THE UNIVERSITY OF ALBERTA

The Control of Germination in *Melia volkensii* Seeds

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled The Control of Germination in *Melia volkensii* Seeds submitted by Patrick B. W. Milimo in partial fulfilment of the requirements for the degree of Master of Science.

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ABSTRACT

Seeds of *Melia volkensii* Gurke. do not germinate when placed under favourable conditions of moisture, air and warm temperature. To study factors which cause and maintain seed dormancy, and conditions that lead to its release, three studies were carried out. These were: (1) to describe flower and fruit external morphology and structure of the ovule; (2) to classify the dormancy type and prescribe seed germination treatments that might break it, and; (3) to propose dormancy mechanisms by which integuments, perisperm and endosperm operate.

Seeds of *M.volkensii* mature in 11 to 13 months, but phases of fruit development lack a seasonal pattern. Change in fruit colour from green to yellowish-green appeared to be the most practical indicator of seed maturity.

Lignification and cutinization of integuments, and their growth to form a caruncle starts early in ontogeny, but major structural changes like deposition of nutrient reserves in the embryo and endosperm, persistence and crushing of the nucellus in mature seed, the thickening of endosperm cell-walls that characterize mature seeds do not occur until late in ontogeny. The embryos and endosperm main constituents are crude fat (54% and 49%, respectively) and crude proteins (35% and 26%, respectively). Integuments are mainly composed of cutin and lignin (60%). These changes appear to play a leading role in establishing and maintaining the state of dormancy.

Seeds with their integuments, perisperm and endosperm removed reached 81% germination, compared to only 9% in intact seeds. The few intact seeds that could germinate probably did so becuase they had small cracks in the seed coats which were not detected on X-rays. These cracks may have developed during seed extraction because the seed coat is quite brittle.

Germination started after seeds with damaged integuments absorbed over 60% water (dry weight basis). Intact seeds can absorb 48% water but most of this is held in the seed coats. Only 15% of this water is taken up by the embryo tissues themselves. This shows that integuments, perisperm and endosperm are not completely impermeable to water, but that they limit rate and amount of water absorbed by the embryo. The endosperm, and/or perisperm seem to be the tissues involved in limiting the amount of water absorbed by the embryo. Consequently, one way in which integuments, perisperm and endosperm inhibit germination is by not allowing the embryo to absorb sufficient water for the start of cell division.

Seeds with damaged integuments could germinate in elevated CO_2 (0.65%) and depressed O_2 (15%), but seeds in 50% O_2 germinated only to 50% while controls (21% O_2) reached 82%. Therefore, gaseous inhibition of germination seems insufficient to account for the failure of intact seeds to germinate.

Normal germination was induced when integuments, perisperm and endosperm were cut longitudinally at the micropylar end, but when cut horizontally in the centre, or longitudinally at the chalazal end, they germinated abnormally. Radicles tended to get trapped in the seed coats. This suggests that the permeability of integuments, perisperm and endosperm to water or gases alone may not account adequately for dormancy in the species. Therefore, in addition to limiting the amount of water absorbed by the embryo, they also restrict radicle protrusion mechanically.

To account for the germination behaviour of seeds subjected to various integuments, perisperm and endosperm treatments and experimental conditions, three mechanisms are proposed: (1) that the endosperm, and/or perisperm do not allow the embryo to absorb adequate water for cell division to start; (2) that the embryo does not acquire sufficient imbibition pressure to break mechanical restraining action of the integuments and/or; (3) that in nature, a mechanical weakening of the integuments is needed in addition to the removal of the perisperm effects, in order that embryos be able to absorb enough water to start cell division.

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1. INTRODUCTION

Kenya has a total area of 580,000 Km² of which 11,200 km² is covered by water. Two thirds of the dry land is semi-arid to arid (Pratt et al. 1966). More than 85% of Kenya's 16 million people live in rural areas, generally concentrated in the fertile high-rainfall regions. The high population density in these areas cannot sustain more growth, and as a result there has been a steady migration to the less densely populated semi-arid areas. The semi-arid areas currently have 20% to 25% of Kenya's population (Midgley 1983; Heuveldop et al. 1982). Vegetation clearing, to create space for agriculture, and overgrazing have diminished the land's crop and animal carrying potential. This has raised public concern, and as a result, a number of reforestation projects have been started to raise, distribute and encourage farmers to plant multi-purpose trees (Buck 1983).

Kenya Forest Department's activities are mainly centered around fast growing exotic plantation species (Midgley 1983). Lack of basic data on which to base management decisions have in the past made most indigenous tree species less favourable for reforestation than exotic tree species(Zumer-Linder 1983). Yet, most of the exotic species are unsuitable for reforestation of semi-arid and arid areas. As a result, reforestation of marginal areas pose a great challenge because in addition to growth rates and suitability of the species to the perceived end uses,

plants must also be tolerant of xeric conditions. *Melia volkensii* Gurke. is such a plant.

The genus *Melia* belongs to the family Meliaceae. There are eight species in this genus, and most of them are indigenous to South Asia, VietNam and Australia. Among them, *M. azedarach* L. has been most widely introduced to other parts of the tropics (Moore 1981). In Africa, apart from *M. azedarach*, which is exotic (Dale and Greenway 1961), *M. volkensii* and *M. bombolo* Melw. are the only known members of the genus recognized as truly indigenous. The former is native to East Africa (Dale and Greenway 1961), and the latter is indigenous to Angola (Mabberley 1984).

M. volkensii is a prized species of semi-arid zones east of the Kenya highlands. It coppices readily, is fast growing, and it sheds its leaves in the dry season to provide mulch (Teel 1985). *M. volkensii's* timber closely resembles that of the African Mahogany (*Khaya anthotheca* (Welw.) C. DC.), which is easy to work, durable and strong (Dale and Greenway 1961). Its large fruits, twigs and leaves make fodder for goats, cattle and sheep (Kenya Forest Research Institute, unpublished records). The species is also among the five most important forage plants for giraffe in Tsavo East National Park, Kenya. It contributes about 2% and 9% forage respectively during the dry and wet seasons (Leuthold and Leuthold 1972). From fruits of its close relatives (*Azadirachta indica* (A. Juss) and *M. azedarach* (L.)) three commercially important chemical compounds have

been isolated. These are: azidirachtin (Butterworth and Morgan 1971), salanin (Hederson et al., 1964) and meliantriol (Levie et al. 1967); The fruits of *M.volkensii* also contain a locust anti-feedant which could be of economic value (Mwangi 1982).

For over 30 years the Kenya Forest Department has attempted to incorporate *M. volkensii* in dry-land reforestation projects, but with no success. Much of the failure is attributed to lack of knowledge on how to propagate the species. Some of the seed pre-germination treatments which have been tested include: endocarp scarification with acids, stratification of seeds, shading of seed-beds and variable watering (Kenya Forest Research Istitute, unpublished records).

Despite the many years of interest, no valid seed pre-germination treatment technique has yet been prescribed (Kenya Forest Research Istitute, unpublished records).

A variety of possible seed dormancy types exist (Nikolaeva 1969). Since at the beginning of this study little was known about how to germinate these seeds, several preliminary studies were carried out to try to determine what types of seed dormancy were involved. The results indicated that integuments, perisperm, endosperm, or all of them may be responsible. That is why only dormancy related to these tissues are addressed in this study.

1.1 STUDY OBJECTIVES

In order to determine and prescribe seed treatments, it became necessary to (1) describe the external morphology of flowers and fruits and relate this to structural and histochemical features of the ovule; (2) classify the dormancy type operating in the species and the techniques for breaking it. These broad objectives include the following specific objectives:

Part I: The objective for phenological study is:

1. To describe external morphological characteristics of mature fruits and seeds that may guide seed collection. The aim of this study was to develop a feel for the correlations between germination morphology and dormancy.

Part II: The objectives for dormancy type classification are:

1. To determine whether integuments, perisperm and/or endosperm or factors within embryos are responsible for germination inhibition.
2. To determine whether integuments, perisperm and/or endosperm are permeable to water and to note the part played by various tissues in the process of water uptake.
3. To determine whether elevated carbon dioxide and depressed oxygen levels affect germination. (This is an indirect test to determine what may happen if the integuments, perisperm and/or endosperms were

impermeable to gases).

4. To determine the effect of cut location in integuments, perisperm and endosperm on the mode of seed germination.
5. To determine an optimum temperature for germination, upon which to base other germination tests.

Part III: The objectives of seed structure, histochemistry and chemical composition analyses investigations are:

1. To determine relative proportions of chemical compounds in different seed tissues and to relate these to their known mechanical or other influences on germination.
2. To describe the structure, organization and localization of cellular material of the ovule.

2. LITERATURE REVIEW

All seeds develop from fertilized ovules consisting of an embryo surrounded by dispersal units (Mayer and Poljakoff-Mayber 1982). Integuments serve to protect the developing ovule by insuring that compressive forces do not affect it. They also protect ovules from drying, predation and other kinds of damages (Esau 1977). Nutritive tissues inside the integuments consist of the endosperm and nucellus. Sometimes, these are also involved in controlling germination by restricting it to periods and conditions most favourable for seedling growth. Thus, they may impose dormancy on the seed as a dispersal unit for the species (Villiers 1972). Such adaptations lead to better survival of the species under difficult environmental conditions (Stebbins 1970, 1971 and Harper 1977).

Dormancy in plants has been defined as "a state in which viable seeds, spores or buds fail to germinate under conditions of moisture, temperature and oxygen favourable for vegetative growth" (Amen 1968). Dormancy in the life cycle of a plant is a necessary adaptation to changing environments (Harper 1977). Plants may withstand adverse seasonal conditions by their ability to perceive and interpret environmental signals. Such dormancy allows seeds to synchronize their life processes with the changing seasons (Villiers 1975).

But the term dormancy is vague and often misleading because it is often used indiscriminately and it lacks

resolution (Nikolaeva 1969). Many environmental and internal factors induce dormancy in seeds (Nikolaeva 1969, Werker 1980). Conditions that break it are equally complex (Nikolaeva 1969). Therefore, to investigate dormancy as a natural phenomenon, it is necessary to start by bringing the available factors into some kind of order by classification, so that common properties and inter-relationships may be seen (Villiers 1975). In some species dormancy may develop at the time of fertilization (Koller et al. 1962), in others seeds acquire dormancy during development or have dormancy thrust upon them (Harper 1957). The first kind, called innate or primary dormancy, describes the dormancy of the seeds at the time of dispersal or harvest; the second type is said to be induced or secondary dormancy, because it is caused by some experience of the seed after ripening, and; the third kind is called enforced dormancy or "quiescence" (Bewley and Black 1982). It implies the arrest or retardation of metabolism and growth due to unfavourable environmental influences. Quiescent seeds will normally germinate when placed under aerated, moist and warm temperature conditions (Nikolaeva 1969, Villiers 1975). In the broadest sense, seeds with innate and induced dormancy will not germinate, even when warm temperatures and adequate water and aeration are supplied. Some special set of conditions has to be experienced before germination can proceed (Villiers 1972, 1975).

Seed dormancy was classified by Crocker (1916) who made a distinction between dormancy or dormancies related to structures external to the embryo (exogenous) and dormancy related to the properties of the embryo itself (endogenous). Removal or injury of integuments, perisperm and endosperm usually permits germination to proceed in seeds with exogenous dormancy. Such dormancy is said to be imposed (Werker 1980). Endogenous dormancy, however, does not allow even excised embryos to germinate (Nikolaeva 1969).

Inhibition of germination by integuments is classified as direct or indirect. The direct inhibition of germination implies: (1) impermeability to water; (2) impermeability to O_2, and; (3) mechanical barrier to radicle protrusion (Crocker 1916; Crocker and Davis 1914; Ballard 1973; Barton 1965). The indirect inhibition of germination implies: (4) impermeability to leakage of germination inhibitors from the embryo (Evenari 1949; Wareing and Foda 1957; Wareing 1965; Maguire 1976, 1977 and 1980); (5) impermeability to CO_2 diffusion from the embryo to the outside, thus narcotizing the embryo (Kidd and West 1917); (6) lack of germination due to inhibitors located in integuments, perisperm and endosperm; (7) integuments, perisperm and/or endosperm acting as light filters, and; (8) osmotic inhibition caused by a hydrostatic pressure resulting from large osmotic potentials in integuments (Evenari 1949, Koller 1955a 1955b and 1957), or a combination of one or more of these, including some form of endogenous dormancy. These mechanisms

may be located in various layers of integuments, perisperm and endosperm either all acting together or separately (Nikolaeva 1969).

As the ovule matures into a seed it usually undergoes changes in form, structure and histochemistry (Singh 1964). Literature on seed dormancy caused by integuments, perisperm and endosperm is well documented (Werker 1980), but information concerning the relationship between histochemistry and the germination physiology is lacking for most species.

Studies on the morphology of the family Meliaceae have been carried out by Narashimhanchar (1936), Nair (1956), Wiger (1936), and; on the genus within the family by Juliano (1934a and 1934b), Nair 1959a and b), Garudamma (1956, 1957), Narayana (1958), Nair and Kanta (1961) and Gavde (1963). From these studies, it appears: (1) that seed structure of *M. volkensii* has not been studied; (2) that detailed study of the specialized areas near the micropyle and chalaza were not included in the work done to date. The structure of these parts has been of great importance in interpreting physiological modes of germination in cotton seed (Simpson 1940) and may be equally important in *M. volkensii*. Against this background it became clear that seed studies must follow a developmental sequence.

3. MATERIALS AND METHODS

Field observations of buds, flowers and fruits were carried out from May to August, 1984. Seed scarification and chemical analyses of seed tissues were carried out in October 1985. Seed tissue structure and histochemistry was studied between March 1985 and April 1986.

3.1 PHENOLOGY

The study of phenology commenced with a reconnaissance of Kenyan districts where the tree grows naturally[1]. Existing bud, flower and fruit phases of development were identified and classified according to Todorov's (1977a, 1977b, and 1982) guidelines. Machakos district was chosen for detailed work (three sites: Kinyambo, Kalulini and Kibwezi Forestry Research station were selected) on the basis of their proximity to Nairobi. Six trees, two per site, were selected and twenty plastic labels used to tag phases of fruit development. Classification was based on visual inspection, but sometimes a hand lens (x10) was used. Also, fruits were cut radially to determine pericarp and stony endocarp hardness. Visual observations included the texture and structure of fruits and seeds; the degree of cork deposition and colour of the fruit surface; length and width of the fruits using hand calipers and the distance from the fruit stock attachment on the branch to the branch tip. Phases of development recorded were vegetative buds,

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[1] The Machakos, Kitui, Taita, Kilifi, Embu and Meru.

reproductive buds, extended and unopened flowers, and senescent flowers. Bud and flower observations were made every other day and fruits were monitored once each month.

3.2 SEED GERMINATION TESTS

3.2.1 SEED SOURCE

Only processed stony endocarps collected from 18 trees at Kibwezi, Kenya in August 1985 and mixed randomly were flown to Edmonton, Canada to be used in germination tests. The selection, collection and processing of the fruits and stony endocarps followed local Kenyan Forestry traditions and were beyond the control of the author. As a result findings in this study refer only to seeds of 18 trees.

3.2.2 SEED GERMINATION: A STANDARD PROCEDURE

The following is a germination procedure that I used in all seed germination tests. Deviations from this standard procedure where applicable are outlined under the specific sections.

The stony endocarps were washed in cold tap water immediately on arrival, in order to remove a copper sulphate fungicide. They were then oven-dried at 25°C for one week. Dried stony endocarps were stored in brown paper bags at about 23°C. Seeds were extracted two to three days prior to the start of germination tests. Extraction involved cracking the stony endocarps (Figures 4 and 5) using a carpenter's

hammer, a pocket knife and sometimes a 3" portable vise. Mechanically damaged seeds were eliminated. Seeds were then randomly divided into four replicates of 25 seeds. Each was X-rayed on a Kodak x-omat TL film (Health Science Markets Division, N.Y.), at 15 KV, 5 mA for 30 seconds in an X-raying chamber (Model M110NH, TF1 Corp-CT), at a distance of about 47 cm from the energy source. By examining the X-ray negatives, seeds with incomplete cotyledons, undeveloped embryos and internally damaged seeds (probably arising during extraction) were excluded in the computation of per cent total germination, as suggested by Muller et al. (1956). Seeds were soaked in water for six hours in a Conviron Growth Chamber (Model G30) set at a constant 30°C, then cut where applicable[2] longitudinally at the micropylar end.

Cut seeds were germinated on moist Kimpak, covered with moist Scott singlefold paper towels (23.9 cm by 26.9 cm) in transparent polystyrene germination boxes. The germination boxes (28.5 cm by 24.5 cm by 7.5 cm) had two 0.5 cm diameter holes mid-way along each of the longer sides. Germination tests were carried out over a period of 14 days in growth chambers set for equal days and nights. Seedlings having a radicle equal or greater than the length of the seed were counted as germinated. Germination was counted each day at the same time and counted seedlings discarded. Total

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[2]A cut is defined as a longitudinal slit through the integuments, the perisperm and the endosperm at the micropylar end of the seed.

germination and germinative rates were computed[3].

3.2.3 SEED GERMINATION TEMPERATURE

The aim was to determine an optimum temperature for germination of *M. volkensii* seeds. The effect of both constant and alternating temperatures were investigated. The procedure was the same as in the standard apart from the fact that seeds were not covered with Paper towels. The five alternating and constant temperatures regimes tested were 25°C days and 22°C nights, 27°C, 32°C, 37°C and 42°C days and 25°C nights; and 25°C, 27°C, 32°C, 37°C and 42°C ± 1°C days and nights. Days and nights were 12 hours each.

High variability in total germination and germinative rates for the temperature tests led to tests where seeds were covered with paper towels. Fluctuations in relative humidity (RH) was suspected to be the cause of the problem. The aim was to determine whether covering seeds, hence stabilizing RH, could reduce variation within replicates. Treatments involved covering four replicates and leaving the other four uncovered.

3.2.4 EMBRYO EXCISION

The hypothesis tested was that excised embryos fail to germinate when placed under favourable moisture, air and warm temperature. The aim was to classify seed dormancy in

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[3] Germinative rate is defined as: the time needed to reach 50% of the total germination of a sample. It was computed in days for each replicate by linear interpolation of the germination curves.

the species. The procedure was the same as in the standard outlined in section 3.2.2.

3.2.5 WHOLE SEEDS AND MOISTURE IMBIBITION

A quantitative study of the course of water absorption by seeds whose integuments, perisperm and endosperm had been variously treated was carried out in order to determine: (1) if intact seeds take up water; (2) if integuments, perisperm and the endosperm separately, or collectively, influence the total amount and rate of water uptake, and; (3) the relative proportion of water absorbed by various seed parts. Fifteen seeds were divided into three groups of five seeds. Seeds in Group One were cut longitudinally at the micropylar end. Those in Group Two had caruncles[4] broken off so as to expose the perisperm and the endosperm at the micropylar end of the seed, and those in Group Three were left intact. Seeds were placed in germination boxes on moist peat moss in a Conviron germinator, set at a constant 30°C. At 12 hour intervals seeds were removed, excess water on the surface drained on kleenex paper tissue, and their weight determined on a Sartorius-Werke balance (Model GMBH, Type 2442 SH). Weighing of one sample took about two minutes from the time the lid of the germination box was removed to when it was returned.

$$\% \text{ M.C.} = \frac{\text{sample weight} - \text{sample oven dry weight}}{\text{sample oven dry weight}} \times 100$$

The experiment was stopped at 144 hours because seeds

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[4]The growth of integuments into a protuberance at the micropylar end of the seed; Esau (1977); Maheshwari (1950).

started to rot. Average gain in fresh weight was then plotted against time. The formula presented above was used to compute per cent gain in fresh weight:

3.2.6 SEED PARTS AND MOISTURE IMBIBITION

Two experiments were carried out. They were: (1) to determine the relative per cent dry weight of different mature seed parts, and; (2) to determine the relationship between dry weight of separate seed tissue and their water absorption. Seeds were divided into seven replicates of five seeds and soaked in water for about three hours. Imbibed seeds were then separated into their constituent parts and dried in a Napko oven (Model 420) at 40°C for 24 hours. To follow gain in fresh weight, seed parts were immersed in tap water in 100 ml conical flasks, and placed in a growth chamber for 24 hours. Per cent increase in fresh weight was then determined by weighing.

3.2.7 OXYGEN CONCENTRATION AND SEED GERMINATION

The effect of various gas concentrations on the germination of cut seeds was investigated. The four oxygen concentrations (by volume) used were: (a) 15% oxygen, 0.65% carbon dioxide and balanced nitrogen; (b) industrial air, or 21% oxygen, and balanced nitrogen (control); (c) 25% oxygen and balanced nitrogen, and; (d) 50% oxygen and balanced nitrogen.

Cut seeds were set to germinate in plexiglass chambers. Each replicate was placed in a single row starting from the inlet to the outlet end. Different gas concentration treatments were investigated in separate growth chambers.

Plexiglass chambers[5] internally measured 50 cm by 35 cm by 7.5 cm, and were fitted with a top. Seams were lined with rubber to ensure air-tight conditions within the chambers. Chamber tops were secured in place by 16 bolts and nuts.

Gas was regulated to flow at a rate of 0.05 l/minute, and was passed through a humidifier before entering the plexiglass chambers. The chambers were opened once daily for a period of about eight minutes, when germinants were counted. The gas flow rate was then re-adjusted to 0.1 l/minute for 30 minutes after which it was set at 0.05 l/minute.

3.2.8 CUT LOCATION AND SEED GERMINATION

The effect of seed-cut location on total germination, germinative rate and mode of germination was investigated. One hundred seeds were sorted by hand into four groups. Group A was cut longitudinally at the micropylar end, Group B was cut horizontally midway between the micropylar and chalazal ends, Group C was cut longitudinally at the chalazal end, and Group D was cut from end to end (Figure 15). Groups A, B and C seeds were approximately 5-10 mm

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[5]Chambers were made by Dr. M. Spencer, Dept. of Plant Science, University of Alberta and lent to me for the duration of my tests.

long, the length of the cut in Group D was depended on the length of the seed. Germination was evaluated and recorded either as normal or abnormal.

3.3 CHEMICAL COMPOSITION OF SEED PARTS

Relative proportions of crude fat (fatty acids), crude proteins (amino acids), cellulose, hemicellulose, cutin and lignin in different seed tissues were quantified. Procedures in this section followed those by Goering and Van Soest (1970).

3.3.1 SAMPLE PREPARATION

Separated seed parts were dried in the oven at 60°C for 24 hours, then ground in a mortar to pass through a 20-30 mesh and stored in vials at about 23°C.

3.3.2 DRY MATTER DETERMINATION

Two grams of freshly ground tissues were weighed in duplicates on a Mettler HK160 balance in aluminum pans and then dried in the oven at 110°C for 4 hours. Per cent dry matter was computed using the formula presented below:

$$(\%)\ \text{Dry matter} = \frac{(\text{wt. dry sample + pan}) - (\text{wt. of pan})}{(\text{wt. wet sample + pan}) - (\text{wt. of pan})} \times 100$$

3.3.3 CRUDE FAT DETERMINATION

Duplicate samples weighing 2.00 g were placed on filter paper, crushed gently in a mortar, and put into numbered thimbles. These were then placed into metallic containers which fitted onto a Goldfish extraction apparatus. Weighed and numbered extraction beakers, containing 40 ml petroleum ether, were fixed onto the extraction apparatus with the water condenser and heat turned on for 14 hours. Petroleum ether was evaporated, the extraction beakers dried in the oven at 100°C for 30 minutes and then weighed. A blank was run by weighing the residue after evaporation of 40 ml of petroleum ether without samples. Per cent crude fat was computed using the following formula:

$$(\%)\ \text{Fat} = \frac{(\text{wt. of beaker + fat}) - (\text{wt. of beaker})}{\text{wt. of fat}} \times 100$$

Constituent fatty acids were determined on a gas chromatograph.

3.3.4 CRUDE PROTEIN DETERMINATION

The Kjeldahl method was used. Duplicate samples were weighed between 1.5 - 2.0 gm in tared plastic containers (weighing < 1 gm). They were folded and dropped into 800 ml Kjeldahl flasks. A blank was run by leaving out the sample. One catalyst package (containing 9.9 gm K_2SO_4, 0.41 gm HgO, and 0.08 gm $CuSO_4$) and 30 ml conc. H_2SO_4 were added to each flask. Samples were digested clear (≃ 30 minutes), cooled, and then 300 ml tap water, 1g zinc metal (20 mesh) and 110

ml of 40% NaOH were added. Flasks were swirled and ammonium distilled into 500 ml Erlenmeyer flasks containing 50 ml aliquots of 4% boric acid solution placed under delivery tubes on the distillation rack. Distillation went on until the Erlenmeyer flasks had collected 200 - 250 mls of ammonium. This was titrated against 0.1067M H_2SO_4 and the amount of nitrogen in the samples determined by the following formula:

$$(\%)\ N = \frac{H_2SO_4 - \text{Blank}}{\text{wt. of sample (gm)}} \times \frac{14\ \text{mg}}{\text{mmole}} \times \frac{NH_2SO_4}{1} \times \frac{100\%}{1} \times \frac{1\ \text{gm}}{1000\ \text{mg}}$$

Per cent nitrogen was converted to percent protein by the formula:

$$\% \text{ Proteins} = \% \text{ N} \times 6.25$$

Acid hydrolysis of proteins for amino acid analysis was done by refluxing samples with 6N HCl for 24 hours. This method is a compromise between obtaining complete hydrolysis of proteins and destruction of some of the amino acids. Analysis of amino acids was done on a Beckman 121MB Amino Acid Analyser.

3.3.5 NEUTRAL DETERGENT FIBRE (NDF)

This procedure analyses total fibre in vegetable feed stuffs. It divides dry matter near the point that separates the soluble constituents from those that are incompletely insoluble and dependent on a microbial fermentation.

Triplicate air-dried, samples between 0.5 and 1.0 g were weighed into beakers of the refluxing apparatus. Neutral detergent solution at room temperature (100 ml), 0.5 g $NaSO_3$ was added and then heated to the boiling point on the refluxing apparatus in 5-10 minutes. Boiling was adjusted to an even level in order to avoid foaming, and refluxed for 60 minutes and timed from onset of boiling. The fibres were then filtered in previously tared and weighed 600 Gooch crucibles on a filter manifold, and a low vacuum used. Samples were rinsed in crucibles, with hot water (90° - 100°C), the vacuum removed, the fibre mat broken and crucibles filled three times with hot water and then twice with acetone and sucked dry. The fibres were dried at 62°C overnight, and then transferred to 100°C for 30 minutes before weighing.

The yield of recovered NDF was determined as a per cent of cell wall constituents. Cell soluble material was estimated by subtracting this value from 100. The residue was ashed for 4 hours at 500° - 550°C, weighed and reported as ash insoluble in Neutral-detergent.

3.3.6 ACID DETERGENT FIBRE (ADF)

This method is used by animal scientists to determine feedstuff lignocellulose material, including silica. The difference between the NDF (cell wall) and ADF is an estimate of hemicellulose. However, this difference does include some proteins attached to cell walls. The ADF is

also used as a preparing step for lignin determination.

Similar procedures and sample preparation as in NDF were employed, except for 100 ml cold acid-detergent solution, which replaced the neutral-detergent solution. The following formula was used in computing (%) ADF;

ADF = (Wo - Wt)(100)/S

where:

Wo = weight of oven dry crucible including fibre

Wt. = tared weight of oven-dry crucible;

S = oven-dry sample weight

To determine the presence of glucose a 0.02N iodine test was carried out (1.27 g iodine and 2.0 g potassium iodide in 500 ml water). Pure glucose was used as a control (Association of Official Analytical Chemists 1980).

3.3.7 ACID DETERGENT LIGNIN (ADL)

ADF removes proteins and other acid soluble material that would interfere with the lignin determination. The ADF residue consists of cellulose, lignin, cutin and acid soluble ash, mainly silica. Treatment with 72% H_2SO_4 dissolves cellulose. Ashing the residue determines the crude lignin fraction, including cutin. Therefore, loss in weight after treatment with 72% H_2SO_4 is equivalent to per cent cellulose in the sample.

Crucibles containing ADF fibre were covered with cool (15°C) 72% H_2SO_4 and stirred with glass rods to a smooth paste, breaking all lumps. Glass rods were left standing in

crucibles, which were refilled three times with 72% H_2SO_4, and stirred at hourly intervals as acid drained. After 3 hours, the acid was filtered by vacuum, contents washed with hot water, and glass rods rinsed and removed. Crucibles were then filled twice with acetone and dried by vacuum, oven-dried at 62°C overnight, and then at 100°C for 30 minutes before weighing. Residues were ignited in a muffle furnace at 500° - 550°C for 4 hours, cooled, and weighed. Acid detergent lignin was computed as follows,

ADF = (L x 100)/S

where:

L = Loss upon ignition after 72% H_2SO_4 treatment;

S = Oven dry sample weight

3.4 SEED STRUCTURE AND HISTOCHEMISTRY

Ovaries of unopened flowers, seven month old fruits, and mature fruits were collected in late January 1985 at Kibwezi, Kenya. These were immediately fixed in Formalin-Aceto-Alcohol (FAA) (Johansen 1940) for seven days after which FAA was drained. Fruits were wrapped in paper towels soaked in FAA and were then sealed in polyethylene bags and mailed by air to the University of Alberta, Canada, where they were stored at 3°C. The three methods used in tissue preparation were: (1) the paraffin method for flowers, immature seeds, embryos, and the endosperm of mature seeds (Johansen 1940, Jensen 1962 , Sass 1940, and O'Brien and McCully 1981); (2) a freezing device was

attached onto a sliding microtome (Model C Reichert Wien No. 2832) for whole sections of mature seed (Farris 1982), and; (3) LKB2218-500 Historesin procedure.

For the paraffin wax method, seeds were separated into embryo, endosperm and integument tissues. Then integuments were boiled for 10 minutes in tap water before fixing in FAA. These were dehydrated and infiltrated with Paraffin (Paraplast Plus, MP 56-57°C) each step taking 24 hours. Four changes were made for the embryos and endosperm during infiltration, but ten changes for the integuments because of poor infiltration due to high lignin content. Embedding, block making and trimming followed Johansen's (1940) procedures.

Slides were deparaffinized in xylene and stained in Periodic Acid Schiff's (PAS)/Light Green (LG); Safranin O/Johansen's Fast Green (FG), and then mounted in Clearmount for light microscopy (O'Brien and McCully 1981; Jensen 1962).

For whole seed sections, seeds were fixed in FAA and softened in "Aerosol O.T." (Fisher Scientific). These were then oriented on a freezing device attached to a sliding microtome and flooded with "Tissue-TEK II Compound" (Farris 1982). To prevent sections from sticking onto the blade, paraffin oil and "Lab Freeze" (Nutritional Biochemical Corporation) were applied frequently. Sections were cut at 21-27 μm thick.

Tissue preparation for the LKB Historesin method involved boiling intact seeds in tap water for 10 minutes, cutting them into very small pieces and fixing them in 3% glutaraldehyde in sodium-cacodylate buffer (at pH 7 overnight in 15 psi vacuum). These were then washed in tap water for about 30 minutes, and dehydrated in 30, 50, 60, 70 and 95% ethanol for about 30 minutes at 4°C each. Dehydration was followed with a change to a 1:1 95% ethanol and Historesin plus a catalyst at room temperature, 100% Historesin plus a catalyst at 15 psi in a vacuum overnight and 100% Historesin plus a catalyst for 4 hours in a vacuum. Tissues were then imbedded (in a mixture of a Historesin, a catalyst and a hardener) in Beem Capsules size 00. These were then left to polymerize (15 ml of Historesin, catalyst and 1 ml hardener) overnight at room temperature, protected from dust. Blocks were cut at 2 μm on an ultramicrotome equiped with a glass knife. Sections were stained in Aniline Blue Black (ABB) and PAS/Johansen's Fast Green (O'Brien and McCully 1981, Johansen 1940 and Jensen 1962).

3.5 ANALYSIS OF DATA

One way analysis of variance and Student t-test were used to compare results among seed treatments (Steel and Torrie 1980; Little and Hills 1977; and Freese 1983). Total percent germination and germinative rates for each treatment were computed based on day 14 from inception of tests.

If there were significant differences among seed treatments at the 5% level, means were compared by the Duncan's New Multiple-Range Test (Steel and Torrie 1980). Regression analysis and coefficients of determination were calculated for total germination and germinative rates.

4. RESULTS

4.0.1 FLOWER AND FRUIT PHENOLOGY

Reproductive buds develop only in terminal branch positions in *M. volkensii*. They are generally larger than vegetative buds. There is considerable variation among trees in development of both buds and flowers. The stalks of the flower buds become extended before flowers were finally developed or before they open. Within six weeks of first being identified as flower buds, flowers begin to wither and petals start to fall off.

After petals fall, tiny fruits (3 to 5 mm in diameter) remain behind. This stage of fruit development is called the "candle stage" (Figure 1.6). In about seven months from the time of flower appearance, the fruits grow to reach 15 to 20 mm in diameter. This stage of fruit development is called "fruit soft" (Figure 1.7). The fruits are light green in colour with a semi-hard endocarp, and a colourless jelly-like substance inside the integuments (Figure 1.7a). Fruits continued to increase in size reaching about 30 mm in diameter at 10 months after fertilization (Figure 1.8). This stage of development is called "fruit hard". It is characterized by exocarp and fruit stalk changing in colour to greyish-green due to the deposit of cork. The mesocarp is firm, the endocarp fully developed and hard, and the outer integument is dark-brown in colour.

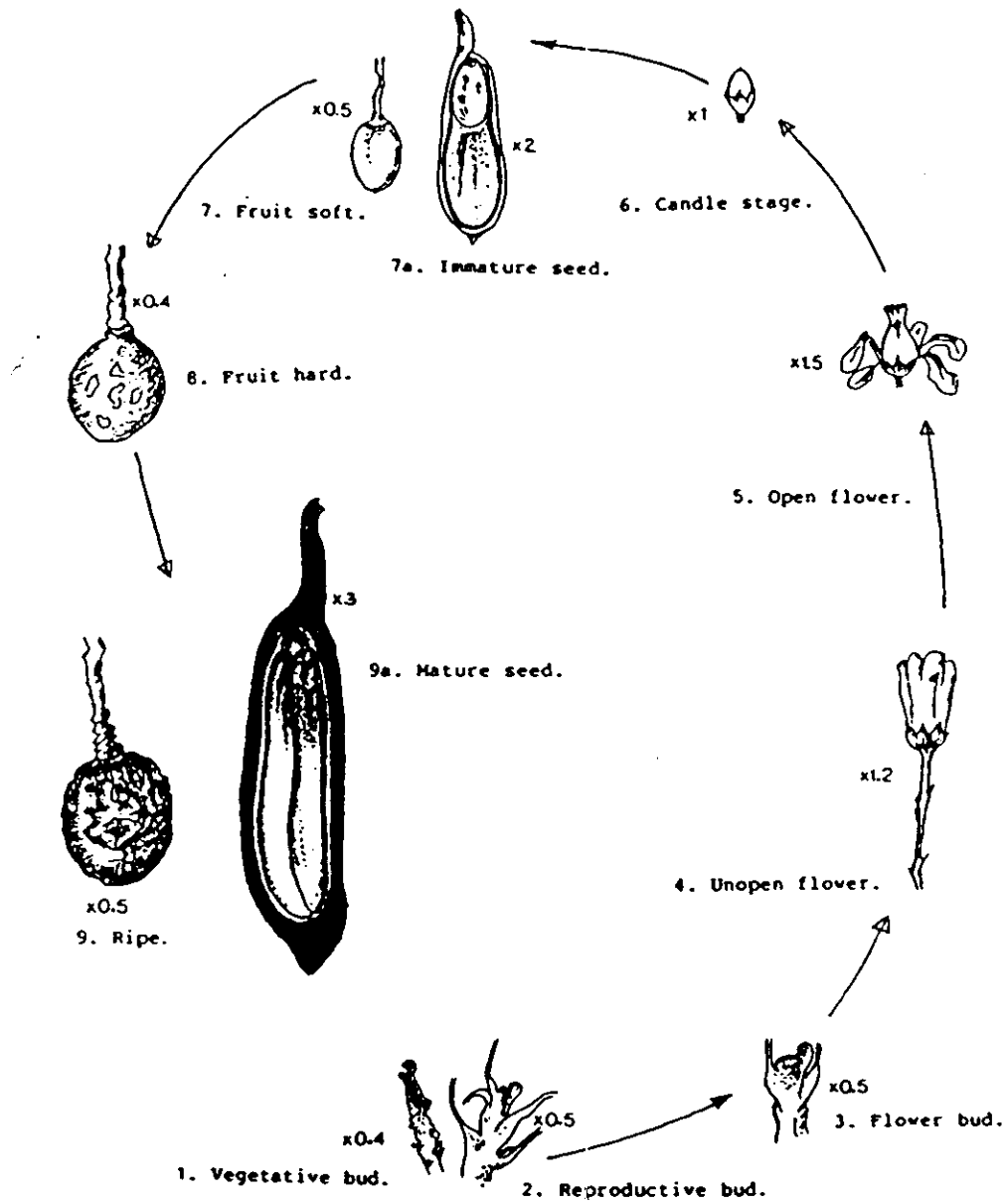


Figure 1. The reproductive cycle of *M. volkensii*. (drawing by P.B. Milimo.)

Plate 1.

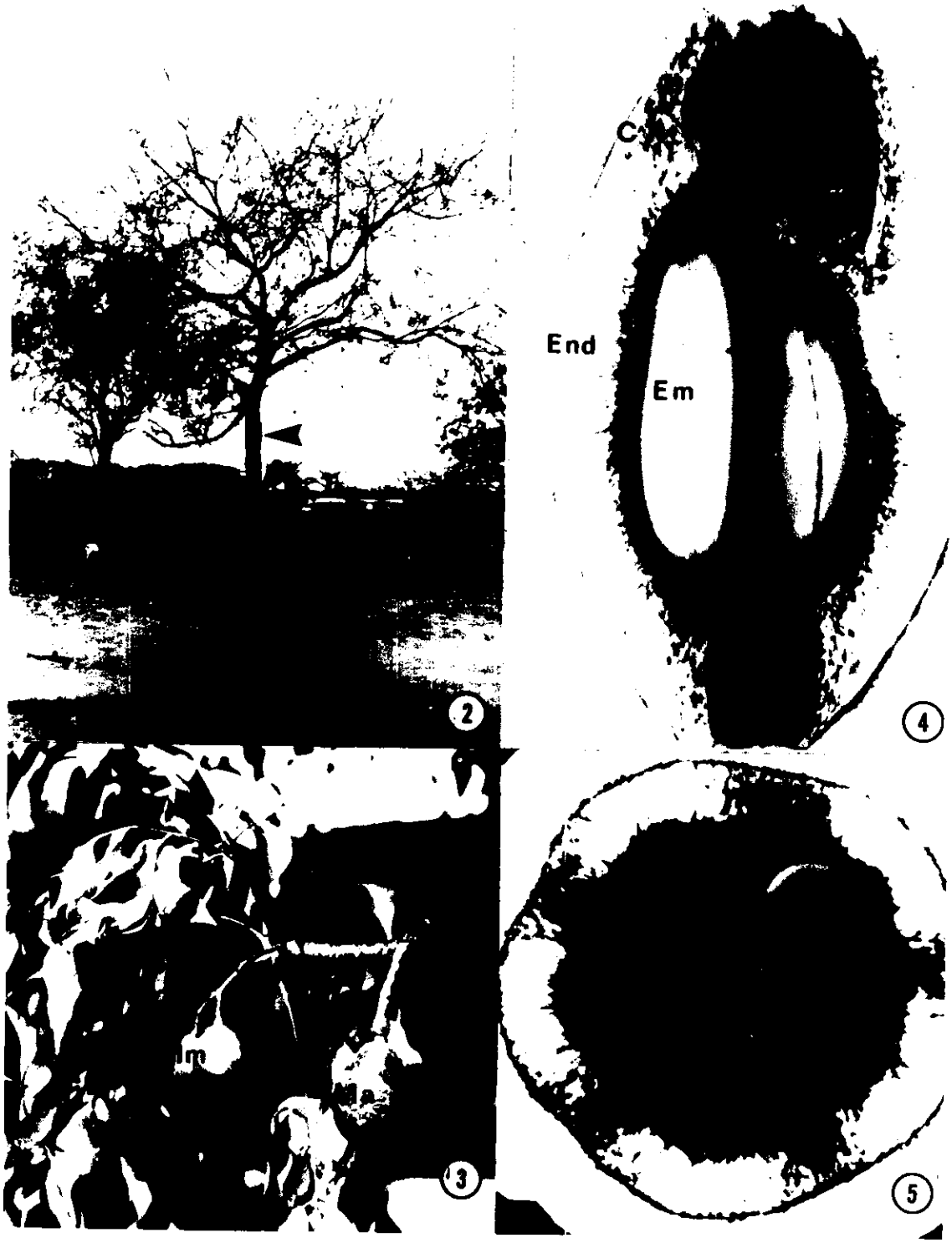
Figure 2. A *M. volkensii* tree with a goat foraging on the fallen fruits. x0.0007.

Figure 3. Immature and mature fruits located on the same branch. x0.4.

Figure 4. LS of a stony endocarp showing seeds and the micropylar orifice. x0.35.

Figure 5. TS of a stony endocarp showing locules and vascular bundles in the centre. x0.35.

Key to Abbreviations: En, endosperm; CR, caruncle; Em, embryo; Im, immature fruit; Ma, mature fruit; TS, Transverse section, and; LS, longitudinal section.


C
End
Em
2
3
4
5

Fruits normally ripen 12 to 13 months from the time of flowering. This final stage of fruit development is called "ripe" (Figure 1.9). Ripe fruits are similar in size to those of the "fruit hard" stage. They are distinguished from hard fruits by the soft mesocarp (pulp) and the yellowish-green colour of the exocarp. Fruits at this stage may be completely covered with cork. Endocarps are very hard and brittle, outer integuments are several cells thick (Figures 25 and 27), it is black in colour and brittle (Figure 1.9a), and abscission rings on fruit stalks are fully developed, allowing the fruit to fall off when lightly disturbed.

Occurrence of any one particular stage of development varied from branch to branch even within trees. Some trees did not produce fruits, while others had one (Figure 3) or all the stages. This type of plant development is considered to be without a seasonal pattern (Todorov 1977a). In cases where more than two stages of fruit development occur, they emerge from a location corresponding to a specific part of the growing season. The youngest emerging from closest to the branch tip and the oldest farthest from the tip.

4.1 FACTORS INFLUENCING GERMINATION

4.1.1 TEMPERATURE AND GERMINATION

Mean per cent total germination under alternating day/night temperatures differed significantly ($P \leq 0.01$),

with the lowest value being 1% for 42°C and 25°C and the highest 78% for 32°C day and 25°C night.

Table 1. Mean per cent total germination in relation to alternating and constant day and night temperatures. (n=~~4~~ 25).

Alternate temp. (°C)	Total germ. (%)	S.D.		Constant temp. (°C)	Total germ. (%)	S.D.	
25/22	59.94	12.36	b	25	55.95	16.99	a
27/25	60.36	16.03	b	27	61.04	6.35	b,c
32/25	77.46	14.52	b	32	83.16	13.12	a
37/25	54.71	9.13	b	37	76.94	14.46	a,b
42/25	1.05	2.10	a	42	0.00	0.0	d

Along each column, means followed by the same letter are not significantly different at $P \leq 0.05$ level. Note: Days and nights were each 12 hours.

Table 2. Mean germinative rates in relation to alternating and constant day and night temperatures. (n=~~4~~ 25).

Alternate temp. (°C)	Germ. rates (days)	S.D.		Constant temp. (°C)	Germinative rates (days)	S.D.	
25/22	9.65	0.25	a	25	6.58	1.52	a
27/25	9.31	0.09	a	27	5.35	0.70	a,b
32/25	7.48	0.42	b	32	4.26	0.58	b
37/25	5.23	0.17	c	37	4.21	0.27	b
42/25	1.15	2.30	d	42	0.00	0.00	c

Along each column, means followed by the same letter are not significantly different at $P \leq 0.05$ level. Note: Days and nights were 12 hours each.

Total germination under constant temperature regimes also differed significantly ($P \leq 0.01$), with the highest value observed for 32°C (83.16%) and the lowest for 42°C day and night (0.00%)(Table 1). The relationships between day temperature and total germination is parabolic (Figure 6), with the maxima at about 31°C ($Y = -525.40 + 38.44X - 0.62X^2$, $R^2 = 0.82$) and

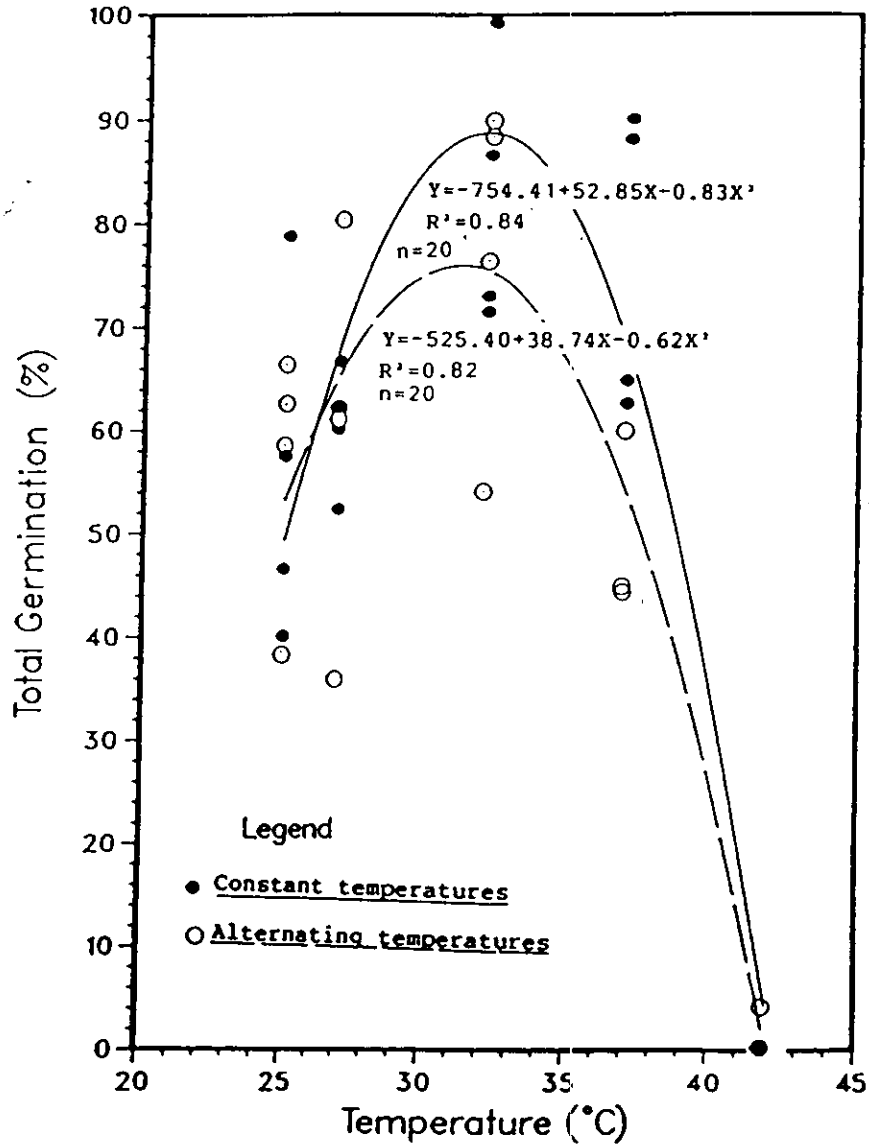


Figure 6. Total germination (%) under alternating and constant day and night temperatures.

32°C ($Y = -754.41 + 52.85X - 0.83X^2$, $R^2 = 0.84$) respectively for alternate and constant temperature regimes.

Germinative rates differed significantly among treatments for both alternating and constant temperatures ($P \leq 0.01$; Table 2, respectively). The fastest germinative rate for alternating temperatures being at 42°C day and 25°C night and the least at 25°C day and 22°C night. Note that total germination corresponding to the highest germinative rate is only 1% (Table 1). For constant temperature, the most rapid germinative rate (4.2 days) was observed at 37°C and the slowest (6.5 days) at 25°C. Germinative rate increases with temperature. The relationship under alternating temperatures is linear ($R^2 = 0.97$, $P \leq 0.01$), but it is curvilinear under constant temperatures ($R^2 = 0.60$, $P \leq 0.01$; Figure 7). Since the highest temperature at which seeds can germinate is not known, germinative rate values for 42°C were excluded during the computation of these relationships.

Total germination of seeds covered with paper towels are presented in Table 3 and the relationship between time and cumulative germination in Figure 8. Seeds covered with paper towels germinated significantly better than uncovered seeds ($P \leq 0.01$), but seed covering had no effect on germinative rates. Lack of significant difference among germinative rates may be due to high variance associated with means.

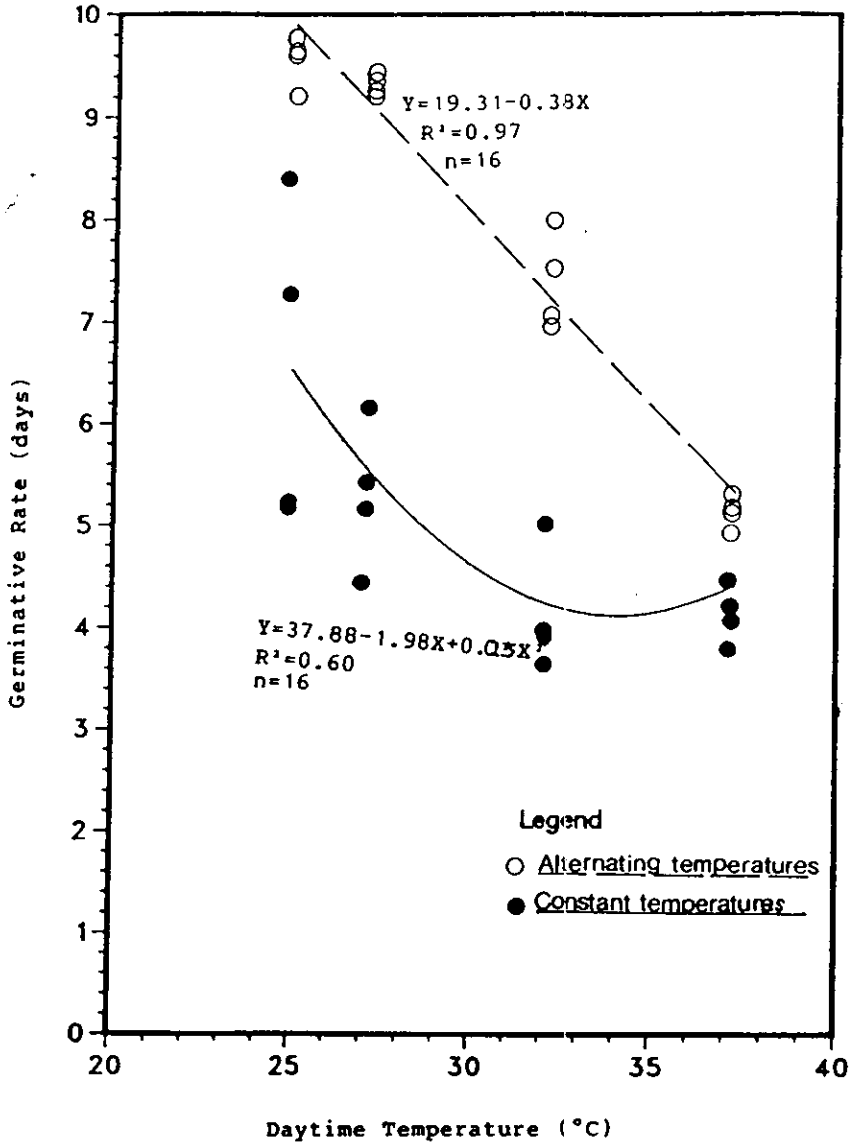


Figure 7. Germinative rates under different alternating and constant day and night temperatures. (In alternating regimes, nights were 25°C except for 25°C day which had 22°C night).

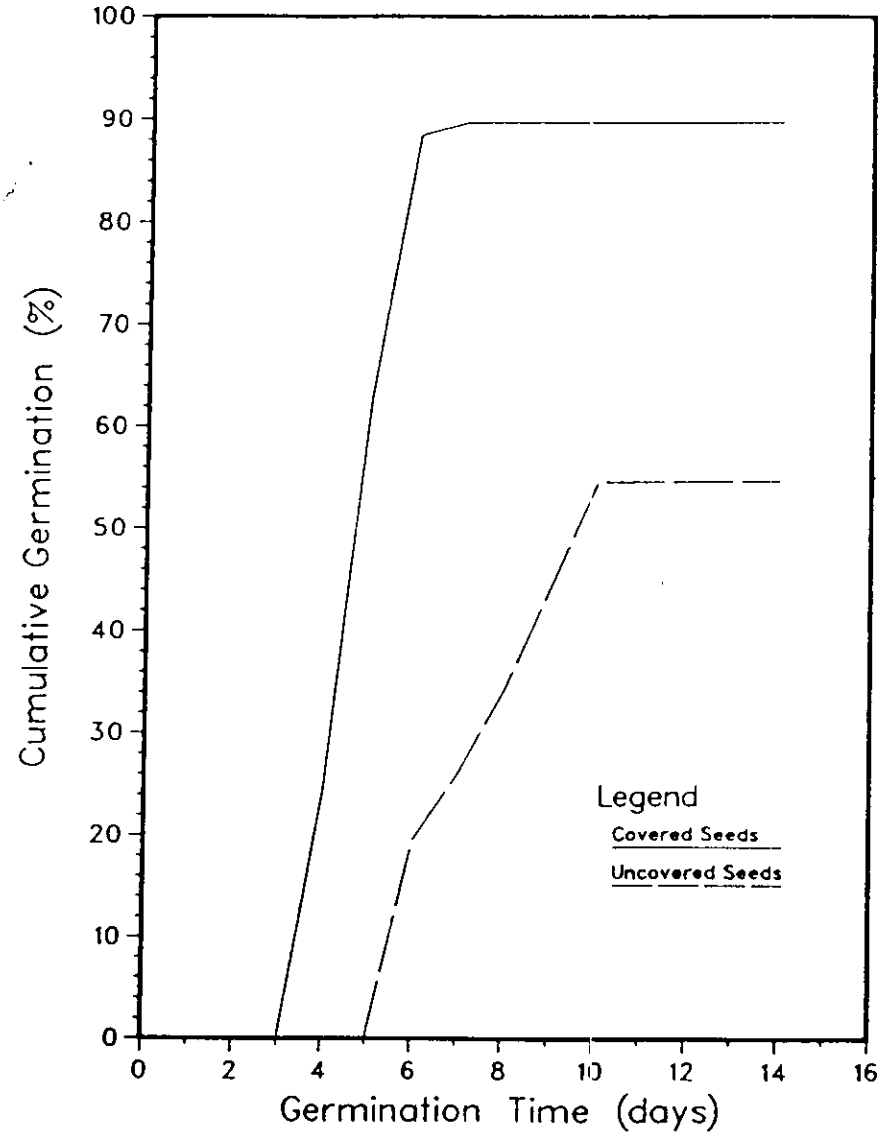


Figure 8. Cumulative germination percentage for covered and uncovered seeds.

Table 3. Mean per cent total germination and germinative rates of covered and uncovered seeds. (n=~~4~~ 25).

COVERED SEEDS				UNCOVERED SEEDS			
germ. (%)	S.D.	rate (days)	S.D.	germ. (%)	S.D.	rate (days)	S.D.
89.72	4.10	4.29	0.03	54.55	0.00	6.63	1.16

4.1.2 EMBRYO EXCISION AND GERMINATION

Excised embryos had significantly better germination (81%) than intact seeds (9%)($P \leq 0.01$; Table 4 and Figure 9). Germinative rates for excised and intact seeds did not differ significantly (Table 4).

Table 4. Mean per cent total germination and germinative rates of excised embryos and intact seeds. (n=~~4~~ 25).

EXCISED EMBRYOS				INTACT SEEDS			
germ. (%)	S.D.	rate (days)	S.D.	germ. (%)	S.D.	rate (days)	S.D.
81.41	8.25	3.92	0.27	8.81	14.34	6.30	1.00

4.1.3 WATER IMBIBITION

4.1.3.1 WATER UPTAKE BY WHOLE SEEDS

Cut seeds absorbed more water at a higher rate than either intact seeds or seeds with broken caruncles (Appendix 1 and Figure 10). The plot between cut seed fresh weight and time is nearly triphasic, but biphasic for intact seeds and seeds with broken caruncles (Figure 10). Phase III of the curve, representing cut seeds,

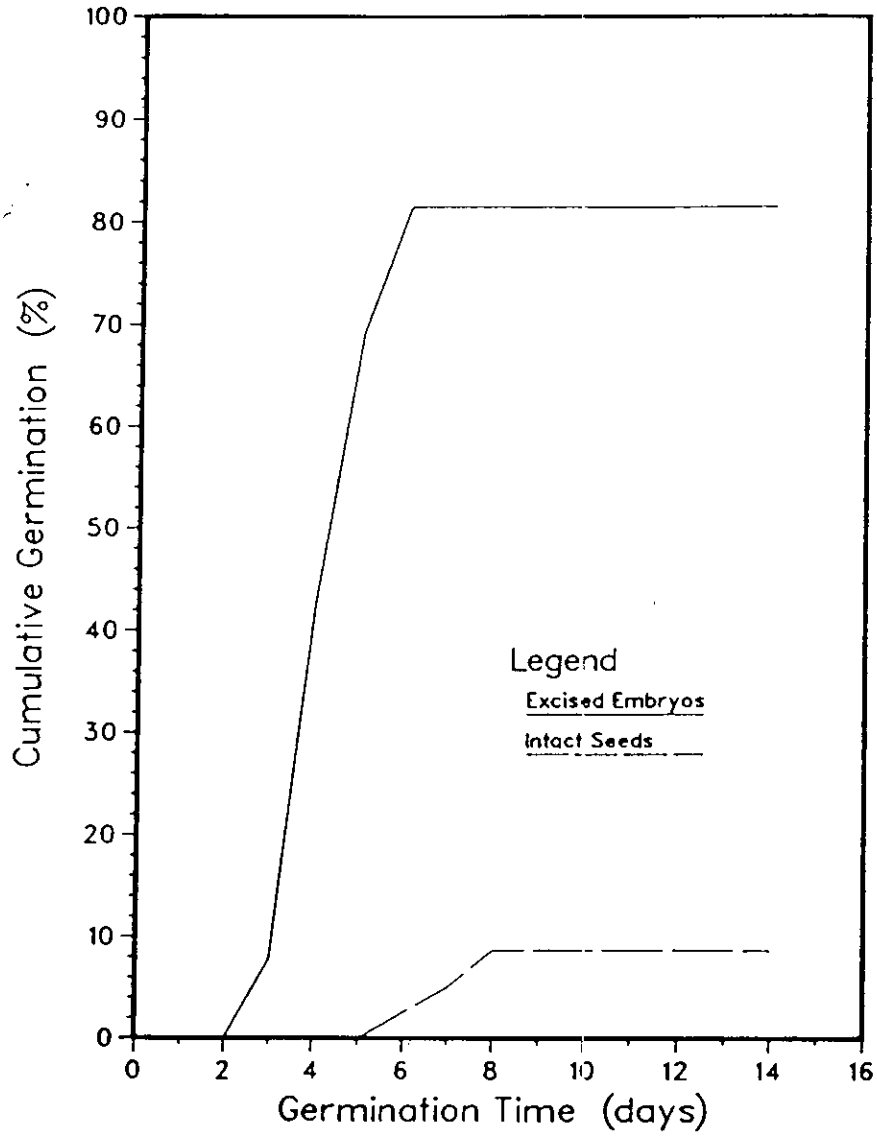


Figure 9. Cumulative germination percentage for excised embryos and intact seeds.

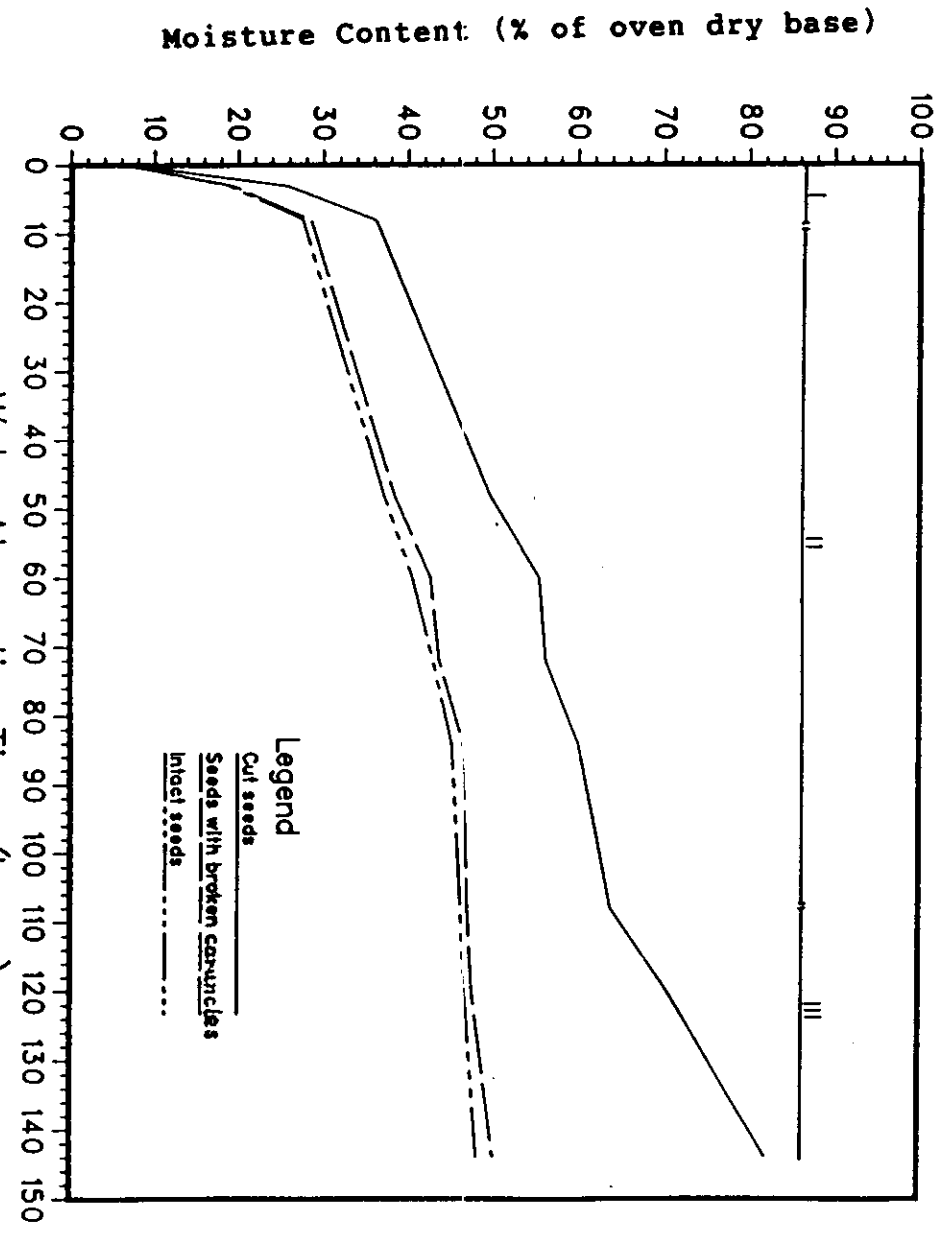


Figure 10. Water imbibition curves for various types of integument treatments.

is the beginning of visual germination (Bewley and Black 1978). Cut seeds started to germinate after about 72 hours but 36 hours before the start of phase III. The explanation for the difference in time may be due to the high standard deviations associated with the means. Intact seeds and seeds whose caruncles had been broken, did not germinate at all, but they took up the same amount of water at the same rate (Paired Student t-test). Therefore, the removal of the caruncle to expose the perisperm and endosperm, with the hope of increasing the amount of water absorbed, did not work. This suggests that the rate and amount of water absorbed by intact seeds is determined by the perisperm and/or endosperm. Integuments did not seem to hinder water absorption.

Integuments, the endosperm and the perisperm, each accounted for 13% and the embryo 74% of the seed dry weight (Table 5 and Figure 11).

Table 5. Per cent dry weight of the integuments, endosperm and embryo tissues, and their relative water uptake. (n=15).

Tissue	Dry wt. (%)	Water uptake (%)
Integuments	13.14±0.56	37.88±2.18
Endosperm	13.24±0.67	46.87±2.95
Embryo	73.62±0.82	15.30±1.35

Note: the perisperm is included with the endosperm.

Water absorption by these tissues seems to bear no relation to their relative weight proportions (Table 5).

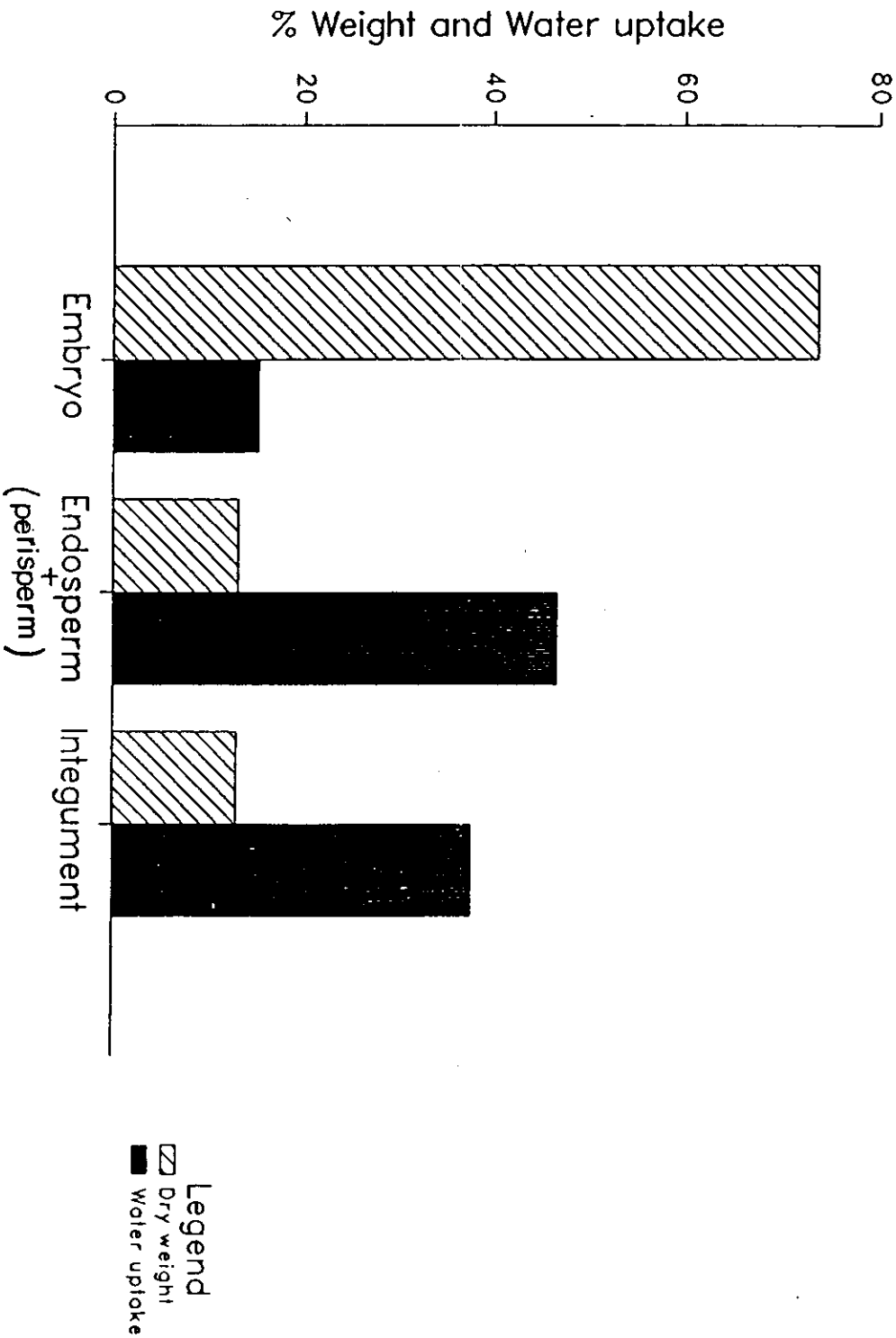


Figure 11. Seed tissue and their relative water absorption.

The embryo, for instance, absorbed only 15%. Integuments and the endosperm absorbed 38% and 47% respectively of the water absorbed by all seed parts combined.

4.1.4 EFFECT OF GAS CONCENTRATION ON GERMINATION

Total germination was significantly different at various O_2 and N_2 levels ($P \leq 0.01$; Table 6). The highest total germination was observed at 21% O_2 (94%), and the lowest at 50% O_2 (42%) (Figure 12). Total germination increased initially with increasing O_2 to a maximum (21% O_2), and then declined thereafter ($R^2 = 0.82$; $P \leq 0.01$; Figure 13).

Germinative rates differed significantly among different gas concentrations ($P \leq 0.01$; Table 6), with the highest observed in 50% O_2, and the lowest in 15% O_2 and 0.65% CO_2. Germinative rates increased with increasing O_2 concentrations.

Table 6. Mean per cent total germination and germinative rates of seeds incubated in various gas types and concentrations. (n=~~4~~ 25).

Gas type concentration (%)	Total germination (%)	S.D.	Germinative rates (days)	S.D.
21% O_2 and 79% N_2	94.24	8.59 c	4.26	0.13 b
15% O_2, 0.65% CO_2 and 84.35% N_2	75.58	9.46 b	4.70	0.20 a
25% O_2 and 75% N_2	72.78	2.56 b	4.86	0.30 a
50% O_2 and 50% N_2	42.06	16.68 a	3.83	0.27 c

Along each column, means followed by the same letter are not significantly different at $P \leq 0.05$ level.

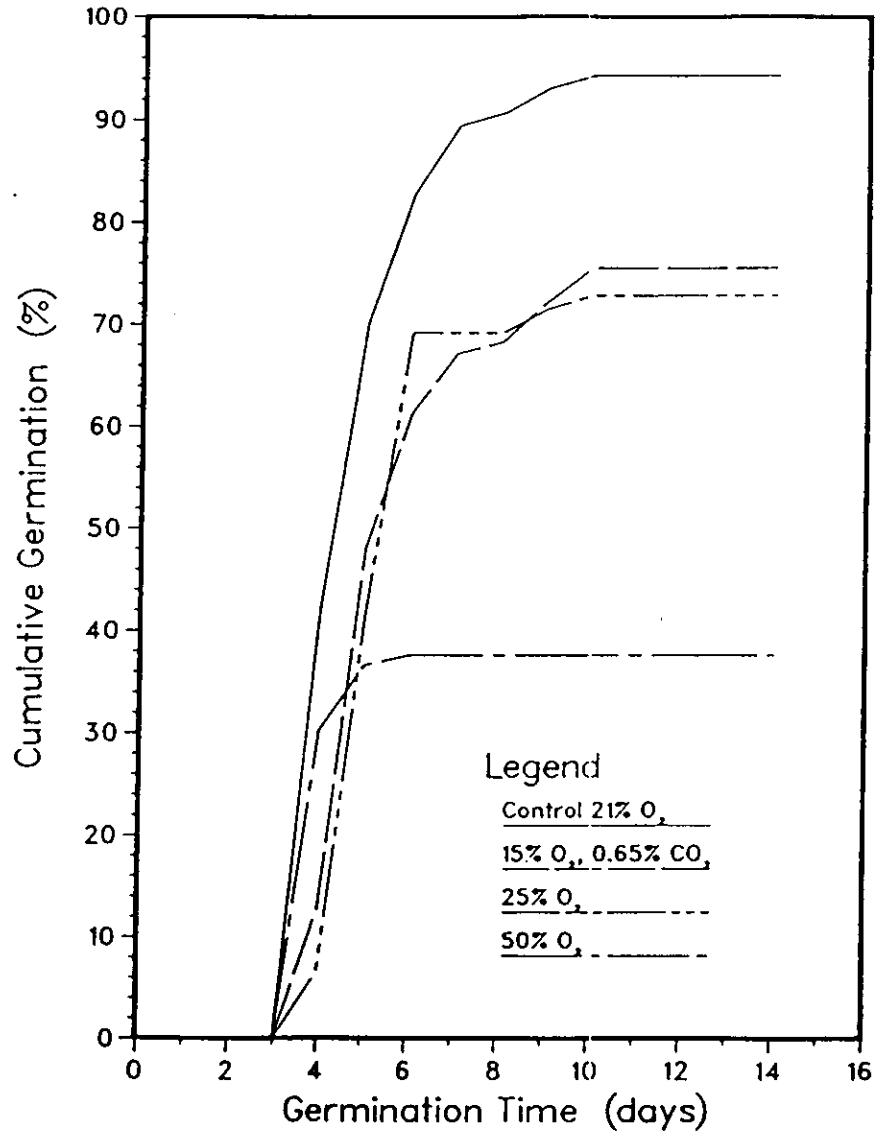


Figure 12. Cumulative germination percentage for seeds incubated at different gas concentrations. (N_2 was the complementary gas in all treatments).

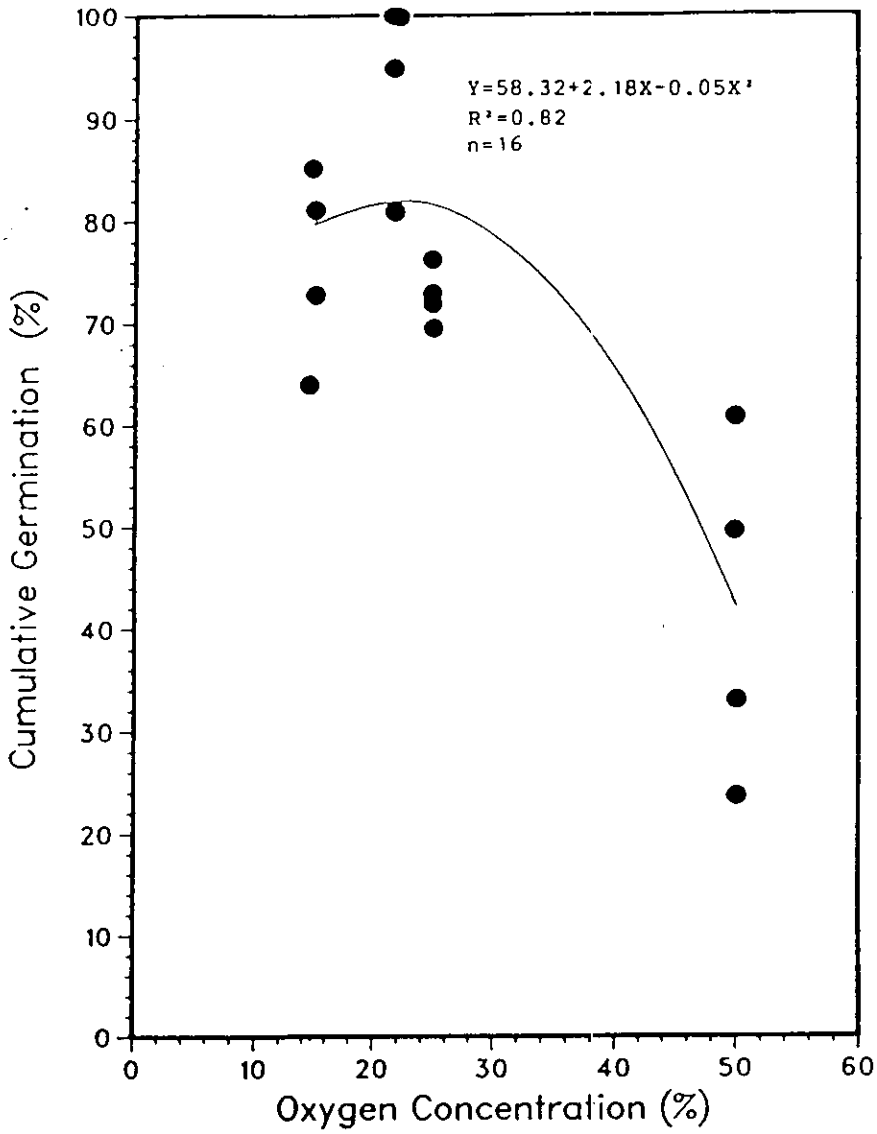


Figure 13. The effect of different gas concentrations on total germination of cut seeds. Note: 15% O_2 also contains 0.65% CO_2; N_2 was the complementary gas in all the treatments.

4.1.5 SEED CUT LOCATION AND GERMINATION

Seeds with cut-locations at A, B, C and D (Figure 15) reached total germination of 92, 39, 63, and 91%, respectively (Table 7). These are significantly different ($P \leq 0.01$). Germinative rates were the same for all the cut locations tested ($P \leq 0.05$; Table 7 and Figure 14).

Table 7. Mean per cent total germination and germinative rates of seeds cut at different locations. (n=~~4~~25).

Seed cut type	Total germination (%)	S.D.		Germinative rates (days)	S.D.	
Micropylar end cut	91.77	7.89	a	6.10	0.75	a
Centre horizontal cut	39.46	13.66	a	5.85	0.31	a
Chalazal end cut	62.86	12.34	b	6.32	0.38	a
Micropylar to chalazal cut	91.20	5.86	c	6.42	0.64	a

Along each column, means followed by the same letter are not significantly different at $P \leq 0.05$ level.

Different cut locations caused various modes of germination. Cuts at A and D induced normal germination, in which the radicle protruded through the slit at the micropylar end. Cuts B and C gave rise to abnormal germination in which radicles were not able to penetrate intact integuments, perisperm and endosperm. Instead, cotyledons were pushed out through the slit. Nobbe (1876), Ikuma and Thimann (1963) called such normal emergence "typical" and abnormal emergence "atypical".

4.1.6 CHEMICAL COMPOSITION OF VARIOUS SEED TISSUES

The main chemical constituents of the embryo and the endosperm are respectively: crude fat (54% and 35%,

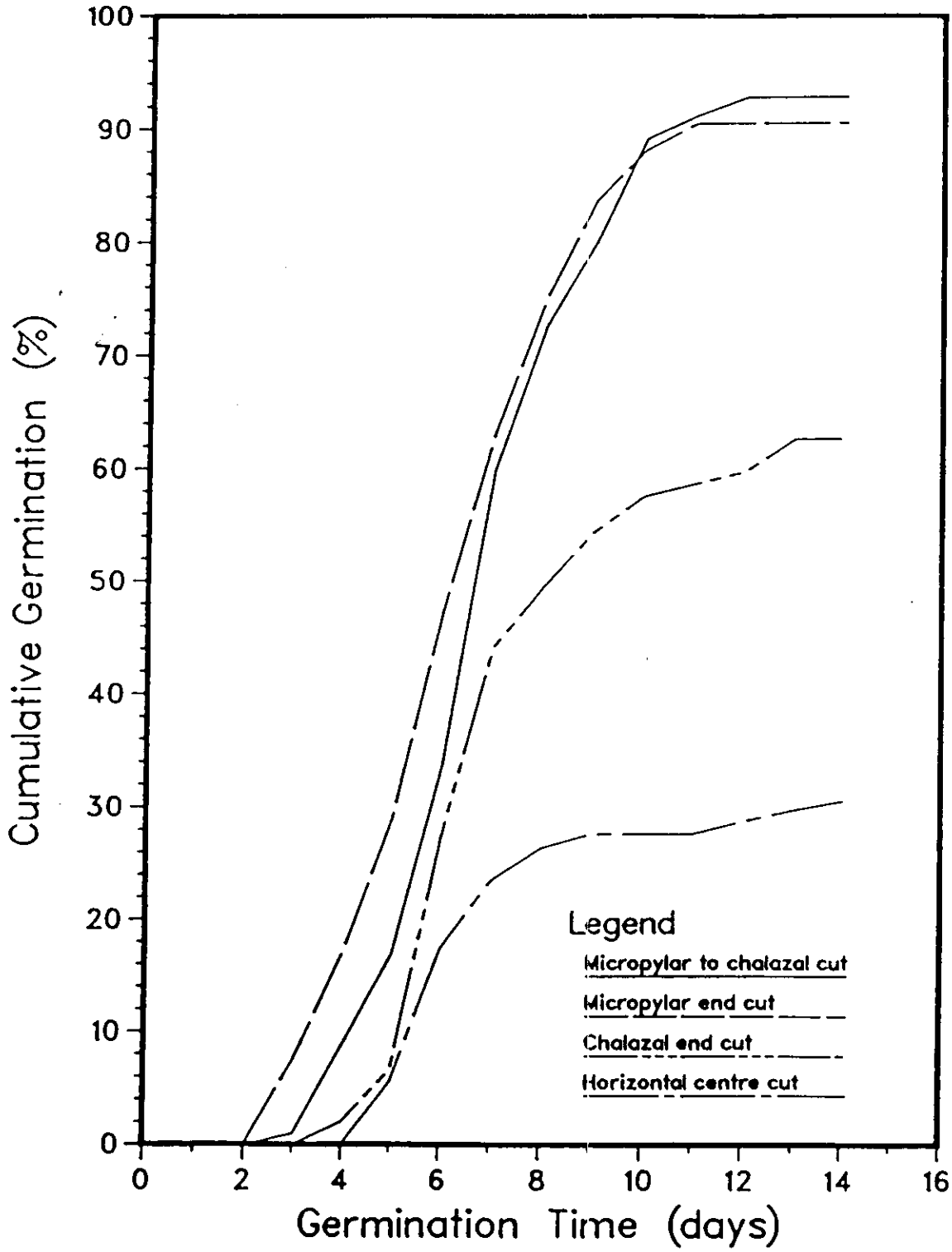


Figure 14. Cumulative germination percentage for seeds when seed coats were cut in different places.

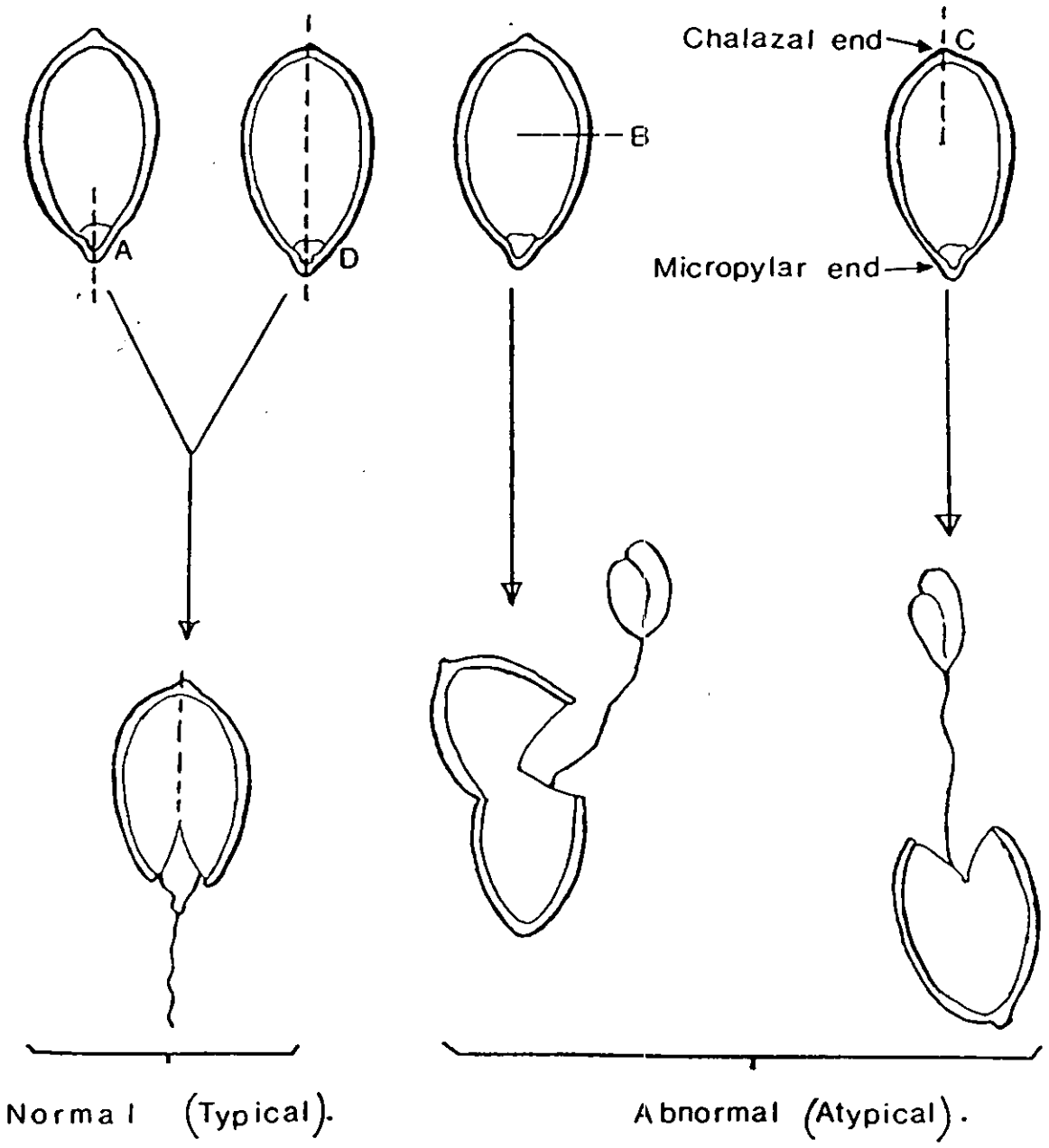


Figure 15. Modes of germination for seeds cut through integuments, perisperm and endosperm at different locations. (Drawing by P.B. Milimo).

respectively) crude proteins (49% and 26%, respectively). Integuments are mainly composed of lignin and cutin (about 60%; Appendix 2 and Figure 16). Oleic and linoleic fatty acids are the main components of crude fat (Appendix 3 and Figure 17). Seventeen amino acids were identified from seed parts and these are classified according to Lehninger (1982) by polarity and location in the structure of globular proteins. About 23% and 20% of the amino acid's composition of the embryo and endosperm proteins, respectively, are hydrophobic. These are located on the exterior and interior of globular proteins (Appendix 4).

The embryo was composed of 3% hemicellulose and 5% cellulose. The endosperm cellulose and hemicellulose made up 5% each. Although an iodine test for the presence of starch gave negative results, a histochemical test with PAS was able to indicate its presence.

4.1.7 SEED STRUCTURE AND HISTOCHEMISTRY

The ovule of *M. volkensii* grows from the placenta as a round protuberance. At origin, the ovule has a symmetrical growth, the funiculus and micropyle have a straight orientation, the ovule is said to be "orthotropous". But during development, the body of the ovule bends (Figure 18) and becomes completely inverted (Figure 20), bringing the micropyle close to the funiculus. This condition is described as "anatropous" (Maheshwari 1950). In the course of maturation various tissues change in thickness,

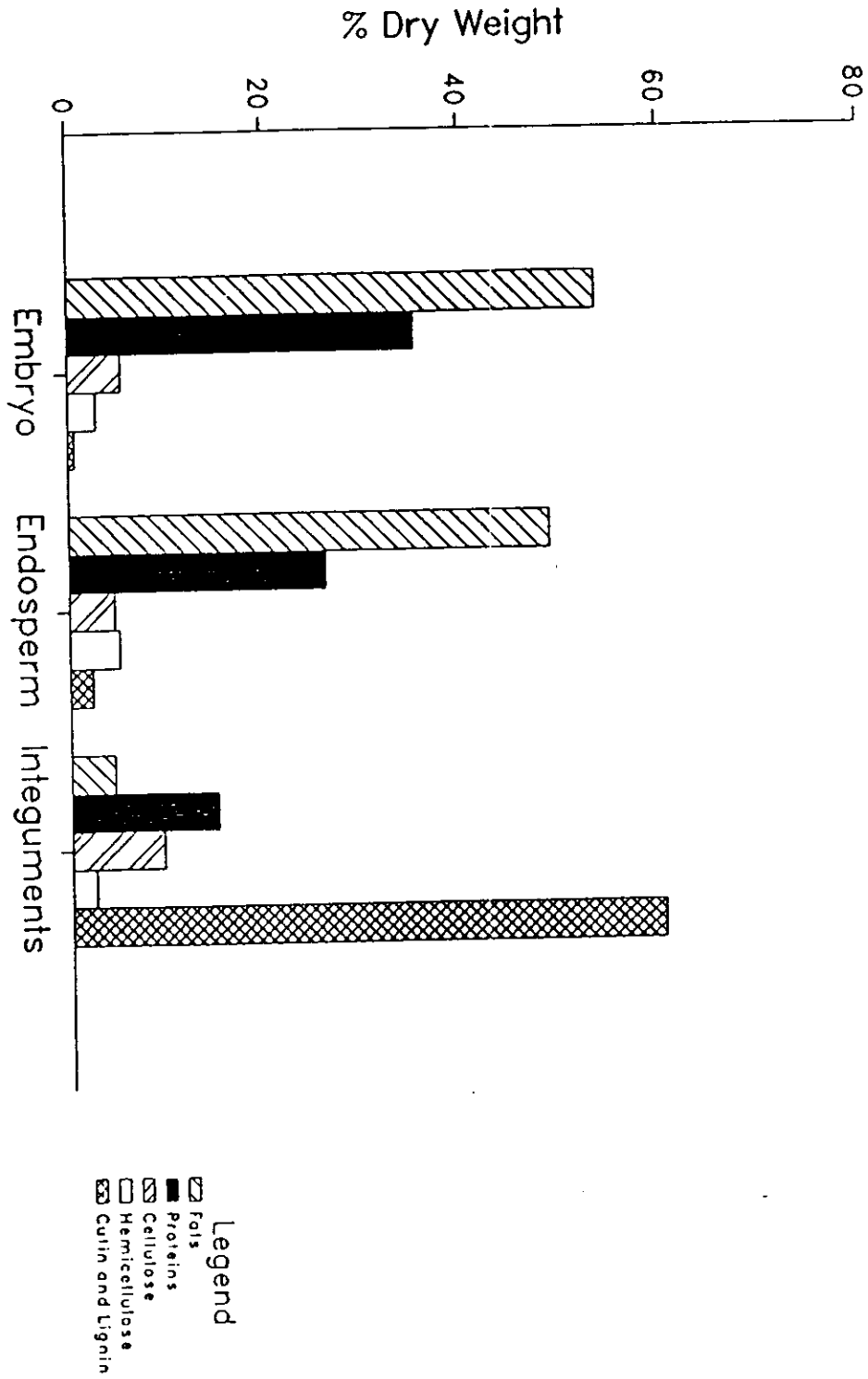


Figure 16. Proportion of chemical compounds in different tissues of the seeds of *M. volkensii*.

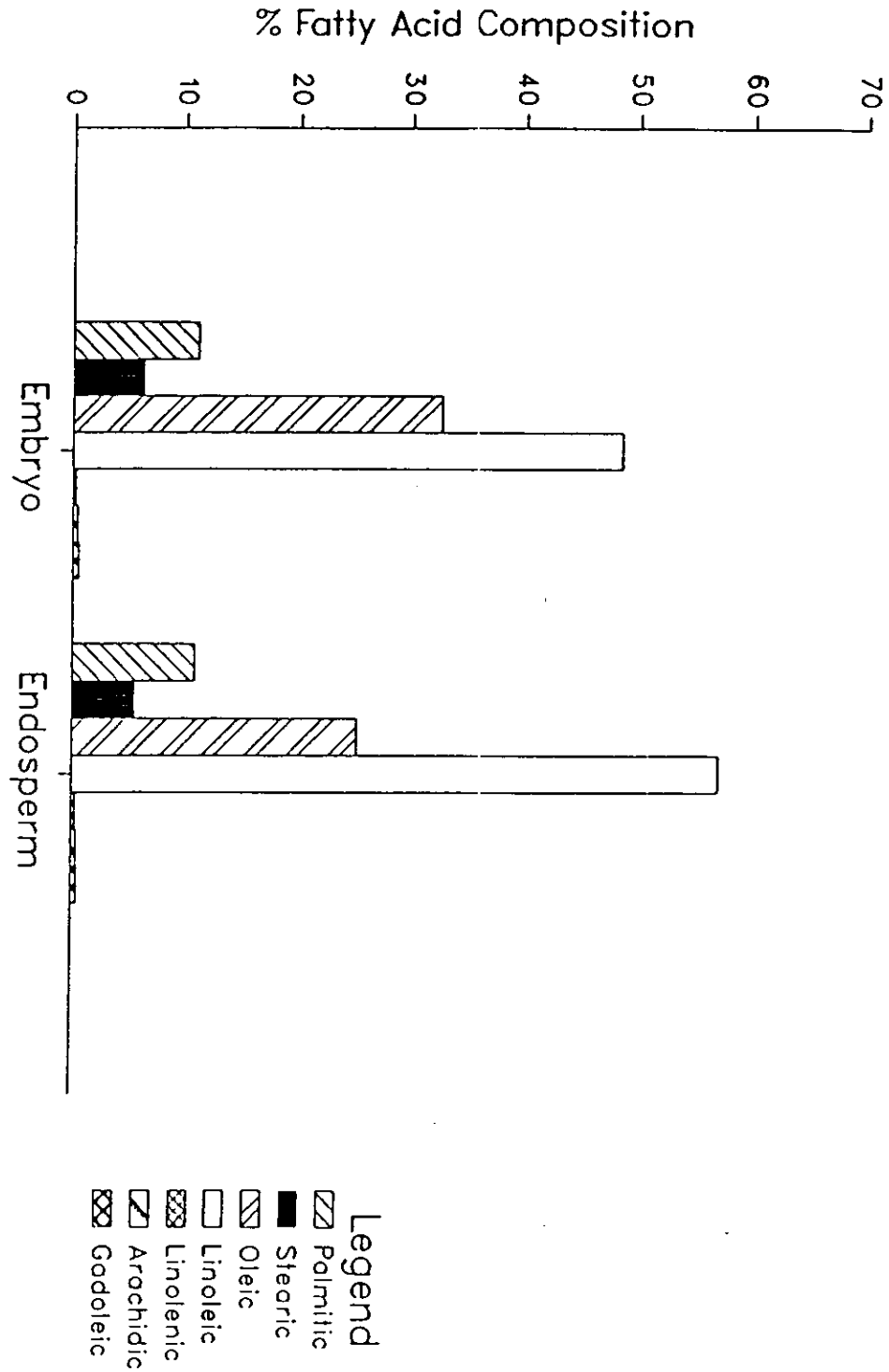


Figure 17. Fatty acid composition of the endosperm and embryo of *M. volkensii*.

and the tip of the integuments develop to form a caruncle. Late in ontogeny the embryo and endosperm start to accumulate storage material. Although the micropylar end is a small part of the total surface of the seed, it is one area which commonly causes germination inhibition and therefore the degree of its specialization justify special consideration in this investigation.

4.1.7.1 INTEGUMENTS

The integuments begin to differentiate before fertilization. The inner integument starts to develop first followed by the outer integument (Figure 18). The outer integument of the pre-fertilization stage is four to six cells thick and the inner integument four cells. Both, however, are much thicker at the micropylar end (Figure 20). The ground tissue at this stage is parenchymatic. The epidermal layer of the outer integument and cells of the inner integument at the chalazal end are vacuolate. The inner cells of the outer integument and the micropylar end of the inner integument are cytoplasmic. Cell walls of the outer integument at the chalazal end stain brownish-red in PAS/FG, while most of those at the micropylar end show an intense green after treatment with PAS and Fast Green. The former is a reaction identifying starch and sometimes lignin (O'Brien and McCully 1981). All cells of the inner integument and those of the nucellus stain a light green with PAS and Fast Green.

PLATE 2:

Figure 18. TS showing an early stage of ovule differentiation. x500.

Figure 19. TS of ovule at the same stage of development as in Figure 20, stained in PAS/FG. x600.

Figure 20. LS of unfertilized ovary stained in PAS/FG. x70.

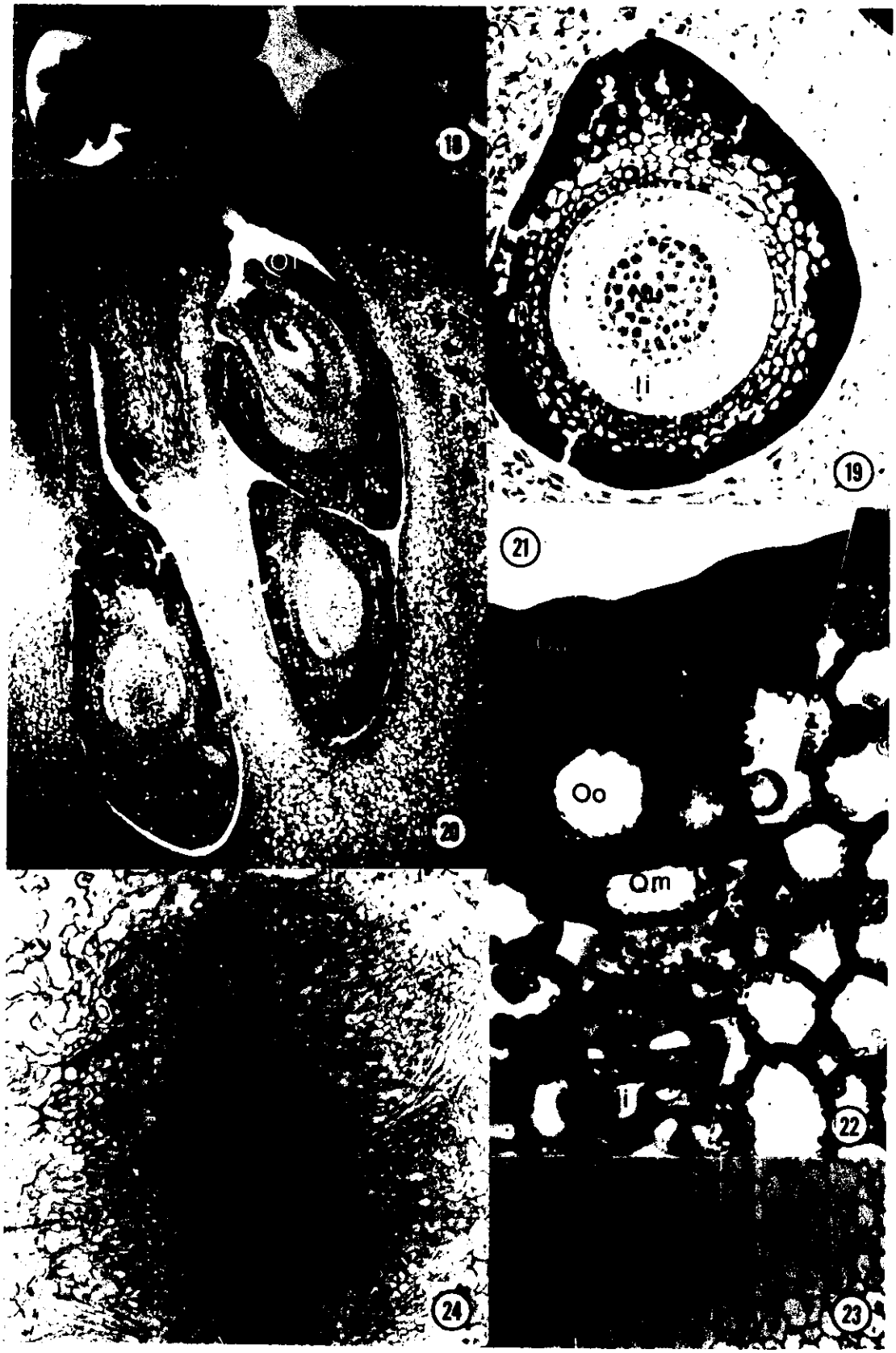
Figure 21. TS taken from the chalazal end, showing the outer and inner integument layers of immature seeds stained in PAS/FG. x650.

Figure 22. LS of the outer integument at the same stage of development as in Figure 21, stained in PAS/FG. x450.

Figure 23. TS of immature cotyledon tissue, stained in PAS/FG x500.

Figure 24. TS through the vascular bundle situated in the caruncle of an immature seed showing an open canal. x500.

Key to Abbreviations: Nu, nucellus; Ii, inner integument; Oi, inner cell-layer of outer integument; Cu, cuticle; Oo, Outer cell-layer of outer integument, and; Om, middle cell layer of outer integument.


18
li
19
21
Oo
Om
li
22
24
23

There is no evidence of a cuticle layer on the outer integument during the pre-fertilization phase.

Epidermal cells of the outer integument are anticlinally elongated, but those of inner cell layers and of the inner integuments are periclinally elongated. This cell orientation does not change much during ovule development, except for two inner cell layers of the outer integument which later become isodiametric (Fiugure 27).

Seven months after fertilization integument cells increase in size, develop large vacuoles and a cuticle (Figure 21). Cell walls of the outer integument are thick and have deposits of crystalline material (Figure 22), while the inner integument is composed of thick spongy parenchyma tissue (Figure 21). The formation of the caruncle is such that the inner integument tips fuse at some distance from the nucellar tip, thus creating a space between integuments and nucellus (Figures 25 and 26).

Figures 25 and 26 represent integument tissue of a mature seed. The two inner cell layers of the outer integument have changed orientation from periclinal to isodiametric, and outer integument cells are more closely packed. The middle cell layer of the outer integument and cells of the inner integument stain light brick-red in PAS and light blue in ABB, reactions identifying lignin, or starch and proteins respectively.

The inner integument tissue also has numerous large oval-shaped secretory cells which do not react with either PAS, or ABB. These cells look like enlarged parenchyma cells (Figure 27).

4.1.7.2 NUCELLUS

The nucellus is the inner part of an ovule in which the embryo sac develops. The nucellus in *M. volkensii* is well developed and is many cells thick, a state refered to as "crassinucellate"; this state is also considered to be primitive (Singh 1964). The pre-fertilization stage of ovule development is characterized by variable nucellar cell thickness, about seven to nine cells thick at the micropylar end (Figure 20). It is fused with integuments at the chalazal end. The pre-fertilization structure, the histochemistry and cell orientation of the nucellus is similar to that of the inner integument.

Post-fertilization changes of the nucellus involve a reduction in cell thickness, reduction in cell wall size, loss of cytoplasm and development of a one-cell-thick cuticular layer. Above the cuticle, but below the inner integument, is a one-cell-thick layer of irregular, sometimes branched, thick walled-cells deposited with a crystalline material (Figure 26) that stained deep-red in Safranin O. On the lower side of the nucellus and bordering the endosperm, is a thick layer of cells probably marking the beginning of compression to be completed before or by maturity.

In sections of a mature seed, the nucellus cuticle and the irregular thick-walled cell layer above it, still persist and the compression of the nucellar tissue is complete (Figures 27 and 28).

4.1.7.3 ENDOSPERM

The first stage of endosperm described here is represented by ovules at the "fruit soft" stage (Figure 1.7 and 1.7a). The endosperm cell-walls have distinct nuclei and cytoplasm (Figure 20), but deposits of nutrient reserves characteristic of cells in mature seed, are not evident. Cell-walls of mature endosperm are thick (Figure 31) and cells contain proteins, starch and lipids. Although a histochemical test to localize lipids was not done, it is clear from chemical analyses that the endosperm is rich in crude fat (Appendix 2 and Figure 16). Between the endosperm and the embryo tissues is a band of mucilage equivalent to several cell thicknesses (Figure 31), it stained positive for starch and proteins. Endosperm thickness is variable at maturity. It is only four cells thick at the micropylar end (Figure 25) and below the raphe (Figure 31), but up to nine cells thick elsewhere in the seed (Figure 28). The sequence of events leading to the reduction in thickness of the endosperm layer are not clear.

Plate 3:

Figure 25. LS of a semi-thick section at the micropylar end, unstained, mature and heavily lignified seed, cut by the freezing microtome. x26.

Figure 26. LS showing the endosperm, nucellus and a layer of granular deposits at the micropylar end at seven months after fertilization, stained in PAS/FG. x108.

Figure 27. TS of the integuments from mature seeds prepared by the Historesin procedure, sectioned at 2 μm and stained in ABB. x150.

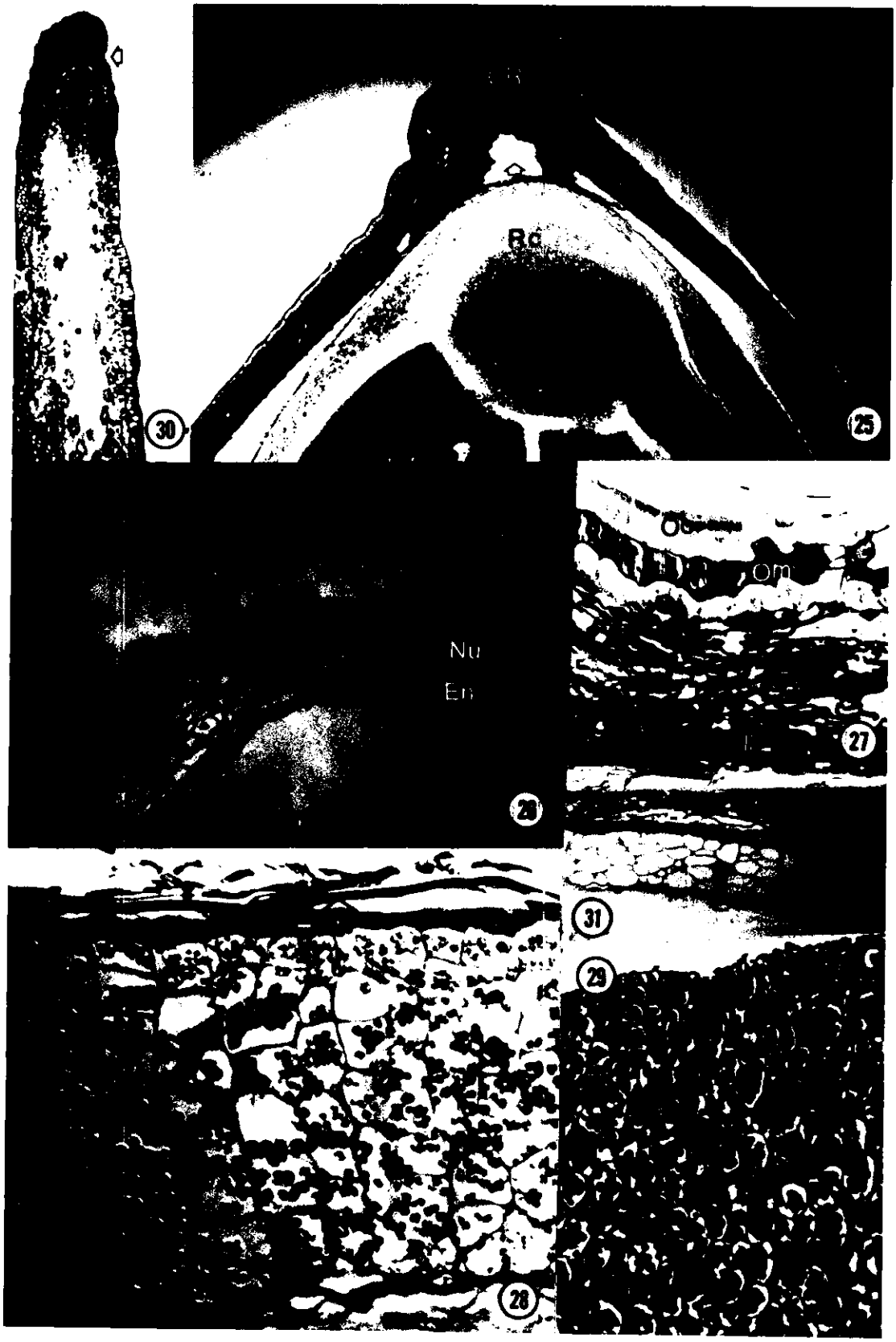
Figure 28. TS by Nomarski-interference optics; showing perisperm and the endosperm, stained in PAS/FG. x210.

Figure 29. TS showing stored material and thin cell walls in the cotyledon of a mature seed, stained in PAS/FG. x500.

Figure 30. LS through the caruncle of an immature seed; showing the micropyle. x75.

Figure 31. TS showing the endosperm, the nucellus and a portion of the mucilage layer of a mature seed stained in PAS. x800.

Key to Abbreviations: CR, caruncle; Rc, root cap; En, endosperm; Ii, inner integument; Nu, nucellus; Oo, outer cell-layer of the outer integument; Om, middle cell-layer of the outer integument; Oi, inner cell-layer of outer integument, and; Pi, compressed perisperm layer.


Rc
Nu
En
Os
Om

4.1.7.4 EMBRYO

The mature embryo of *M. volkensii* seed consists of two fleshy cotyledons and an axis bearing the plumule and a radicle (Figure 4).

At "fruit soft" stage, cell walls are thin and have no nutrient reserves (Figure 23). At maturity, a root cap is well developed (Figure 25). The cytoplasm and vascular bundles are filled with storage material: proteins, lipids and probably starch (Figure 29). Cell walls stain a light brick-red in PAS and a light blue in ABB as does the storage material.

5. DISCUSSION

5.1 MORPHOLOGICAL AND STRUCTURAL DEVELOPMENT OF THE FRUIT AND SEED

Seed and fruit development is without a seasonal pattern in *M. volkensii* in Kenya taking up to 13 months to mature (Figure 1). Many reproductive stages of development can be found on a single branch of the same tree at most times of the year. This makes it difficult to ensure that only mature seeds are collected in bulk for germination purposes. But different exocarp colour and mesocarp structure may serve as indicators of stages of maturity. Accordingly, when exocarp colour changes from green to yellowish-green this is a fairly reliable indicator of maturity (based on seed germinability). However, fruit colour at maturity also depends on the amount of cork on the fruit. When the deposition is heavy, fruit colour varies from greyish-yellow to grey. As a result, the ability to distinguish between mature and immature fruits is dependent on the knowledge of the reproductive cycle, and the morphological characteristics pertinent to each of these phases. The ovule structure appear to be closely correlated to the external development of the fruit.

Some of the striking features of the ovule development are growth of the integument to form a caruncle, the persistence and crushing of the nucellus, the reduction in the thickness of the cell layers of the endosperm at the

micropylar end and below the raphe, and the deposition of nutrient reserves in the endosperm and embryo tissue.

The development of a caruncle, its lignification and cutinization are initiated and completed early in ontogeny of the ovule. That the micropyle is crushed during the caruncle formation is inferred from the fact that a continuous micropyle is not evident in mature seeds. At the time when the caruncle development is complete, the micropyle is formed by the spongy parenchyma cells of the inner integument. The inner integument tissue is arranged such that it fills the central core of the caruncle from base to tip and the outer integument covers it (Figure 30). A canal running the full length of the seed is evident within the raphe (Figure 24). This may serve as a direct avenue for the entry of gases and water.

During development of the embryo sac and embryo, the nucellus is partly re-absorbed and crushed. In a mature seed it persists as a thick layer of compressed perisperm having a distinct cuticle.

Accumulation of nutrient reserves in the endosperm and embryo, and the development of a mucilaginous layer between the endosperm and the embryo start after the "fruit soft" stage. If the assumption that these changes are the major factors underlying the dormancy mechanism in the species, then there may be a period in the course of ovule development when seeds may be collected and made to germinate without a pre-germination requirement.

5.2 TEMPERATURE

The Kimpak was always kept wet and air circulation between the growth chamber and germination boxes maintained through ventilation holes. Therefore, temperature was the only uncontrolled environmental variable.

The optimum temperature was the same for total germination and germinative rate for both temperature regimes. Although germinative rates for higher temperatures above the optimum are better (Table 2), the corresponding total germination is very low (Table 1). Results of this study indicate that seeds of *M. volkensii* are capable of germinating between day temperatures 25° to 37°C. This may suggest that temperature is not the limiting factor in germination of intact seeds. Since neither air nor water were limiting, it is concluded that seeds are dormant and not just quiescent. This view is supported further by high total germination observed when seeds were cut and incubated under optimum temperatures. The fact that total germination at 42°C for both temperature regimes were very low show that imbibed seeds may not be able to tolerate exposure to high temperatures, even for short period of time (Table 1).

5.3 EMBRYO EXCISION

Two main categories of seed dormancy are distinguished: those related to structures external to the embryo, and those related to the properties of the embryo itself (Crocker 1916, Nikolaeva 1969). Excision of embryos from

seeds with physical dormancy (impermeability to water and gases), or from chemical dormancy (impermeability of seed coats to leachable chemical inhibitors), or from mechanical dormancy (prevention of radicle protrusion) allows germination to proceed (Nikolaeva 1969).

Excised embryos germinate significantly better (81%) than do intact seeds (9%) ($P \leq 0.01$). The fact that 9% of intact seeds germinated is probably an indication that germination of intact seeds was made possible because of cracks in embryo covers developed during extraction.

A much more widespread and better documented dormancy mechanism is the impermeability of integuments to water, or "seed hardness" (Ballard 1973; Barton 1965; Copeland 1976; Hamilton and Carpenter 1975; Rolston 1978). The term "hard seed" was introduced by Nobbe (1876) to represent seeds which are unable to imbibe water. In other cases integuments, perisperm and endosperm are impermeable to leachable endogenous inhibitors (Evenari 1949; Wareing and Foda 1957; Wareing 1965; Maguire 1976; Nikolaeva 1977; Maguire 1977; Maguire 1980), and to gases causing a deficiency in O_2 for respiration. In barley, O_2 supply to the embryo is restricted. In wheat, dormancy is attributed to the tensile properties of the pericarp and integuments in unripe grain, and of the pericarp epidermis in ripening grain (Durham and Wellington 1961).

5.4 PERMEABILITY OF INTEGUMENTS TO WATER

The first stage in the process of seed germination is water absorption. The three factors influencing this process are availability of water in liquid or gaseous form, permeability of integuments, perisperm and endosperm to water and the chemical composition of the seed of the seed (Mayer and Poljakoff-Mayber 1982, Ching 1972). The role of integuments, perisperm and endosperms in water absorption is discussed in relation to permeability and chemical composition of the seed.

Both intact and cut seeds absorbed water when soaked, but intact seeds absorbed only a small amount compared to cut seeds. This shows that integuments, perisperm and endosperm are not completely impermeable to water, but that they reduce the rate and total amount absorbed (Figure 10). When caruncles are broken off to expose the perisperm and endosperm, seeds absorb the same amount of water as that absorbed by intact seeds. This means that the seed absorbs the same amount of water whether integuments are intact or damaged. This may be interpreted as suggesting that integuments do not limit water absorption. This leaves the perisperm and endosperm, suggesting that either separately or together they account for the difference in water absorbed between intact and cut seeds.

The fact that embryos of intact seeds were stained is proof that the perisperm and endosperm allow at least some water to enter the seed. When the stain path was traced, it

became clear that water entered at the tip of the caruncle and then through the caruncle to the embryo (Figure 24). Cut seeds initiated visible germination after increasing in fresh weight above 64%. Intact seeds absorbed only 48% water. Intact seeds and seeds without caruncles failed to initiate visible germination. Failure of intact seeds to germinate may therefore be explained by the fact that the amount of water (48%) absorbed is not sufficient for the beginning of cell division.

The necessity for water in seed germination has long been taken for granted; but the actual amount of water needed, the mechanisms by which it is absorbed and its distribution have been given comparatively little attention (Stiles 1948). Water relations of seed tissues are also influenced by their chemical composition (Ching 1972). The main storage tissues in *M. volkensii* seeds are the cotyledons and the endosperm; their main nutrient reserves being crude fat and crude proteins. Oleic and linoleic acids are the major components of the crude fat (Figure 17). About 23% and 20% of the amino acids in the embryo and endosperm respectively are classified as hydrophobic (Lehninger 1982).

The process of germination involves water uptake and swelling which results in the production of considerable imbibition pressure. The pressure is of great importance in the process of germination as it leads to the breaking of integuments, perisperm and endosperm (Mayer and Poljakoff-Mayber 1982). If the embryo cannot imbibe water to

bring about sufficient imbibition pressure to surmount the mechanical restraint of integuments, perisperm and endosperm, then it must remain ungerminated.

Storage proteins of dry seeds are usually in crystalline form. About 80% of these are in storage organelles called protein bodies. The remaining 20% are distributed in nuclei, mitochondria, proplastids, microsomes, and cytosol (Larson and Beevers 1965; Newcomb 1967; Ching 1968.). Amino acids are classified as either polar or non-polar compounds, this classification also influencing their location in the structure of globular proteins.

In the pea and bean seeds, water penetration into large storage tissues is from the periphery to the interior. At the cellular level, the areas next to the cell wall and the spaces between the storage organelles become hydrated first then tissues swell and absorb more water until 67% to 150% water content on a dry weight basis is reached. Since starch granules, protein bodies and lipid bodies are water insoluble, metabolically functional organelles are the first to absorb water (Ching 1972).

Against this background, it is likely that water entry into the embryos of intact seeds is controlled by a self regulating mechanism. This view is supported by the fact that water absorption stops after the first 80 hours (Figure 10). In some legume seeds, water absorption is restricted because seeds posses a modified micropyle which operates

with a valve-like action (Corner 1951). The valve allows water vapour to diffuse out of the seed in a dry atmosphere, but closes firmly to prevent water entry in a humid atmosphere, or in liquid water (Villiers 1975).

Although it seems obvious that the micropyle in *M. volkensii* is not completely blocked, a gap in the perisperm and endosperm at the micropylar end has not been observed. Although water absorption mechanism is not clear, it is proposed that initial water absorption takes place via intercellular spaces, functional organelles, and via surfaces of hydrophilic storage bodies which may then swell. Swelling fills up inter-organelle spaces and hence stops further water absorption.

5.5 PERMEABILITY OF INTEGUMENTS TO GASES

Germination of seeds require expenditure of energy provided by oxidation (Lehninger 1982; Mayer and Poljakoff-Mayber 1982; Goodwin and Mercer 1983). Oxygen for this process must be taken in and carbon dioxide must be expelled. But, sometimes integuments, perisperm and endosperm restrict gaseous exchange. In this study high O_2 concentrations above the ambient (25% and 50%) failed to stimulate significantly higher total germination than in 21% oxygen. Seeds could also germinate in an environment with elevated CO_2 (0.65%) and depressed O_2 (15%), however, it is not whether this is due to high CO_2 or due to a drop in O_2. These observations are not unusual, as similar responses

have also been reported in seeds of *Stachys alpina* (L.), which not only failed to germinate significantly better at elevated O_2 concentrations than in air, but also could germinate in 100% N_2 (Pinfield et al. 1972). But seeds of many other species respond to elevated O_2 concentration by a significant increase in total germination. Among these are *Elaeis quineensis* (Jacq.) (Hussey 1958), and *Pisum sativum* L. (Spragg and Yemm 1959). As a result, integuments may be compared to a solution of phenolics which by absorbing O_2 act as a physicochemical O_2 barrier for the embryo (Coumans et al. 1976).

Based on these results, gaseous inhibition of germination seems insufficient to account for the failure of intact seeds to germinate. Since the endosperm and perisperm are not completely impermeable to water, it is likely that they also are permeable to water-dissolved O_2.

Germinative rates increased with increases in O_2 concentration. Therefore, the O_2 concentration seem to be the limiting factor for the germination of cut seeds under ambient conditions because when seeds were exposed to higher O_2 concentrations, they responded with increases in germinative rate. However, this increased germinative rate does not correspond to increased total germination. In fact the relationship between total germination and O_2 concentration is negative ($R^2 = 0.82$, $P \leq 0.01$; Figure 13).

5.6 MECHANICAL RESISTANCE TO EMBRYO GROWTH

Seeds which are in a state of physical dormancy are capable of normal growth when integuments, perisperm and endosperm are cut or removed (Ching 1972). Seeds which are cut at the micropylar end can germinate normally, but when cut at the chalazal end, or in the centre of the seed, then germination did not proceed normally. It does not make any difference to total germination whether seeds are cut at the micropylar end or whether the cut is extended from the micropylar to the chalazal end (cut A and D; Figure 11). In both cases germination proceeded normally and quickly. When integuments are damaged in other places germination could proceed but, instead of radicles emerging first, the cotyledons emerged first. From this premiss, it is clear that aspects of integuments, perisperm and endosperm permeability to water and gases alone do not adequately account for dormancy in this species. Observed modes of seed germination seem to suggest that mechanical properties of integuments, perisperm and endosperm also play a role. Integuments not only appear to limit the rate and the amount of water absorbed by seeds, but they also prevent the radicle from protruding mechanically (i.e. only a cut at the micropylar end brings about normal germination).

Information on mechanisms of mechanical dormancy in seed germination is meager (Villiers 1975) and the little that is available is seldom of direct value; such dormancy is inferred from the effects of external factors such as

specific environments, of treatments and of substances (Nikolaeva 1969). Crocker and Davis (1914) were the first to attempt to measure mechanical restriction of integuments on radicle emergence. In *Xanthium pennsylvanicum* Wallr., dormancy exists because the embryo cannot swell and exert adequate pressure to rupture the integuments (Esashi and Leopold 1968). More recently, Tao and Khan (1979) determined changes in the strength of lettuce endosperm during germination. They observed that these changes were not directly related to those of the radicle protrusion but in *Lactuca sativa* L. (Grand Rapids lettuce), red light initiates germination by production of an enzyme whose action enables the tip of the radicle to penetrate the endosperm (Ikuma and Thimann 1963). The enzyme was proposed to work by degrading the endosperm cell wall as a nutrient source for the growing embryo (Halmer et al. 1976).

Mechanisms for the release of radicle growth resistance in nature for *M. volkensii* seeds are not known, but it seems likely that injury acts by breaking or weakening integuments, perisperm and endosperm so that the imbibed and swelling radicle can emerge. Therefore, to account for mechanical restriction of integuments, perisperm and endosperm to embryo growth, two mechanisms are proposed: that the embryo does not acquire sufficient imbibition pressure to surmount the mechanical restraint of the integuments, perisperm and endosperm, and, that a chemical weakening of the perisperm, or endosperm, or both in

addition to the imbibition force of the embryo, is needed.

6. CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

1. Seeds take 11 to 13 months to reach maturity. Stages of fruit development lack a seasonal pattern, as a result it is difficult to differentiate mature from immature fruits. However, this study has shown that change in fruit colour may be a reliable indicator of seed maturity. Ripe fruits are yellowish green and sometimes grey or greyish yellow.
2. Seeds fail to germinate when left intact, but they germinate normally when excised. Therefore dormancy in intact seeds is imposed and maintained by integuments, perisperm and/or endosperm. There was no evidence of embryo related dormancy in seeds used in this study, but this conclusion does not rule out the possible need for an after-ripening period.
3. Integuments prevent germination partly by not allowing the embryo to absorb sufficient water for the beginning of cell division. This is shown by the fact that intact seeds absorb only 48% water (dry weight basis), and only 15% of this is absorbed by the embryo. Yet, visible germination start when excised seeds absorbed over 60% water. Therefore, although integuments, perisperm and endosperm may not be permeable to water they limit rate and total amount absorbed.
4. When the endosperm and perisperm are exposed by breaking

off the caruncle, no improved water absorption above that observed in intact seeds is observed. The presence of a canal in the raphe and staining of embryos after imbibing them in Eosin Red-yellowish is an indication that integuments are not completely impermeable to water. It is therefore concluded that the endosperm, and/or perisperm may be responsible for limiting water absorption.

5. When integuments, perisperm and endosperm are cut longitudinally at the micropylar end of the seed, normal germination can proceed. But, a horizontal cut in the centre, or a longitudinal cut at the chalazal end induce abnormal germination in which the radicle elongates but fails to shed the integuments, perisperm and endosperm. This may suggest that aspects of integument permeability to water or gases alone do not account adequately for dormancy in *M. volkensii*. Therefore, even if embryos in intact seeds were able to absorb sufficient water for growth to start, they might still fail to initiate visible germination because integuments, perisperm and endosperm restriction to radicle protrusion is not overcome. Consequently, integuments, perisperm and endosperm in addition to limiting the amount of water absorbed by the embryo, also mechanically prevent the radicle from emerging.

6. During transformation into seed, the ovule undergoes changes in form, structure and histochemistry. All these

changes occur late in ontogeny of the ovule. These changes include: endosperm cell wall thickening, compression and persistence of the nucellar tissue with its distinctive cuticle in mature seed and the accumulation of insoluble, non-polar and non-swelling storage material in the embryo and endosperm. Against this background, it is therefore inferred that (a) much of the water that needs to be absorbed by the seed for it to germinate is prevented entry by the perisperm, endosperm, or both, (b) the embryo does not acquire sufficient imbibition pressure to overcome the mechanical restriction of integuments, perisperm and endosperm. (c) a chemical weakening of the perisperm and/or endosperm in addition to the imbibition force of the embryo is needed.

7. Evidence for a germination inhibition mechanism through impermeability of integuments, perisperm and endosperm to gases was not found. Seeds could germinate at depressed O_2 and elevated CO_2 levels. Also, high O_2 (50%) concentrations fail to induce significantly better germination than that observed in air; besides, some water-dissolved O_2 must get in with the limited amount of water. Therefore, gaseous inhibition seems insufficient to account for the failure of intact seeds to germinate.

6.2 RECOMMENDATIONS

This study has shown that *M. volkensii* seeds can be germinated easily, as long as the integuments, perisperm and endosperm at the micropylar end are cut or removed. For operational purposes, such as in nurseries or in the field, mature fruits should be processed, seeds extracted and mechanically scarified, before sowing.

6.2.1 FRUIT COLLECTION

1. Collect mature ripe fruits (yellowish-green) for raising out-planting stock.
2. Best germination results were obtained when seeds were placed at temperatures between 30°C and 37°C. Therefore, seeds are to be sowed in nurseries where mean ground temperatures are within this range.

6.2.2 SEED EXTRACTION

1. Place a dry stony endocarp horizontally on a large cross-cut tree stump (about 30 cm in diameter).
2. Place a sharp pocket-knife blade mid-way across the endocarp and gently apply several hammer blows until a crack develops. The knife-blade must not penetrate deeper than the stony endocarp wall, otherwise seeds will be damaged.
3. Carefully penetrate and open the crack with the sharp edge of the knife-blade by pushing and twisting. The aim is to separate the two stony endocarp halves.

4. Pull out seeds from their locules in the stony endocarp.
5. Inspect seeds visually and discard those that are mechanically damaged. Seeds without seed coats or with partly removed seed coats will germinate well as long as embryos have not been damaged physically as well.

6.2.3 SCARIFICATION

1. Break the caruncle off the seed to expose the endosperm at the micropylar end. This will help in estimating the approximate position of the radicle and hence avoid cutting it.
2. Soak seeds in water at room temperature for six hours in order to soften the seed coats.
3. Cut longitudinally through integuments, perisperm and endosperm from the seed centre to the micropylar end (Figure 15).

6.2.4 SOWING

1. Place scarified seeds directly into containers filled with a germination medium. Keep the medium moist all the time. Visible germination should start by the fifth day after sowing.

6.3 FURTHER WORK

In order for people in dry areas to derive maximum benefits from *M.volkensii*, all possible end uses of the tree at all stages of development must be exploited, now that its

seeds can be germinated. In order to realise maximum benefits from the various potential uses of the tree, further studies on the following are recommended:

1. To determine the relationship between fruit maturity and different ecological zones of the country. This will provide data for planning of reforestation projects and help in estimating seed yield.
2. To develop a method for large scale seed scarification.
3. To carry out progeny and provenance studies with the aim of selecting populations with desired characteristics.
4. To investigate potentials for the manufacture of animal feed from fruits, since they are rich in crude fat and crude proteins.
5. To quantify various chemical compounds with potential industrial uses and relating these to various stages of fruit development.

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8. APPENDICES

Appendix 1. Water uptake by intact seeds, seeds with broken caruncles and seeds with cuts through integuments and endosperm at the micropylar end.

Time (hours)	Type of seed coat treatment					
	Intact seeds	Std. dev.	Broken caruncles	Std. dev.	Cut seeds	Std. dev.
0	7.17	4.29	5.66	3.60	7.95	7.39
3	18.77	7.14	19.30	6.26	25.70	11.36
8	27.58	8.12	28.55	6.27	36.20	11.75
48	37.25	9.16	38.49	7.36	49.72	10.44
60	40.65	9.87	42.78	7.43	55.50	12.74
72	43.06	10.18	43.80	7.20	56.28	12.77
84	45.29	10.46	46.63	7.63	60.16	14.08
108	46.35	10.14	47.12	8.40	63.96	15.63
120	46.86	10.20	47.71	9.15	70.61	14.48
144	48.31	10.06	50.27	11.22	82.24	21.08

Appendix 2. Chemical composition of different seed parts of M. volkensii (percent composition).

	Type of tissue					
	Embryos	Std. dev.	Endosperm	Std. dev.	Integuments	Std. dev.
Dry Matter	96.96	0.32	97.62	0.13	96.22	0.12
Fat	53.80	0.61	49.09	1.86	4.54	0.28
Protein	35.36	0.82	26.23	0.90	15.09	0.02
Neutral Detergent Fibre	8.92	0.91	12.08	1.12	62.38	0.84
Acid Detergent Fibre	6.07	0.62	6.93	0.19	69.90	2.01
Cellulose	5.45	0.36	4.66	0.06	9.50	0.72
Hemicellulose	2.85	--	5.15	--	2.48	--
Cutin and Lignin	0.61	0.27	2.33	0.13	60.37	1.52

Appendix 3. Fatty acid methyl esters in the cotyledons (T) and endosperms (E) of M. volkensii (% composition). (C = length of carbon chain : number of double bonds)

	Palmitic (C 16:0)		Stearic (C 18:0)		Oleic (C 18:1)		Linoleic (C 18:2)		Linolenic (C 18:3)		Arachidic (C 20:0)		Gadoleic (C 20:1)	
	T	E	T	E	T	E	T	E	T	E	T	E	T	E
	10.96	10.87	6.18	5.49	33.17	25.14	48.31	57.46	0.40	0.30	0.50	0.25	0.50	0.50
	11.04	10.97	6.07	5.54	33.23	25.18	48.46	57.18	0.20	0.31	0.50	0.23	0.50	0.58
	11.89	10.92	6.03	5.52	30.16	25.31	50.61	57.11	0.42	0.29	0.41	0.32	0.49	0.53
	11.65	10.79	5.98	5.47	30.33	25.28	51.01	57.25	0.21	0.38	0.31	0.44	0.49	0.38
	10.84	10.88	6.44	5.44	33.33	25.29	48.26	57.33	0.20	0.20	0.41	0.85	0.51	0.50
	11.05	10.81	6.35	5.41	33.26	25.19	47.92	57.37	0.22	0.30	0.55	0.38	0.66	0.53
	10.82	--	6.43	--	33.26	--	48.48	--	0.23	--	0.34	--	0.45	--
	10.87	--	6.37	--	33.26	--	48.63	--	0.22	--	0.33	--	0.33	--
	11.01	--	6.63	--	33.64	--	47.60	--	0.10	--	0.31	--	0.71	--
	11.09	--	6.50	--	33.68	--	47.77	--	0.10	--	0.26	--	0.60	--
MEAN	11.02	10.87	6.30	5.48	32.73	25.23	48.71	57.28	0.23	0.30	0.39	0.33	0.52	0.50
STD. DEV.	0.36	0.07	0.22	0.05	1.32	0.07	1.16	0.13	0.12	0.06	0.10	0.23	0.12	0.07

Appendix 4. Per cent amino acid composition of seed tissues.

	Amino Acid	Embryo Mean	Embryo Std. dev.	Endosperm Mean	Endosperm Std. dev.
1	Aspartic	8.04	0.20	7.73	0.04
2	Glutamic	25.08	0.12	25.08	0.21
3	Lysine	2.71	0.12	3.32	0.02
4	Arginine	12.55	0.13	11.08	0.00
5	Histidine	2.32	0.03	2.09	0.08
6	Phenylalanine	4.98	0.05	4.34	0.02
7	Leucine	6.95	0.04	6.02	0.06
8	Isoleucine	3.84	0.06	3.62	0.06
9	Methionine	2.14	0.03	2.14	0.02
10	Valine	4.70	0.07	4.12	0.10
11	Proline	2.34	0.03	2.98	0.14
12	Threonine	2.43	0.01	2.34	0.01
13	Serine	4.86	0.01	4.41	0.07
14	Cysteine	1.82	0.05	0.00	0.00
15	Alanine	2.70	0.08	2.02	0.02
16	Glycine	4.52	0.00	8.16	0.01
17	Tyrosine	2.45	0.06	2.74	0.04
18	Ammonia	1.84	0.10	1.80	0.04
% recovery (Protein)		96.27	1.00	90.96	0.33

Amino Acid

1 to 5 Highly hydrophilic, nearly always found on the outer surface of globular proteins.

6 to 10 Highly hydrophobic, found largely in the interior of globular proteins.

11 to 17 Amino acids of intermediate polarity, found in both the interior and exterior of globular proteins. (Source: Lehninger, 1982)