d	24 ± 1.4b	35 ± 6.8d	11 ± 3.2f
Complete removal of testa (S_2)	0	5 ± 2.0a	5 ± 2.0a	0.1 ± 0.1a	24 ± 3.0c	25 ± 3.0a	1 ± 0.0a
	2	16 ± 3.3b	16 ± 3.3b	0.6 ± 0.2a	22 ± 1.5c	30 ± 0.6b	8 ± 1.9d
	4	10 ± 7.7a	10 ± 7.7a	0.3 ± 0.1a	22 ± 2.0c	29 ± 6.5b	7 ± 2.3cd
	6	9 ± 7.6a	9 ± 7.6a	0.3 ± 0.2a	24 ± 3.0c	27 ± 2.6b	3 ± 3.4b
	8	9 ± 3.8a	9 ± 3.8a	0.2 ± 0.1a	24 ± 2.6c	29 ± 3.5b	5 ± 3.3c

Data represents a mean of four replicates ± Standard Deviation.

* Values in the same column and within a scarification treatment followed by the same letter do not differ significantly based on Duncan's Multiple Range test ($P < 0.05$).

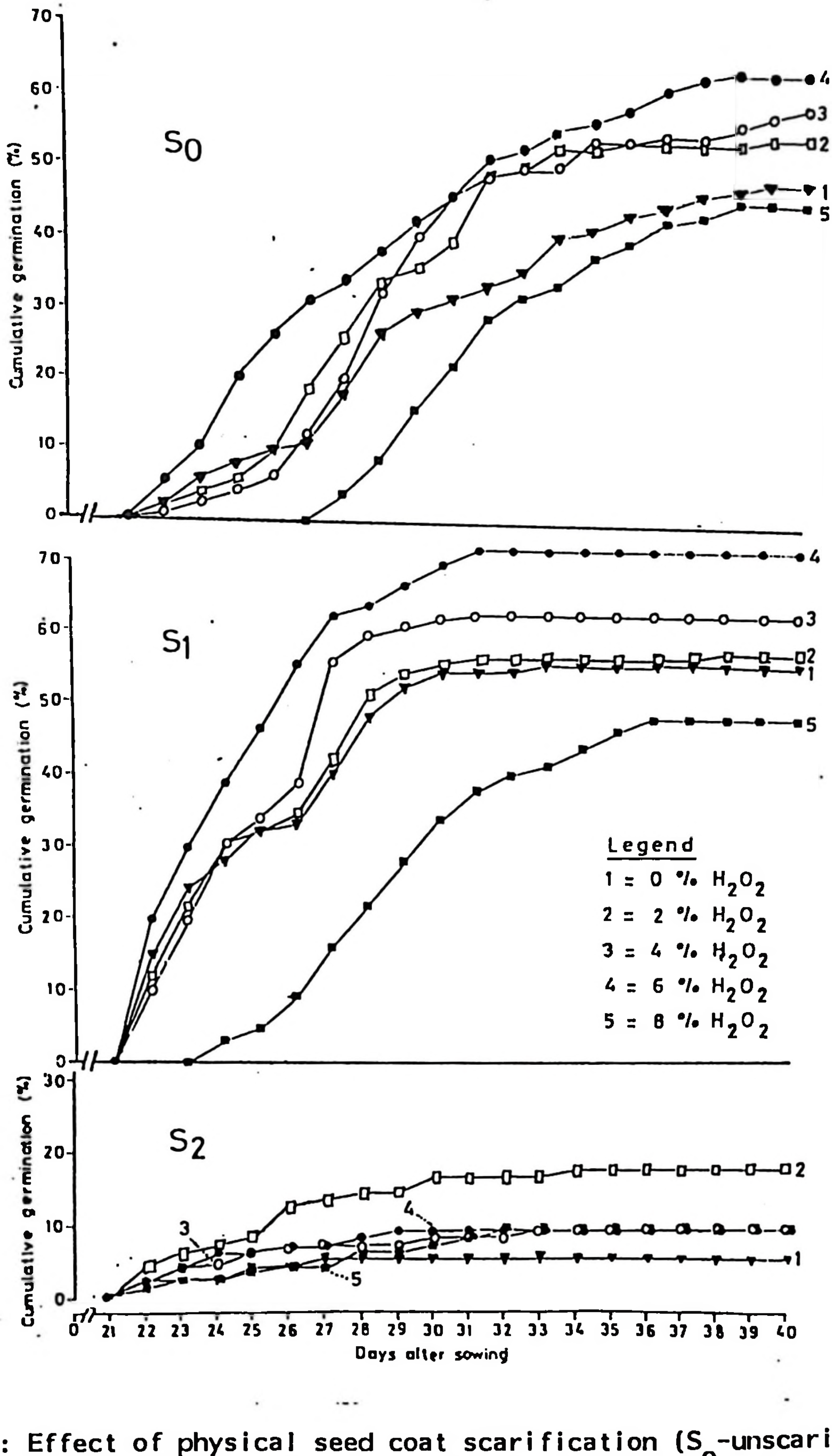

Fig. 16 : Effect of physical seed coat scarification (S_o-unscarified; S_1-partial removal of testa by abrasion; S_2 - complete removal of testa) and concentrations of H_2O_2 on cumulative germination of *Vangueria infausta* seed in the laboratory.

show significance for all the treatments employed.

Germination energy results followed the same trends as for cumulative germination. Based on the tetrazolium test, at the termination of the experiment all ungerminated seeds were apparently non - viable.

4.7.1.2 Germination value

Germination value (GV) was significantly affected by both physical seed coat scarification and application of H_2O_2 (Table 14). Germination value was highest in seeds involving partial removal of testa by abrasion (S_1), intermediate in unscarified seeds (S_o) and lowest in seeds with complete removal of testa (S_2) (Figure 17).

Application of 2 - 6% H_2O_2 significantly increased GV in unscarified seeds and in seeds with partial removal of testa by abrasion. However,GV was reduced when the concentration of H_2O_2 was 8%. Hydrogen peroxide effects on seeds with complete removal of testa were not significant (Table 15).

As for cumulative germination, linear regression analysis between GV and H_2O_2 concentrations did not show significant relationships for all the treatments studied.

4.7.1.3 Dormancy

Both scarification and H_2O_2 treatments produced significant effect on dormancy periods (Table 14). All dormancy periods were longer in unscarified seeds than in scarified ones (Table 15). Application of 8% H_2O_2 significantly increased the complete dormancy period from 22 days (control) to 26 days for unscarified

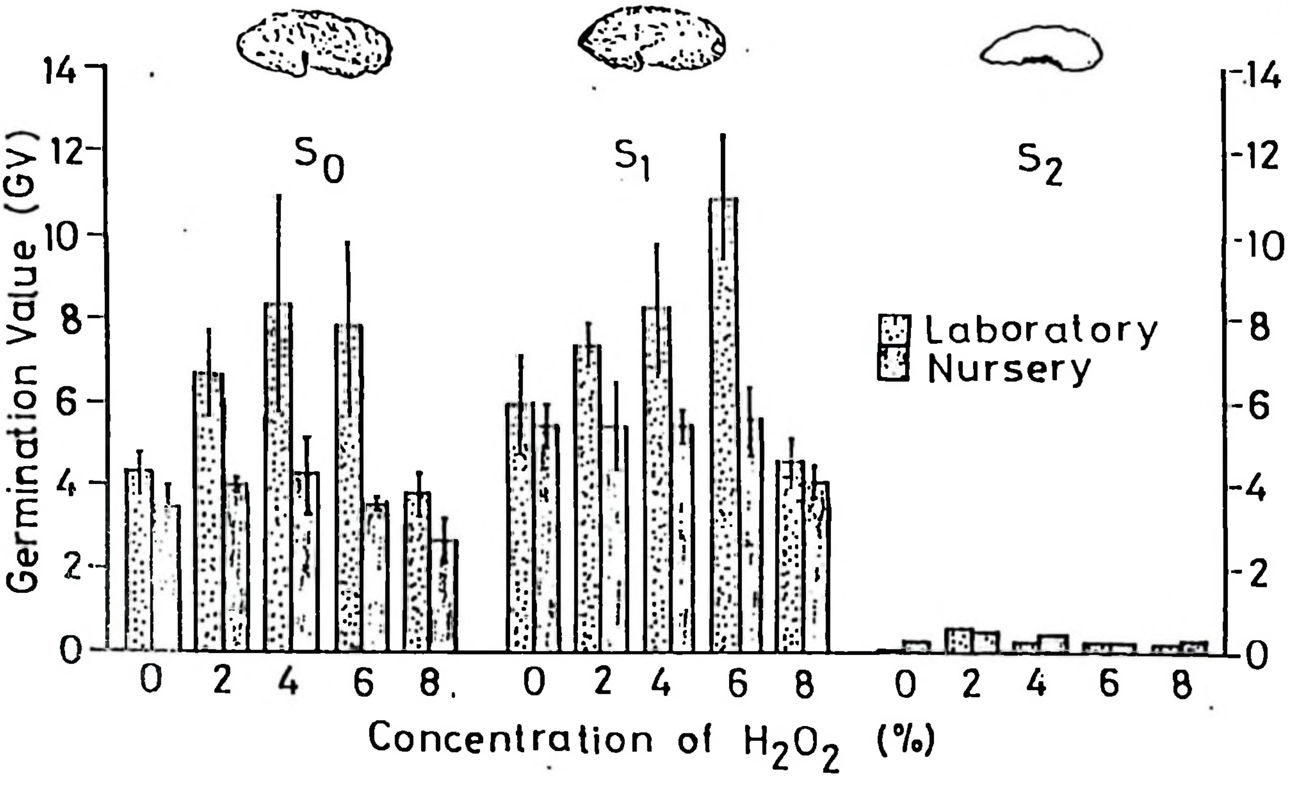

Fig. 17 : Influence of physical seed coat scarification and H_2O_2 concentrations on the germination of Vangueria infausta seed in laboratory and nursery experiments. (S_0) unscarified, (S_1) partial removal of testa by abrasion, (S_2) complete removal of testa. Each bar is a mean of four replicates. Vertical lines indicate ± standard deviations. A bar without a line denotes that the standard deviation is nearly zero.

seeds, and from 21 days (control) to 24 days for those with partial removal of testa by abrasion. Where the testa was completely removed, application of H_20_2 had no significant effect on complete dormancy period.

In unscarified seeds and seeds with partial removal of testa by abrasion, application of H_20_2 had no effect on the total dormancy period. However, where the testa was completely removed, application of H_20_2 significantly increased the total dormancy period. Similarly application of H_20_2 had significant influences on the differential dormancy period for all types of seed scarification studied.

4.7.2 Germination in the nursery

4.7.2.1 Cumulative germination and germination energy

Physical seed coat scarification and H_20_2 treatments had significant effects on cumulative germination (Table 16). At final assessment cumulative germination percent was highest in seeds with partial removal of testa, followed by unscarified seeds and lowest in seeds with complete removal of testa (Table 17 and Figure 18). Hydrogen peroxide had no significant effects on unscarified seeds. Application of 6% H_20_2 significantly increased cumulative germination for seeds with partial removal of testa by abrasion. Concentration of 8% H_20_2 was harmful to the seeds. Application of 2% H_20_2 in seeds complete removal of testa showed a minor increase in cumulative germination from 10 - 16%. Concentrations of 4% - 8% slightly increased the cumulative germination as compared to no treatment, but the increase was not significant.

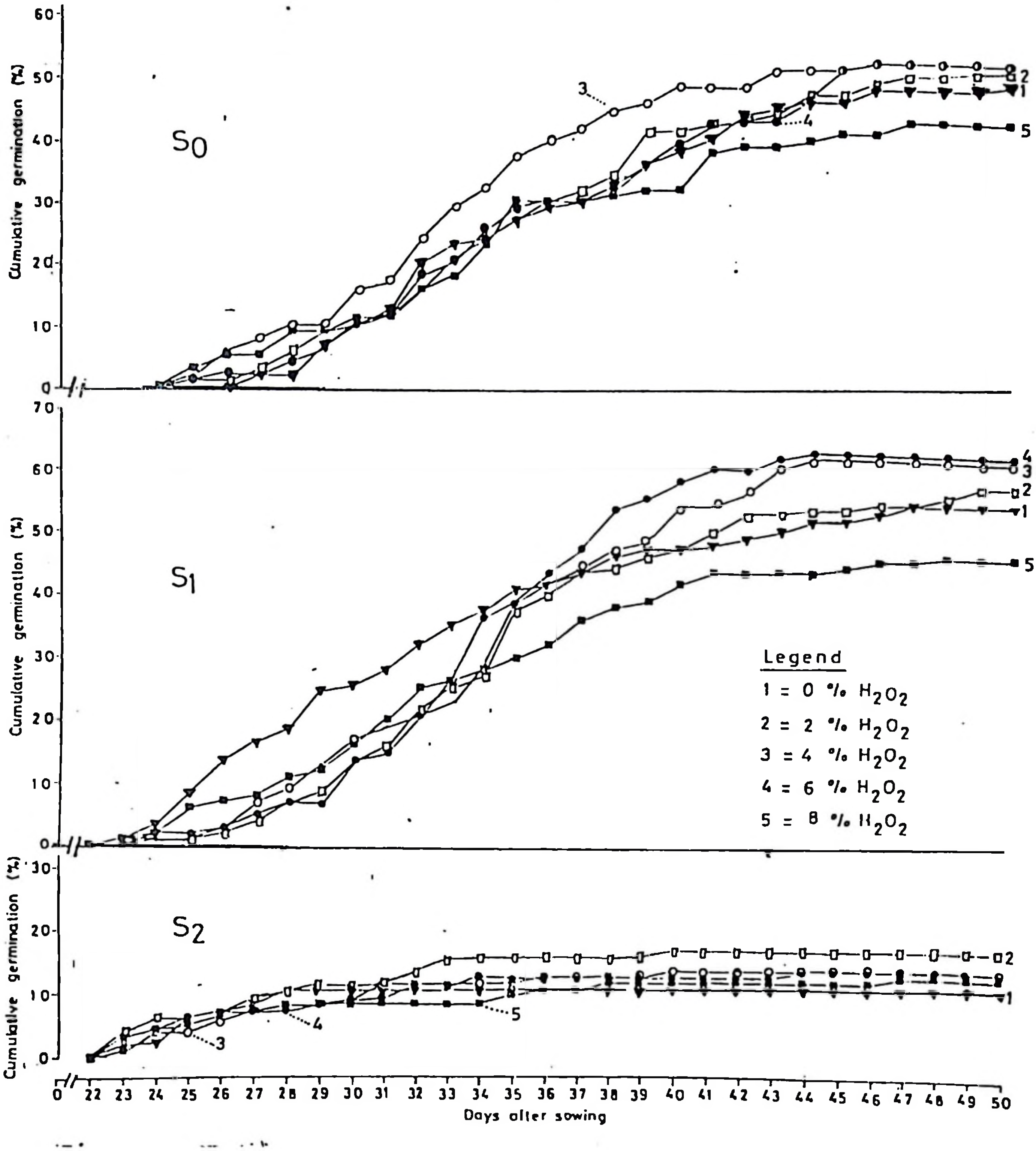

Fig. 18 : Effect of physical seed coat scarification (S_o-unscarified; S_1-partial removal of testa by abrasion; S_2 - complete removal of testa) and concentrations of H_2O_2 on cumulative germination of Vangueria infausta seed in the nursery.

Linear regression analysis between cumulative germination percent and H_2O_2 concentration did not show significant relationships in all treatments. Germination energy results followed the same trends as for cumulative germination. At the termination of the experiment all ungerminated seeds were found not viable.

4.7.2.2 Germination value

Physical seed coat scarification produced significant effects on germination value (GV) (Table 16). Germination value increased progressively from seeds with complete removal of testa, unscarified seeds to seeds with partial removal of testa (Figure 17). Treating the seeds with H_2O_2 did not give significant influences on GV (Table 17). As for cumulative germination, linear regression analysis between germination value and H_2O_2 concentrations did not show significant results.

4.7.2.3 Height of seedlings

Trends in height of seedlings were similar to germination value (Table 17). Seed scarification had highly significant effects on height of seedlings, whereas application of H_2O_2 had no effect. The tallest seedlings were produced by seeds with partial removal of testa, intermediate in unscarified seeds and shortest in seeds with complete removal of testa.

4.7.2.4 Dormancy

While seed scarification had highly significant effects in dormancy periods, H_2O_2 had little or no effect (Table 16). All dormancy periods were shortest in seeds whose testa was removed

Table 16 : F -values from differenti ANOVA tables for the effect of physical seed coat scarification and H_2O_2 concentrations on the germination of *Vangueria infausta* seed in the nursery

Source of variation	Cumulative germination	Germination energy	Germination value	Seedling height	Dormancy periods		
					Complete	Total	Differential
Replicates	1.407 NS	0.452 NS	1.917 NS	0.625 NS	0.219 NS	2.444 NS	1.857 NS
Physical scarification	238.026^{**}	183.631^{**}	105.048^{**}	462.000^{**}	15.49^{**}	17.439^{**}	23.353^{**}
Hydrogen peroxide (H_2O_2)	3.445^{*}	2.633^{*}	2.490 NS	2.250 NS	2.463 NS	1.786 NS	1.764 NS
Physical scar. x H_2O_2	0.809 NS	0.926 NS	0.949 NS	0.250 NS	2.031 NS	1.524 NS	1.948 NS

* = Significant at $P < 0.05$

** = Significant at $P < 0.01$

NS = Not significant

and longest in unscarified seeds (Table 17).

4.7.3 Comparisons between laboratory and nursery germination results

Unscarified seeds and seeds whose testa was partly removed by abrasion and sown in the laboratory acquired higher cumulative germination, germination energy and germination values compared to those sown in the nursery. Where the testa was completely removed, opposite trends were shown (Tables 15 and 17). Seeds sown in the laboratory attained shorter dormancy periods than nursery ones. In the laboratory the most dormant seeds completed their total dormancy period after 38 days, while those in the nursery completed after 45 days.

Correlation analysis between laboratory and nursery tests for all scarification treatments did not show significant relationships between laboratory and nursery germination results.

4.8 Effect of physical seed coat scarification and IAA treatments on the germination *Vanqueria infausta* seed

4.8.1 Germination in the laboratory

4.8.1.1 Cumulative germination energy

Scarification and treatment with indole acetic acid (IAA) showed significant improvements on cumulative germination in *Vanqueria infausta* seed (Table 18). Seeds with the testa partly split (i.e. cracking part of the testa) at the radicle end (S_1) attained the highest cumulative germination, followed by unscarified seeds (S_0) and lowest in

Table 17 : Effect of physical seed coat scarification and H_2O_2 concentrations on the germination of *Vangueria infausta* seed 50 days after sowing in the nursery

Treatments						Dormancy period (Days)		
Physical scarification	Chemical H_2O_2 (%)	Cumulative germination (%)	Germination energy (%)	Germination value (GV)	Seedling height (mm)	Complete (C)	Total (T)	Differential (T - C)
Unscarified (S_o)	0	49 ± 12.8f*	46 ± 14.0e	3.5 ± 1.5c	49 ± 2.1c	26 ± 0.5c	45 ± 3.9c	19 ± 3.9c
	2	51 ± 6.8f	49 ± 7.6e	3.6 ± 0.5c	53 ± 11.2c	27 ± 1.9c	45 ± 4.2c	18 ± 5.7c
	4	52 ± 7.3f	49 ± 3.8e	4.3 ± 1.8c	55 ± 13.7c	25 ± 0.6c	43 ± 2.4c	18 ± 2.9c
	6	52 ± 4.6f	51 ± 6.0e	3.4 ± 0.4c	56 ± 11.4c	27 ± 2.6c	44 ± 2.9c	17 ± 4.6c
	8	43 ± 8.2f	43 ± 8.2e	2.6 ± 0.9c	46 ± 5.8c	25 ± 1.5c	44 ± 2.8c	19 ± 3.7c
Partial removal of testa by abrasion (S_1)	0	55 ± 8.2cd	54 ± 10.1d	5.3 ± 0.9b	59 ± 5.9b	24 ± 2.2b	42 ± 4.0b	18 ± 3.7b
	2	58 ± 13.2d	52 ± 13.9cd	5.4 ± 2.3b	62 ± 5.7b	26 ± 1.0b	45 ± 2.9b	19 ± 3.1b
	4	62 ± 7.7de	62 ± 7.7d	5.4 ± 0.8b	65 ± 3.6b	25 ± 2.5b	44 ± 0.6b	19 ± 2.5b
	6	63 ± 8.2e	61 ± 8.9d	5.6 ± 1.7b	69 ± 6.5b	25 ± 2.4b	42 ± 2.1b	17 ± 4.2b
	8	47 ± 5.7c	43 ± 5.0c	4.3 ± 0.7b	53 ± 10.6b	23 ± 0.6b	45 ± 2.9b	22 ± 2.9b
Complete removal of testa (S_2)	0	10 ± 2.3a	10 ± 2.3a	0.3 ± 0.1a	9 ± 3.2a	23 ± 1.2a	37 ± 1.5a	14 ± 1.7a
	2	16 ± 3.3b	16 ± 3.3b	0.5 ± 0.2a	12 ± 2.1b	22 ± 0.5a	34 ± 5.3a	12 ± 5.3a
	4	13 ± 3.8ab	12 ± 3.3ab	0.3 ± 0.1a	11 ± 1.7b	23 ± 0.5a	36 ± 6.3a	13 ± 6.5a
	6	13 ± 3.8ab	11 ± 3.3ab	0.3 ± 0.1a	11 ± 3.9b	23 ± 0.5a	36 ± 7.5a	13 ± 7.0a
	8	12 ± 3.2ab	11 ± 3.8ab	0.2 ± 0.1a	9 ± 1.7a	23 ± 0.8a	37 ± 9.3a	14 ± 8.3a

Data represents a mean of four replicates ± Standard Deviation.

* Values in the same column and within a scarification treatment followed by the same letter do not differ significantly based on Duncan's Multiple Range test ($P < 0.05$).

Table 18 : F - values from different ANOVA tables for the effect of physical seed coat scarification and IAA concentrations on the germination of *Vangueria infausta* seed in the laboratory

Source of variation	Cumulative germination	Germination energy	Germination value	Dormancy periods		
				Complete	Total	Differential
Replicates	0.176 NS	0.180 NS	0.103 NS	0.714 NS	0.222 NS	0.247 NS
Physical scarification	35.979**	29.116**	30.063**	15.971**	3.767*	1.393 NS
Indole acetic acid (IAA)	5.179**	3.637*	6.757**	1.086 NS	1.289 NS	0.157 NS
Physical scar. x IAA	0.177 NS	0.259 NS	0.360 NS	1.229 NS	0.333 NS	0.758 NS

* = Significant at $P < 0.05$

** = Significant at $P < 0.01$

NS = Not significant

seeds with the testa partly removed by chipping (i.e. cutting off a portion of the testa) at the radicle end (S_2) (Figure 19). In both unscarified treatments, application of 50-100 ppm IAA produced a significant increase in cumulative germination (Table 19).

Seed scarification and IAA treatments also showed significant effects on germination energy (Table 18). In unscarified seeds and with partial splitting of testa, application of 75 ppm or 100 ppm IAA produced a significant increase in germination energy. In seeds with partial removal of the testa by chipping the effect of IAA was significant only at 100 ppm (Table 19). Linear regression analysis of cumulative germination and germination energy on the concentrations of IAA is presented in Table 20.

4.8.1.2 Germination value

Germination value (GV) was highly influenced by both seed scarification and IAA treatments (Table 18). As for cumulative germination, GV was highest in seeds involving splitting part of the testa (S_1), intermediate in unscarified seeds (S_o) and lowest in seeds with partial removal of the testa by chipping (S_2) (Figure 20). Application of 25 - 100 ppm IAA produced a significant increase in GV for unscarified seeds and seeds partly split on the testa. Linear regression analysis of germination value on the concentrations of IAA was significant in unscarified seeds and seeds involving splitting part of testa (Table 20).

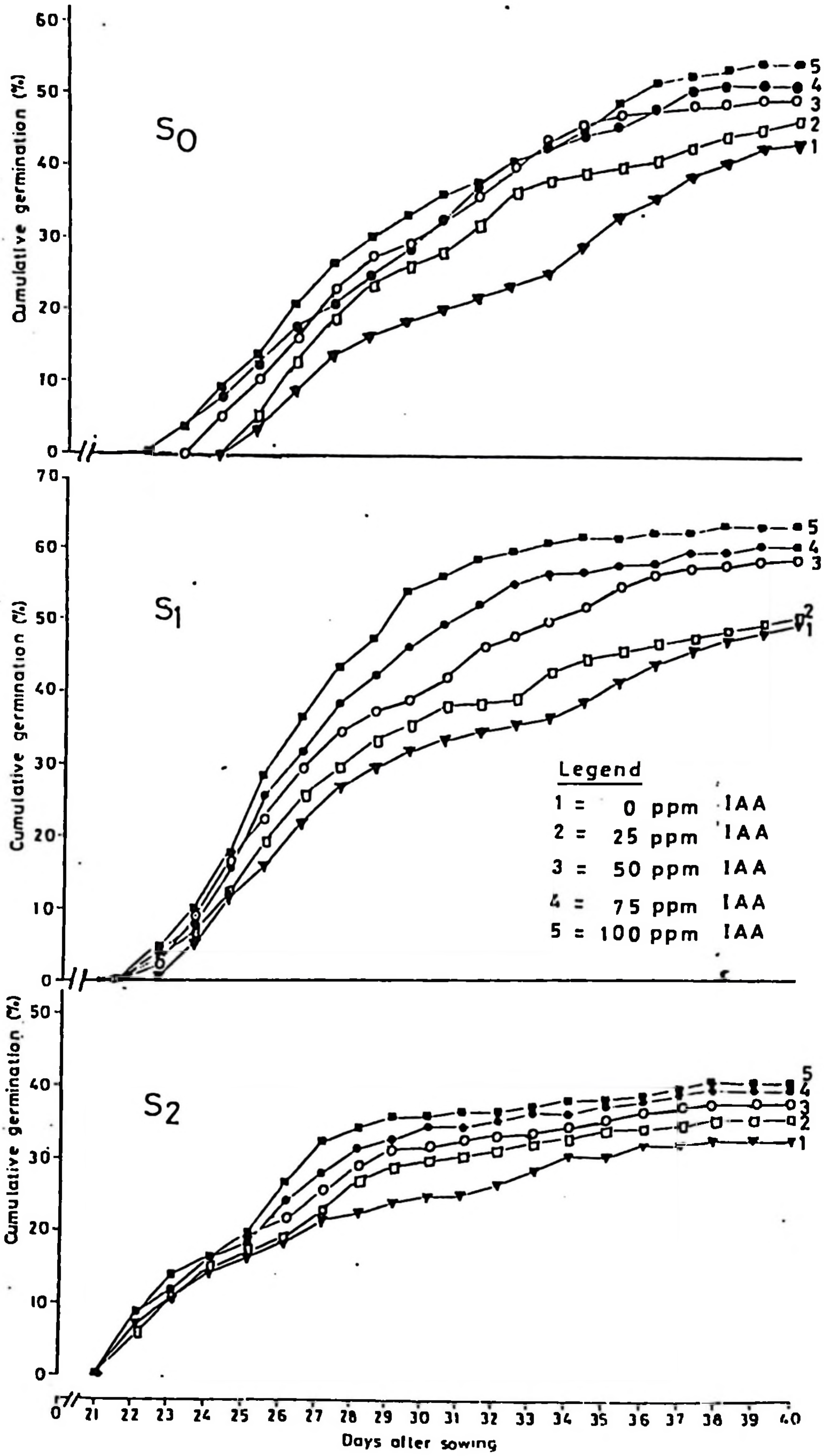

Fig. 19 : Effect of physical seed coat scarification (S_o-unscarified; S_1-splitting part of testa; S_2-partial removal of testa by chipping) and concentrations of IAA on cumulative germination of <u>Vangueria infausta</u> seed in the laboratory.

Table 19 : Effect of physical seed coat scarification and IAA concentrations on the germination of *Vangueria infausta* seed 40 days after sowing in the laboratory

Treatments					Dormancy periods (Days)		
Physical scarification	Chemical IAA (ppm)	Cumulative germination (%)	Germination energy (%)	Germination value (GV)	Complete (C)	Total (T)	Differential (T-C)
Unscarified (S_o)	0	47 ± 5.2f*	46 ± 5.2e	3.6 ± 0.8e	24 ± 0.5c	38 ± 1.3c	14 ± 1.4c
	25	50 ± 6.8fg	47 ± 10.0ef	5.3 ± 1.1fg	24 ± 0.0c	38 ± 1.8c	14 ± 1.8c
	50	57 ± 5g	56 ± 3.3ef	5.5 ± 0.5fg	23 ± 0.0c	37 ± 1.4c	14 ± 1.4c
	75	56 ± 3.8g	54 ± 8.9ef	5.8 ± 0.5g	22 ± 0.5c	38 ± 1.3c	16 ± 1.0c
	100	58 ± 9.2g	57 ± 10.5f	6.6 ± 2.1g	22 ± 0.0c	37 ± 1.8c	15 ± 1.7c
Splitting part of testa (S_1)	0	51 ± 8.9c	50 ± 9.5c	5.0 ± 1.3b	22 ± 0.0b	37 ± 2.6b	15 ± 3.3b
	25	54 ± 10.6cd	52 ± 11.3cd	6.0 ± 2.6c	22 ± 0.5b	37 ± 3.6b	15 ± 3.3b
	50	60 ± 3.3de	56 ± 6.5cd	7.4 ± 0.5cd	22 ± 0.5b	37 ± 1.4b	15 ± 1.7b
	75	62 ± 5.2de	62 ± 5.2d	7.8 ± 1.4d	21 ± 0.0b	34 ± 3.2b	13 ± 3.2b
	100	65 ± 6.0e	62 ± 5.2d	8.3 ± 1.6d	21 ± 0.0b	35 ± 2.2b	14 ± 2.2b
Partial removal of testa by chipping (S_2)	0	35 ± 8.9a	33 ± 12.4a	2.6 ± 1.0a	21 ± 0.0a	35 ± 2.6a	14 ± 2.6a
	25	38 ± 5.2 ab	34 ± 5.2a	3.2 ± 0.8a	21 ± 0.0a	36 ± 2.8a	15 ± 2.8a
	50	40 ± 7.3b	38 ± 5.2ab	3.6 ± 1.0a	21 ± 0.0a	35 ± 3.3a	14 ± 3.3a
	75	42 ± 5.2b	38 ± 5.2ab	4.0 ± 1.0a	21 ± 0.0a	35 ± 3.6a	14 ± 3.6a
	100	43 ± 8.6b	43 ± 8.6b	4.3 ± 1.4a	21 ± 0.0a	33 ± 5.9a	12 ± 5.9a

Data represents a mean of four replicates ± Standard Deviation.

* Values in the same column and within a scarification treatment followed by the same letter do not differ significantly based on Duncan's Multiple Range test ($P < 0.05$).

Table 20 : Relationships between laboratory germination and IAA concentrations for *Vangueria infausta* seeds given different physical seed coat scarification treatments

Physical seed coat scarification	Cumulative germination (Arcsin $\sqrt{\%}$)	Germination energy (Arcsin $\sqrt{\%}$)	Germination value
Unscarified (S_o)	$Y = 43.760 + 0.074x$ $R^2 = 0.372$ $F = 10.7^{**}$	$Y = 42.830 + 0.060x$ $R^2 = 0.200$ $F = 4.5^{*}$	$Y = 4.093 + 0.026x$ $R^2 = 0.421$ $F = 13.1^{**}$
Splitting part of testa (S_1)	$Y = 45.845 + 0.079x$ $R^2 = 0.349$ $F = 9.6^{**}$	$Y = 44.830 + 0.078x$ $R^2 = 0.320$ $F = 8.5^{**}$	$Y = 5.257 + 0.063x$ $R^2 = 0.229$ $F = 5.2^{*}$
Partial removal of testa by chpping (S_2)	$Y = 36.445 + 0.052x$ $R^2 = 0.192$ $F = 4.3$ NS	$Y = 34.515 + 0.059$ $R^2 = 0.193$ $F = 4.3$ NS	-

Each regression is based on 20 data points

Y = Parameter indicated

x = IAA concentration (ppm)

* = Significant at $P < 0.05$

** = SIgnificant at $P < 0.01$

NS = Not significant

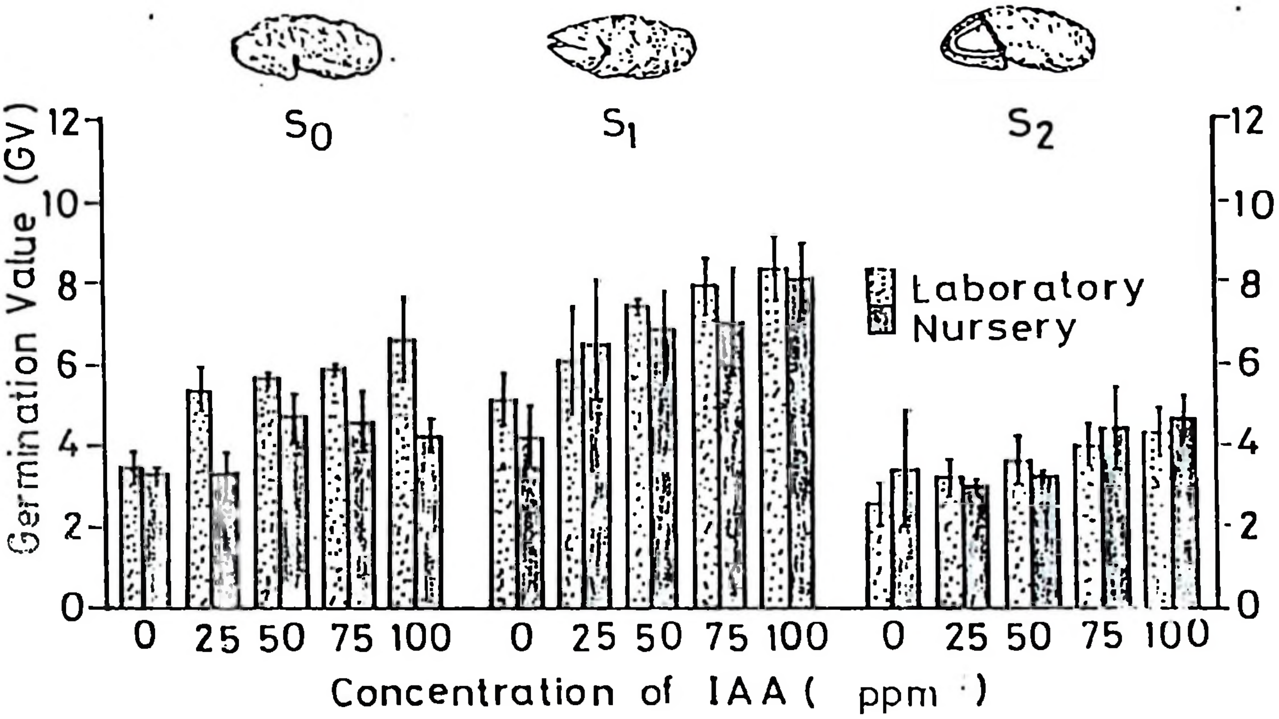

Fig. 20 : Influence of physical seed coat scarification and IAA concentrations on the germination of Vangueria infausta seed in laboratory and nursery experiments. (S_o) unscarified, (S_1) splitting part of testa, (S_2) partial removal of testa by chipping. Each bar is a mean of four replicates. Vertical lines indicate ± standard deviations.

4.8.1.3 Dormancy

Physical scarification significantly reduced the complete and total dormancy periods, but had no significant effects on the differential dormancy period. Complete and total dormancy periods were longest in unscarified seeds (S_0), intermediate in seeds partly split on the testa (S_1) and shortest in seeds with partial removal of the testa (S_2) (Table 19). Application of indole acetic acid was insignificant.

4.8.2 Germination in the nursery

4.8.2.1 Cumulative germination and germination energy

Seed scarification and indole acetic acid (IAA) had significant influences on cumulative germination of *Vangueria infausta* seed (Table 21). At the termination of the experiment, cumulative germination was highest in treatments involving partial splitting of the testa (S_1), followed by unsacrified seeds (S_0), and lowest in seeds whose testa was partly removed by chipping (S_2) (Table 22 and Figure 21).

Application of 50 ppm IAA significantly increased cumulative germination of both unscarified and scarified seeds (Table 22). Linear regression analysis did not show significant relationships for all scarification treatments studied.

Germination energy results followed the same trends as for cumulative germination (Table 22), except that linear regression analysis showed a

Table 21 : F - values from different ANOVA tables for the effect of physical seed coat scarification and IAA concentrations on the germination of *Vangueria infausta* seed in the nursery

Source of variation	Cumulative germination	Germination energy	Germination value	Seedling height	Dormancy periods		
					Complete	Total	Differential
Replicates	3.625*	4.052*	1.594 NS	1.024 NS	0.953 NS	0.614 NS	1.197 NS
Physical scarification	20.861**	19.789**	43.757*	3.954*	14.435*	35.851*	10.176*
Indole acetic acid (IAA)	3.748*	3.273*	4.658**	7.544*	1.118 NS	0.772 NS	0.916 NS
Physical scarif. x IAA	0.305 NS	0.337 NS	0.679 NS	0.330 NS	1.129 NS	1.327 NS	1.861 NS

* = Significant at $P < 0,05$

** = Significant at $P < 0,01$

NS = Not significant

Table 22 : Effect of physical seed coat scarification and IAA concentrations on the germination of *Vangueria infausta* seed 50 days after sowing in the nursery

Treatments						Dormancy periods (Days)		
Physical scarification	Chemical IAA (ppm)	Cumulative germination (%)	Germination energy (%)	Germination value (GV)	Seedling height (mm)	Complete (C)	Total (T)	Differential (T - C)
Unscarified (S_o)	0	50 ± 5.2e*	50 ± 5.2e	3.4 ± 0.7e	51 ± 1.8a	30 ± 1.9c	48 ± 1.5c	18 ± 1.7c
	25	51 ± 6.8e	51 ± 6.8e	3.6 ± 1.5e	53 ± 2.4a	27 ± 2.2c	47 ± 3.4c	20 ± 3.1c
	50	58 ± 10.6f	56 ± 12.8f	4.8 ± 1.2e	58 ± 5.7b	26 ± 2.8c	49 ± 1.0c	23 ± 3.5c
	75	58 ± 8.3f	58 ± 8.3f	4.6 ± 1.4e	59 ± 4.0bc	26 ± 1.3c	47 ± 2.2c	21 ± 2.7c
	100	59 ± 3.8f	58 ± 2.3f	4.2 ± 0.8e	65 ± 2.2c	25 ± 1.7c	43 ± 3.4c	18 ± 4.2c
Splitting part of test (S_1)	0	53 ± 11.9c	53 ± 11.9c	4.9 ± 1.5b	57 ± 7.0d	25 ± 1.7b	42 ± 5.9b	17 ± 1.0b
	25	56 ± 10.3c	52 ± 6.5c	6.4 ± 3.3bc	60 ± 3.6d	25 ± 2.6b	40 ± 5.2b	15 ± 4.9b
	50	64 ± 9.8d	62 ± 11.4d	6.8 ± 1.9cd	68 ± 5.7e	24 ± 1.0b	44 ± 3.7b	20 ± 3.9b
	75	66 ± 14.8d	64 ± 14.3d	7.0 ± 2.8cd	70 ± 4.8e	24 ± 0.6b	46 ± 3.9b	22 ± 3.9b
	100	70 ± 7.7d	68 ± 11.3d	8.1 ± 1.8d	73 ± 2.6e	24 ± 0.6b	39 ± 1.7b	15 ± 2.2b
Partial removal of testa by chipping (S_2)	0	40 ± 14.2a	39 ± 14.4a	3.4 ± 2.7a	53 ± 7.1f	22 ± 1.0a	36 ± 3.2a	14 ± 2.2a
	25	43 ± 3.8ab	41 ± 2.0a	3.6 ± 1.4a	57 ± 6.7g	21 ± 0.5a	37 ± 5.2a	16 ± 5.6a
	50	44 ± 5.7ab	43 ± 6.8ab	3.8 ± 0.5a	59 ± 7.1gh	22 ± 0.6a	35 ± 5.4a	13 ± 5.3a
	75	47 ± 10.5b	44 ± 8.6ab	4.4 ± 2.0a	63 ± 4.8hi	22 ± 1.0a	34 ± 2.2a	12 ± 1.4a
	100	48 ± 7.3b	47 ± 6.8b	4.6 ± 1.3a	68 ± 5.1i	23 ± 0.5a	34 ± 2.3a	12 ± 2.3a

Data represents a mean of four replicates ± standard deviation

* Values in the same column and within a scarification treatment followed by the same letter do not differ significantly based on Duncan's Multiple Range test ($P < 0.05$).

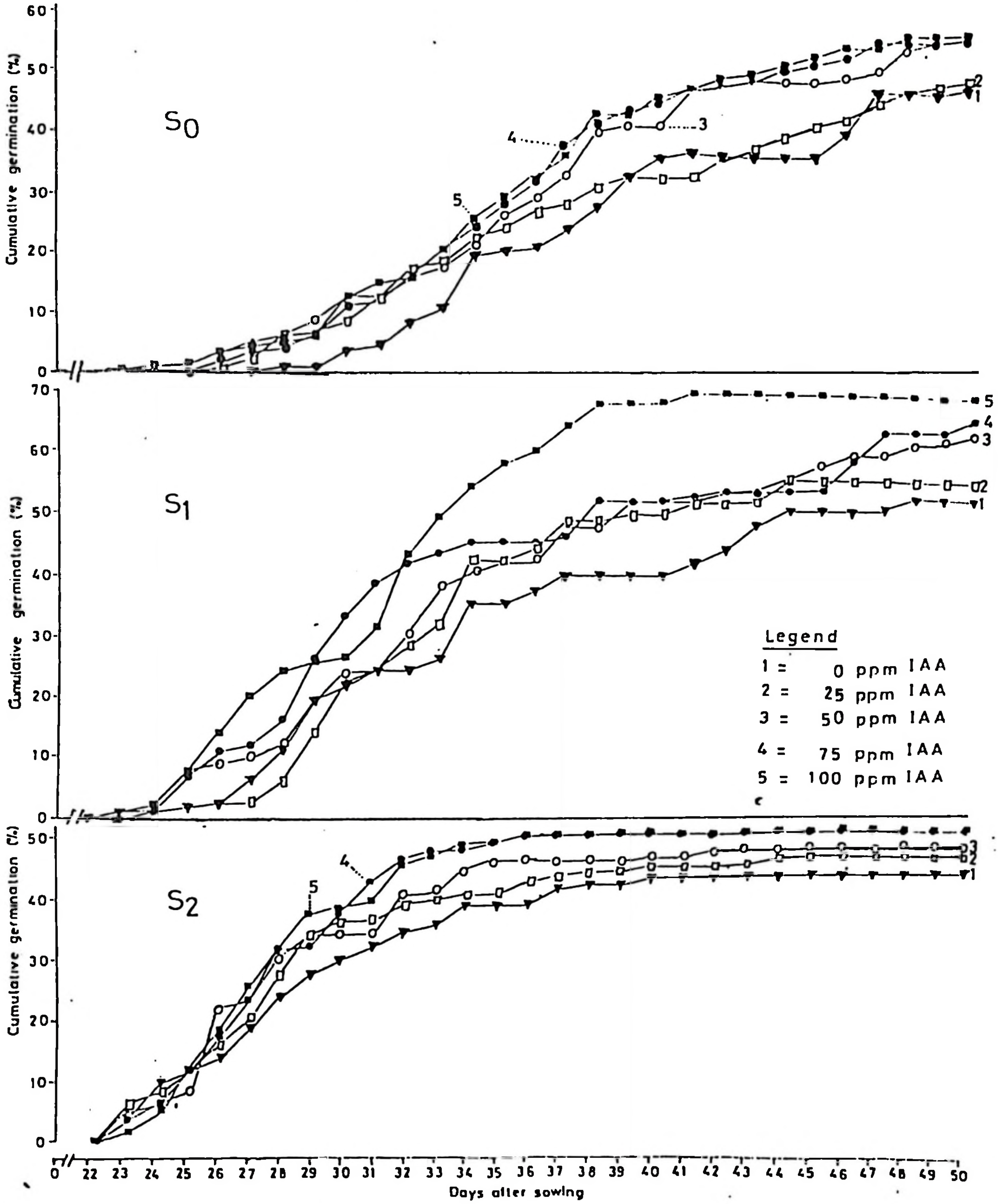

Fig. 21 : Effect of physical seed coat scarification (S_o - unscarified; S_1 - splitting part of testa; S_2 - partial removal of testa by chipping) and concentrations of IAA on cumulative germination of *Vangueria infausta* seed in the nursery.

significance only in the treatment involving partial splitting of the testa (S_1).

4.8.2.2 Germination value

Germination values (GV) for all treatments are illustrated in Figure 20. As for cumulative germination, GV was highest in seeds with treatments involving partial splitting of the testa (S_1), intermediate in unscarified seeds (S_0) and lowest in seeds with the testa partly removed by chipping (S_2) (Table 22). Application of 50 - 100 ppm IAA significantly increased GV in seeds involving partial splitting of the testa. Indole acetic acid had no significant effects on GV in other scarification treatments.

Regression analysis between GV and IAA concentrations showed a significance only in the treatment involving partial splitting of the testa (Table 23).

4.8.2.3 Height of seedlings

The initial height of the seedlings was influenced significantly by both seed scarification and IAA concentrations (Table 21). Splitting part of the testa (S_1) induced the highest initial growth and unscarified seeds (S_0) the lowest. For each scarification treatment, the height of the seedlings increased progressively with increasing IAA concentration. Application of IAA at concentrations of 50 - 100 ppm in unscarified treatments (S_0) and in treatments involving partial splitting of the testa (S_1) caused significant increases in

Table 23 : Relationships between nursery germination and IAA concentrations for *Vangueria infausta* seeds given different physical seed coat scarification treatments

Physical seed coat scarification	Germination energy (Arcsin $\sqrt{\%}$)	Germination value	Seedling height ($\log_{10}$ mm)
Unscarified (S_o)	$Y = 44.725 + 0.053x$ $R^2 = 0.117$ $F = 3.9$ NS	-	$Y = 1.704 + 0.001x$ $R^2 = 0.685$ $F = 39.2^{**}$
Splitting part of testa (S_1)	$Y = 45.805 + 0.101x$ $R^2 = 0.257$ $F = 6.2^{*}$	$Y = 4.797 + 0.034x$ $R^2 = 0.240$ $F = 5.7^{*}$	$Y = 1.175 + 0.001x$ $R^2 = 0.635$ $F = 31.3^{**}$
Partial removal of testa by chipping (S_2)	$Y = 38.585 + 0.045x$ $R^2 = 0.115$ $F = 2.3$ NS	-	$Y = 1.718 + 0.001x$ $R^2 = 0.459$ $F = 15.3^{**}$

Each regression is based on 20 data points

y = Parameter indicated

x = IAA concentration

* = Significant at $P < 0.05$

** = Significant at $P < 0.01$

NS = Not significant

height of seedlings. In the case of partial removal of the testa by chipping(S_2), a significant increase in height of the seedlings occurred at concentrations of 25 - 100 ppm IAA (Table 22).

Regression analysis of height of the seedlings on IAA concentrations showed highly significant relationships for all the studied treatments (Table 23).

4.8.2.4 Dormancy

Data on complete dormancy, total dormancy and differential dormancy periods are shown in Table 22. Analysis of variance showed seed scarification treatments to have highly significant effects on all dormancy periods, and IAA treatments to have no significant effects (Table 21). All dormancy periods were shortest in seeds whose testa was partly removed by chipping (S_2), intermediate in seeds involving partial splitting of the testa (S_1) and longest in unscarified seeds (S_o). The longest total dormancy period of 49 days was attained by unscarified seeds treated with 50 ppm IAA, while the shortest total dormancy period of 34 days was attained by seeds whose testa was partly removed by chipping and treated with 75 - 100 ppm IAA (Table 22).

4.8.3 Comparisons between laboratory and nursery germination results

In all scarification treatments, seeds sown in the nursery attained higher cumulative germination and germination energy than those sown in the laboratory (Tables 19 and 22). Generally, germination values were higher in seeds sown in the laboratory than those sown in the nursery except for a few

IAA treatments where the testa was partly removed by chipping (Figure 20).

In both tests cumulative germination, germination energy and germination value increased progressively with increasing in IAA concentrations and at 50 ppm IAA and above, a significant increase occurred.

In the laboratory, regression analysis showed significant relationships between these parameters and IAA concentrations (Table 20). While in the nursery the only significant relationship was observed for germination energy and germination value (Table 23).

In unscarified seeds (S_0) and seeds involving splitting part of testa (S_1) laboratory tests resulted in shorter dormancy periods compared to the nursery tests. In the case of treatments involving partial removal of testa by chipping of testa (S_2), dormancy periods were practically the same for laboratory and nursery tests (Tables 19 and 22).

CHAPTER 5

DISCUSSION

5.1 Seed morphology and structure

The aril and seed coat of Trichilia emetica seeds have a vital role in germination and survival under natural conditions. The colourful aril attracts monkeys and many species of birds. These animals eat the whole seed and others eat only the aril. Seeds which are undamaged following these animal encounters can germinate over time if suitable conditions for germination occur.

As the aril is light, it makes it possible for seed dispersal by run-off or in small streams. It is considered that this process provides scarification for the few seeds that germinate in nature. This may also explain in part the reasons why the species generally grows in riverine areas.

The extreme "hardness" of Vangueria infausta seeds posses the question on how these seeds are rendered permeable to water and gases in nature. It is possible however that the seed coat is cracked by rodents which eat the kernels. Those seeds which are cracked without killing the embryo can possibly germinate under suitable conditions.

The seed coat of Vangueria infausta seed may also be scarified by fires during the long spells of dry weather in the miombo woodlands where the species occur.

5.2 Seed moisture content and viability

Seeds of Trichilia emetica, like those of many tropical rain forest trees have a relatively high moisture content at seed fall. They are typical of recalcitrant seeds whose moisture content at this stage is 20 - 50% of fresh weight (Roberts, 1979). At room temperature Trichilia emetica seeds can be stored only for a short period (Shehaghilo, 1979). It is possible that this loss of viability is caused by the high moisture content of the seeds. Under natural conditions, the seeds must germinate within a few days. If conditions are however unsuitable for germination they lose viability and die. This characteristic is common among members of the Meliaceae family and especially among Azadirachta, Cedrela, Entandophragma, Khaya and Lovoa species (Ezumah, 1986).

The relatively high moisture content of Trichilia emetica seed correlates with the fact that the species is relatively more abundant in wet riverine - alluvial soils in lowland tropical rain forests. It is absent in miombo and dry screlophylous thickets. The species has also adapted itself to produce seeds during the rain season when conditions are cundusive for immediate germination (high humidity and temperature).

In contrast, the relatively low moisture content of Vangueria infausta seeds at ripening may be an adaptation to the environmental conditions where it grows naturally. During the dry season, ripe fruits dry up and are persistant on the tree for the whole season without the seeds inside losing their viability.

5.3 Seed germination in Trichilia emetica

The germination characteristics in Trichilia emetica seeds is illustrated in Figure 6. The rapid growth of the primary and lateral roots ensures supplies of water and nutrients to the developing seedling. The hypocotyl and cotyledons also start to photosynthesise very early so that the seedling becomes established before the onset of dry weather.

There are some conflicting views of how to obtain the best germination of Trichilia emetica seed. Watkins (1960) finds that the germination is fair and that it is advisable to remove the aril from the base of the seeds. On the other hand, Forest Division (1984) considers that Trichilia emetica seeds need no pretreatment. The latter findings are difficult to compare with the present findings as no germination figures are given.

In the present study, it was found that seed germination was very poor when seeds were untreated. Significant germination improvements occured following physical seed coat scarification involving partial removal of seed coat or complete removal of seed coat. This may be attributed mainly to the impermeability of the aril and seed coats to fluids.The exchange of gases through the hilum as in normal seeds (Hyde, 1954; Ballard et al., 1976) is not possible for Trichilia emetica seed as the hilum is concealed completely by the aril.

The results of the present study supports those obtained from earlier studies in other species with similar seed coat dormancy problem. In Santalum album the seed coat was either less permeable to water or possesed some inhibitory principle making the seed liable to poor germination (Nagaveni and Srimathi, 1981). Inhibitory chemical in the seed coat or aril may not be the cause of poor and delayed germination in Trichilia emetica seed because even seeds which received only partial scarification germinated satisfactorily in the presence of the remaining part of the aril and seed coat. In Erythrina caffra (Small et al., 1977) and Cucumis melo (Pesis and Ng, 1986) the seed coat acted as barriers to oxygen uptake. In both cases stimulation of germination was obtained by partial or complete removal of the seed coat. This might also be as in the case with Trichilia emetica.

Little or no stimulation of germination of Trichilia emetica seeds was brought about by application of gibberellic acid (GA_3) in both laboratory and nursery experiments. This suggests that scarification alone is necessary to overcome dormancy in these seeds. In fact, very high germination figures were obtained in seeds where the seed coat was completely removed and not treated with GA_3. The non significant effects of GA_3 on dormancy periods in all scarification treatments also suggest that application of GA_3 is unnecessary in germinating seeds of Trichilia emetica.

The seedlings which emerged from GA_3 treated seeds were slender and weak. This is suggestive that the longitudinal growth of the seedlings was extended at the expense of strength. Consequently such GA_3 treated seeds produced abnormal seedlings. Since gibberellins stimulate cell division and elongation (Jones and Phillips, 1966 ; Cohen and Kende, 1986; MacDonald et al., 1986), application of GA_3 may have created excessive quantities of the chemical within the germinating seedling.

Generally potassium nitrate (KNO_3) promotes seed germination in a number of species (Ogawara and Ono, 1961; ISTA, 1985). In these studies however, this did not happen when Trichilia emetica seeds were soaked in the chemical. Similar results have also been reported for many tree species (AOSA, 1955; Msanga and Maghembe, 1986; Curran and McCarthy, 1986).

It is possible that the adverse effects related to concentrations of KNO_3 higher than 0.2% were due to high osmotic pressure created by the chemical in the substrate. It has been reported that high concentrations of solutes increase osmotic pressure of the germination solutions and make imbibitions difficult. Such solutes therefore retard germination in many plant species (Rodgers et al., 1959; Bonner, 1968).

In the present study the concentrations of KNO_3 in the substrate did not affect the average height of seedlings. This may have been so because both KNO_3 treated and untreated seeds took nearly the same period to start and complete their germination. Consequently the seedlings attained nearly the same average seedling height in both untreated and KNO_3 treated seeds although relatively fewer seedlings emerged from the KNO_3 treated seeds.

5.4 Seed germination in Vangueria infausta

The germination characteristics in Vangueria infausta seed as illustrated in Figure 15 suggest that radicle elongation is preceded by cotyledon expansion. This expansion of the cotyledons enables the radicle to emerge easily from within the seed coat. In Vangueria infausta the young seedling can establish itself by obtaining food from three sources: the endosperm, cotyledons and through photosynthesis. This ensures that the radicle develops into a stout vigorous taproot and form lateral roots ready to tap water and nutrients from the germination medium within a short period. This feature is important for survival in dry areas.

Detailed germination studies demonstrated that much of seed coat of Vangueria infausta seed was impermeable to water and gases and that these fluids could only penetrate the embryo very slowly through the hilum. The swelling embryo in turn built up pressure gradually on the inside of the seed coat and eventually cracked it and released the developing radicle. It was apparent therefore that delayed germination in Vangueria infausta seed was caused by seed coat dormancy due to mechanical obstruction of the seed coat. Unlike for Trichilia emetica, therefore, dormancy in Vangueria infausta was not due to impermeability of the seed coat. However, in both species the delay in germination can be accounted for by the extra time required by the seeds to imbibe water for the growth phase of germination to begin.

The results showed that partial removal of the testa at the radicle end by abrasion could overcome the mechanical resistance to the embryo growth and increase water uptake. This in turn promotes germination. The results for this pre-treatment agree with that reported by Ahmad (1978) for seeds of Dialium mainganji and Sindora coriaceae which also have hard seed coats. The results further showed that splitting the testa at the radicle and or partial removal of testa by chipping was harmful to the seed. The portion of the embryo which was exposed after chipping swelled more than the rest of the seed enclosed in the seed coat. This differential swelling caused the embryo to fracture along the line bordering the cut edges of the seed coat and contributed to death in the majority of seeds before germination.

Seeds with complete removal of the testa swelled upon imbibition of water until the endosperm got fractured and bursted. The poor germination in seeds that received this treatment was therefore due to physical injury in addition to a probable loss of nutrient materials and hormones from the endosperm and cotyledons through the fractures. Previous work done on other seeds such as Pisum sativum (Powell and Matthews,1978; 1980) has shown that the existence of cracks in the seed coat leads to rapid water uptake. This causes death of cells of the cotyledons and results in high levels of solute leakage and leads to poor field emergence. This so-called imbibition damage (Powell and Matthews, 1978) has also been observed by Oliveira et al. (1984) in the embryo of Glycine max. The poor germination results attained by seeds of Vangueria infausta with splitted, chipped or completely removed testa suggest that imbibition damage could be a major cause of poor seed germination as it is in Pisum sativum and Glycine max.

Complete removal of testa did not only give poor germination but also the seedlings that emerged from seeds with complete removal of testa exhibited poor initial growth. Poor initial growth has also been seen as a consequence of imbibition damage in Pisum sativum seeds (Powell and Matthews, 1978) and in Glycine max (Obendorf and Hobbs, 1970). These workers explained this observation in terms of reduced mobilization of food reserves from the cotyledons to the axis when cotyledonary cells had been damaged by rapid imbibition. This might also be the explanation for the reduced seedling growth reported in the present work.

In the present study the optimum concentration of hydrogen peroxide (H_2O_2) required to stimulate germination in Vangueria infausta seed was 6%. It can be postulated that at concentrations above this level, the rate of respiration and oxidation of reserve materials (protein, fats and carbohydrates) in the endosperm and embryo was so rapid that the products were not fully mobilized for the nutrition of the developing embryo. This assumption explains the decline in germination when the concentration of $H_2 0_2$ was increased above 6%.

The significant increase in dormancy periods by application of $H_2 0_2$ in seeds which were sown in the laboratory was a sign of disinfectant properties of the $H_2 0_2$. Seeds which were treated with $H_2 0_2$ stayed for a relatively longer time before they started to rot compared to untreated seeds. The $H_2 0_2$ treated seeds maintained viability until germination started. Most seeds which were untreated with $H_2 0_2$ attained shorter dormancy periods not because they germinated faster, but because they died immediately after sowing. This is in agreement with observations of Riffle and Springfield (1968) in their studies on the effect of $H_2 0_2$ on microflora on seed of woody species.

The positive influence of indole acetic acid (IAA) which was observed in the germination percentages was probably associated with extension of growth in the developing embryo after germination had started and not the actual involvement in seed germination. This view is supported by the fact that IAA had no influence on dormancy periods. Earlier reports on the effect of IAA on seed germination are not consistent and infact, some are contradictory (Van Staden et al., 1972 ; Ferreira and Small, 1974; Patil and Ballal, 1974). However, various authors have confirmed that IAA activities include both stimulation (principally cell elongation) and inhibition of growth. Detailed observations have demonstrated that individual cells may exhibit opposite responses depending on the concentration of IAA (Scott and Briggs, 1960; Atmans and Wareing, 1975; Oppenoorth, 1978; Friedman et al., 1979).

Generally, the present findings show that the controlled conditions in the incubator were more suitable for germination compared to those in the nursery.

The effects of physical seed coat scarification and H_2O_2 treatments on height of seedlings followed nearly the same trends as for germination value (GV). The manifestation of the GV results on the height of seedlings shows how GV can be a powerful tool in assessing germination.

The effect of IAA was more pronounced in the height of seedlings compared to germination percentages and GV. These results again indicate that IAA stimulated growth in the developing seedling after germination had started and not the actual involvement in seed germination.

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