INFLUENCE OF TEMPERATURE AND MOISTURE CONTENT ON THE VIABILITY AND VIGOUR OF *ELEUSINE CORACANA* L. AND *AMARANTHUS HYBRIDUS* L. SEEDS DURING STORAGE.

BY

EVANS MUTEGI
B.Sc. (HONS.) NAIROBI

A THESIS SUBMITTED IN PARTIAL FULFILMENT FOR MASTERS OF SCIENCE
AT
THE UNIVERSITY OF NAIROBI

JANUARY 1998

DECLARATION

This thesis is my original work and has not been presented for a degree in any other university

EVANS MUTEGI

SIGNATURE 30/10/98

This thesis has been submitted for examination with our approval as supervisors

1.Dr. A.K. MISRA

BOTANY DEPARTMENT

UNIVERSITY OF NAIROBI

SIGNATURE .....

2.MR. D.K. KIAMBI

INTERNATIONAL PLANT GENETIC RESOURCES INSTITUTE (IPGRI)

REGIONAL OFFICE FOR SUB-SAHARAN AFRICA

SIGNATURE 30/10/98

DEDICATION

This work is dedicated to my parents; Mr. E. Nthambara and Mrs. L. Nthambara, who have worked tirelessly in support and encouragement at all stages of my studies.

ACKNOWLEDGEMENT

I am very grateful to Dr. A.K. Misra (Botany Department, University of Nairobi), for his role in ensuring that this study took off by facilitating the transport of some equipment from the Department of Botany to the National Genebank of Kenya, Muguga. His generosity in offering constructive comments, criticism and suggestions at all stages of this work can not be overemphasized. I also wish to convey my sincere gratitude to Mr. D. Kiambi (IPGRI-SSA) for his role in identification of the problem of study in this work, and for his supervision, creative criticism, suggestions, advice and encouragement which helped a great deal in the entire study. I would like to acknowledge the timely intervention by Mr. H. Kamau (IPGRI-SSA) who actually catalyzed my interest in the area of plant genetic resources conservation. He was instrumental in facilitating a collaborative working formula between, IPGRI-SSA, National Genebank of Kenya- KARI and Botany Department, University of Nairobi. In this regard, I am very grateful to the following : Dr. A.F. Attere (Director, IPGRI-SSA), for approving the collaboration and financial assistance towards this study; Prof. T.K. Mukiama (Chairman, Botany Department) for his support towards the collaboration; and Mr. E. Seme (Officer in-charge, National Genebank of Kenya - KARI) for allowing me to use their seed conservation facilities. I am deeply indebted to Mr. D.O Nyamongo (Head; Seed Technology Section - National Genebank of Kenya), who closely monitored my progress in the seed technology laboratory and was prepared at all times to offer practical suggestions, advice and criticism. My thanks also go to all the other staff at the National Genebank of Kenya for their co-operation and support. I especially convey my gratitude to Mr. J. I. Kamau (characterization section) and Mr. C. Ndiege (seed technology laboratory) for their involvement during my data acquisition.

I am very grateful to Ir. H. de Koeijer (IPGRI-SSA) for his assistance in the search and acquisition of the relevant literature, and also for his help in typesetting of this work. The final printout of this work was done at IPGRI – SSA, and so I am very grateful to all the member of staff involved.

My acknowledgement list would be incomplete without a mention of the following: 1. Dr. Richard Coe (ICRAF, Biostatistics) and Mr. P. Wanjohi (ICRAF, Computer applications), for their assistance in probit analysis. 2. Dr. Robin J. Probert (Seed conservation section - Royal Botanic Gardens, Wakehurst Place, UK) and Prof. J. Chweya (Crop Science Department - University of Nairobi) for accepting to read my thesis work and especially for their separate comments and suggestions which were very useful. 3. Mr. P. Kamau (Katumani Dryland Research Station - KARI, Machakos) for providing finger millet seeds used in this study. 4. Mr. Mugambi G.K (National Museums of Kenya) for his assistance time and again in computer applications. 5. The Director Nuclear Physics Institute, University of Nairobi, for allowing me to acquire double distilled water from the institute for my work. 6. Ms. Mary Kimani (National Genebank of Kenya - KARI) and Ms. Pauline Mbuguah (Botany Department - University of Nairobi) for their dedication in typing different parts of this work. 7. All friends and fellow post-graduate students who stood with me in any way during this study through encouragement and prayers. To the Almighty God be all Glory and Honour.

Last but not least, I am grateful to the Board of Post-graduate Students - University of Nairobi, for nominating and awarding to me a scholarship in support of the entire masters programme.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND SYMBOLS

AOSA	Association of Official Seed Analysts
cm	centimetres
Deg. C	Degrees centigrade
DF	Degrees of freedom
FAO	Food and Agriculture Organization of the United Nations
g	Gramme(s)
IBPGR	International Board for Plant Genetic Resources
ICRAF	International Centre for Research in Agroforestry
IPGRI	International Plant Genetic Resources Institute
ISTA	International Seed Testing Association
KARI	Kenya Agricultural Research Institute
m	Metre(s)
mm	Millimetre(s)
m.c	Moisture content
p	Probability
r	Correlation coefficient
SSA	Sub-Saharan Africa
Temp.	Temperature
Var.	Variety
$\mu Scm^{-1}g^{-1}$	Micro Siemens per centimetre per gramme

ABSTRACT

The present study was carried out at the National Genebank of Kenya-KARI, Muguga between September 1996 and August 1997. The study aimed at determining the progress and extent of deterioration in seeds of two traditional crops at various combinations of constant storage temperature and moisture content, and to quantify the resultant viability decline, with a view to predicting longevity at alternative storage conditions.

Seeds of finger millet (*Eleusine coracana* L. Gaertn. Var. KAT/FM-1) and those of Amaranth (*Amaranthus hybridus* L. Ssp. *cruentus*) were stored in hermetically sealed laminated aluminium foil packets, for up to 252 days at different constant environments, which included combinations of temperatures ranging from 15°C to 40°C and moisture contents ranging from 5.3% to 17.3% (fresh weight basis).

To determine the progress and extent of seed deterioration at each of the different combinations of temperature and moisture content, seeds were sampled periodically from storage for vigour and viability determination. Two procedures were used to evaluate vigour. These were; percentage normal (first count); germination and electrical conductivity of seed leachate. Viability was expressed as a percentage of the total germinated normal seedlings at final count.

At most of the storage combinations, both viability and vigour declined during storage, for the two species. At relatively higher storage temperatures ($\geq$ 25°C) and moisture contents ($\geq$11.3%), depending on the species, viability and vigour declined completely during storage, at rates dependent on the treatment combinations. The decline was generally rapid at higher temperature and/ or moisture content combinations. For both species, the electrical conductivity test was unable to detect significant deterioration of seeds during storage.

The effect of storage conditions on viability was quantified using the simple viability equations. Seed survival curves (percentage normal germination, plotted against storage period) were fairly described by negative cumulative normal distribution. For each of the two species, the estimated periods for viability to fall to 50% (p_{50} period) was shorter the higher the storage temperature and /or moisture content.

Simple viability constants were for each of the two species obtained, and used to predict longevity at various non-extreme hypothetical storage conditions. At relatively low moisture content ($\leq$10%) and temperature($\leq 15^{o}$C) combinations, seeds of *A. hybridus* were predicted to store much longer than those of *E. coracana.* At storage combination of 5% moisture content and a temperature of 5^{o}C for example, seeds of the former are predicted to have a 28-fold greater longevity than the latter. The constants obtained in the present study could be applied in predicting storage life of seeds of the two species, especially in the short- and medium-term storage conditions.

CHAPTER ONE

1.0 INTRODUCTION

The importance of seed storage has been recognised ever since the days of primitive agriculture, when man began settling down from the usual hunter and gatherer lifestyle. As domesticating wild plants started for various needs, the need for storing seeds arose. Ancient literature and religious scriptures are full of references to quality and proper seed storage methods. It was not until the advent of organised agricultural practices that seed storage technology became fully significant to man. Since then, seeds have been stored for varying periods of time and reasons.

Seeds may be stored by farmers in such a way that their levels of germination and vigour are least affected at the time of sowing in the forthcoming or subsequent season(s). For this, agricultural seeds need to be stored for one or two cropping seasons or years. Secondly and equally important, some quantity of seed may be stored for 2 to 3 years (or more) for utilization in commercial seed production, the so called "carry over stock" by seedmen. The third and the much more difficult task in seed storage is for the conservation of plant genetic resources in genebanks for utilization in crop improvement programmes by present and future generations. In this, seeds of a wide range of species and cultivars are stored for prolonged periods of time, normally extending to hundreds of years.

While factors that influence the lifespan of a seed lot (population) during storage are the same for the above three categories of seed storage, the stringency of measures taken to minimise seed deterioration vary. The basic objective, however, is to maintain genetic integrity by minimising the loss of seed viability and vigour for a desired period of time. All seeds lose viability during storage with a loss of vigour preceding the loss in germination (Agrawal, 1986). There is considerable evidence that damage to chromosomes, some of it resulting in heritable changes, takes place as seeds loose their viability. Deterioration of seeds under storage is, therefore, an important issue to consider. Especially so, in relation to the time and the storage environment. This is because the success in using

seed germplasm for plant breeding or other purposes will depend on their genetic integrity. For the conservation of plant genetic resources therefore, the genetic integrity should be preserved in storage for as long a period as possible.

Temperature and moisture content are the two most important factors which influence the deterioration of seeds in storage (Delouche *et al.*, 1973; Harrington, 1970). It is important, therefore, to investigate and understand the storage physiology of seeds, which are held for conservation in genebanks, in relation to temperature and moisture content of storage. This will be a fundamental step towards their effective management under long-term conservation.

1.1 Conservation of plant genetic resources

1.1.1 The importance of conserving plant genetic resources

Today the world is well endowed with a rich diversity of plant genetic resources, an accumulation of the past and present biological heritage. From the early days of primitive agriculture, farmers have been selecting crops for various reasons, such as yield, adaptability and nutritional value. The term plant genetic resources has often been used to describe the total genetic diversity of cultivated species and their wild relatives, much of which may be valuable to breeders (Jackson and Ford-Lloyd, 1990). The role played by plant genetic resources so far in developing new crop varieties through breeding, cannot be overemphasised. Recent progress in genetic improvement of crops has resulted in dramatic environmental adaptability and increased yields of many food crops including rice, wheat and maize, thus avoiding worldwide food catastrophes and famines. Moreover, plant genetic resources have also played major roles in combating major diseases and crop pests.

The estimated number of plant species in the world exceeds a quarter of a million (Wilson, 1988) and many species have yet to be discovered, classified and documented systematically. Although, the earth is currently supporting this great wealth of plant genetic resources, loss of global biodiversity has been identified and considered as one of the most critical and important environmental issue of the 1990's. Many estimates draw the attention to an alarming rate of loss of plant species at

every level, posing a real threat to biodiversity.

1.1.2 Genetic erosion

Throughout the world, widespread deforestation, in addition to over-exploitation of species, destruction and modification of natural habitats, have resulted in the extinction of many plant species. Moreover, natural calamities such as desertification, drought, floods, landslides and fires have contributed to disappearance of many plant cultivars. With modern agricultural practices and an ever increasing human population, the emphasis is on high yields. This has led to the genetic erosion within many important traditional food crops as farmers shift to monoculture and consequently replacing landraces (or traditional non-improved varieties) with modern high yielding varieties of crops. Inter- and intra-specific diversity is thus unwittingly being replaced by uniformity. Germplasm is disappearing at a very alarming rate and unless conserved now, valuable genes will not be available when required to respond to new agricultural challenges in the future. Traditional knowledge on the local varieties of landraces and wild plants and their use is also disappearing fast. This is mainly due to changes in the social economic structures of many local communities living in the rural areas. Such knowledge is of great importance especially during collection of landraces and their wild relatives for conservation, and should thus be tapped now.

The future agricultural problems are not predictable. New diseases and pests may arise and changes may occur in the atmosphere such as the 'greenhouse effect'. In the event of such changes, it is plant genetic resources that will provide genes, capable of conferring disease and pest resistance and adaptation to changing environments. Therefore, plant genetic resources have to be collected now and conserved for use today and in the future.

1.1.3 Global conservation efforts

Plant genetic resources conservation is not exactly new, but now worldwide attention has been focused on the significance of biological diversity. For example, as early as in the 1970's, an FAO

linked International Board for Plant Genetic Resources (IBPGR) was established with a mandate to further the study, collection, conservation, documentation and evaluation of genetic diversity of important crop plants and their wild relatives. Recently in 1994, an autonomous organization, IPGRI, was established from IBPGR to advance the conservation and use of plant genetic resources for the benefit of present and future generations. The establishment of IBPGR and now IPGRI, was only after the realisation that valuable genes were being lost through genetic erosion. Both IBPGR and IPGRI have contributed immensely to the conservation of crop genetic resources, leading to considerable progress in germplasm collection and conservation in Africa and the rest of the world.

Most recently (1996) an International Technical Conference on Plant Genetic Resources, was held in Leipzig, Germany. This led to negotiations and approval by 150 countries, of the first Global Plan of Action for conservation and use of plant genetic resources. This was prompted by the urgency needed to protect the world's rapidly shrinking supply of plant genetic resources for food and agriculture. The conference, in addition, adopted the "Leipzig Declaration", a political statement stressing the link between world food security and conservation, and sustainable use of plant genetic resources. Issues of priority towards achieving this goal were identified and are aimed at strengthening; *in situ* conservation and development; *ex situ* conservation; utilization of plant genetic resources; and; institution and capacity building.

It is apparent, therefore, that plant genetic resources are critically important to agricultural progress in the coming millennium, and that they shall play a major role in ensuring meaningful food security. Between 2010 and 2025 the world population is projected to grow by over one thousand million, nearly a 97% growth in developing countries (Population Reference Bureau, 1997), already affected by poverty and malnourishment. Such population increase requires an increase in food production, never achieved before. More intensive, medium and high in-put farming methods will contribute to increase productivity in many parts of the world. For a large number of developing countries, however, this will only come with improvements in low in-put agriculture, under difficult

environmental conditions. Plant genetic resources will most certainly play a key role in achieving these new levels of production, chiefly through plant breeding and development of currently under-utilised and neglected crops. For all practical purposes, therefore, genetic resources of plants are the raw materials for future food security, and should be conserved now.

1.1.4 Conserving African indigenous crop germplasm

Much of Africa's agricultural development and cultivation is based mainly on subsistence farming. In this form of farming, families grow crops to meet almost all their food needs. A wide diversity of food crops including both cultivated and weedy species, have been grown under such mixed cropping systems. This ensures stability of total yields since each crop species has different growing requirements at different times of growing season thus buffering any environmental stresses(Chweya,1994). Included in the wide diversity of crops used, depending on locality, are several important cereals, roots and tubers, legumes and many vegetable crops (Chweya,1994; Okigbo,1983;1994). Okigbo(1983), listed 150 food crops consumed by man worldwide, 115 species of which, are indigenous African food crops, that contribute significantly (at least locally) to the subsistence requirements of many rural populations. Thus, Africa is a centre of origin and diversity of many important crops including; chickpea, finger millet, (Ethiopian centre of diversity), African rice and tuber crops (e.g. yams) (West African centre of diversity).

Much of the wide diversity in African indigenous crops is, however, threatened with erosion, due to several factors. In the recent past, for instance Africa has strived to accommodate and feed a rapidly increasing human population. Current figures do not show any let-up in this alarming trend of human population increase. In fact, it is estimated that Africa will have to feed and accommodate a doubled human population in the next 25 years (Population Reference Bureau, 1997). Much of this increase is bound to affect the Sub-Saharan Africa, already experiencing a lot of pressure in accommodating more than 80% of Africa's population. Previous increases in human population have led to widespread destruction of natural environment and resources, as many people expand their farms

and/ or grazing grounds. Agricultural systems on the other hand, have changed from the originally diversity based mixed systems to high yielding monocultural systems. Consequently, most farmers throughout Africa, have been abandoning traditional varieties of crops in preference to introduced high yielding varieties. In total, great genetic erosion of important food crops has thus occurred both in their traditional areas of cultivation and wild relatives in their indigenous niches.

Indigenous crop genetic diversity is of vital importance to future efforts of providing sustainable increases in agricultural production, especially through genetic manipulations. Great efforts should, therefore, be geared towards conservation of these germplasm, for present and future utilization. It is in recognation of this fact that a number of African governments are initiating programmes intended to acheive this noble goal.

Currently, only a limited number of indigenous African crop species are undergoing routine breeding/ or research of sufficient scope. Many crops of regional or local importance have been relatively ignored by most scientific studies. Ironically, though, this goes on against a rich background of information on the vital role these indigenous crops have played in feeding the majority of rural populations throughout Africa. Included within this rich diversity of indigenous crops are important cereals such as, finger millet(*Eleusine coracana* (L.) Gaertn.) and leafy vegetables such as, amaranth(*Amaranthus hybridus* L.).

1.1.4.1 Finger millet

Finger millet (*Eleusine coracana* (L.) Gaertn.), is a member of Gramineae. It is also known as African millet, Koracan (English) Wimbi, Ulezi (swahili) and ragi (India). It is a tufted annual crop, growing 40 - 130 cm tall, taking between 2.5 and 6 months to mature with narrow grasslike leaves and many tillers and branches. The head consists of a group of digitally arranged spikes (and probably the source of the name; "finger" millet).

The genetic resource potential of finger millet is immense, as necessitated by its long ancestral history and wide geographical distribution. It is said to have probably originated in the highlands of

Uganda, with its center of diversity in Ethiopia. The crop is now cultivated in many parts of the world.

Various finger millet landraces are reported to possess genes for blast resistance, robust growth, early vigour, large panicle size, high finger number and branches, and high density grain (Kempanna, 1975; National Research Council, 1996). Moreover, some water efficient and long glume types, important for semi-arid conditions and increasing grain size respectively, have been reported (National research Council, 1996). These and more are important genotypes that could transform this crop, for the present and future utilization, in feeding the world's ever increasing human population.

Finger millet is a versatile cereal with several major uses as both human food and animal feed. Nutritionally finger millet grains could be of great value as a preventive against malnutrition. For example, its main protein fraction (elusinin) is reported to have good amounts of nutritionally crucial amino acids such as; tryptophan, cystine and methionine (National Research Council, 1996). These amino acids are deficient in most other cereals. Finger millet grains are in addition a rich source of minerals such as calcium, phosphorus and iron.

The small grains are usually ground into flour and boiled into either a thick porridge "Ugali" or a thin one "Uji", two delicacies for many people in East Africa. The flour may also be used in making bread and other baking products. Finger millet malt (sprouted seeds) is often produced as a food in a few places, particularly recommended for infants and elderly, owing to its nutritional value and easy digestibility. Much of the finger millet malt in Africa, however, is used to make traditional beer. When popped, the grains of finger millet are widely enjoyed in a tasty form especially in India. Finger millet straw makes good fodder - better than that from pearl millet, wheat or sorghum, and is said to contain up to 61 percent total digestible nutrients (National Research Council, 1996).

However, finger millet is neglected scientifically, both locally and internationally, compared to research lavished on wheat, rice or maize. Worse still, even many countries that grow it, have left it to languish in uncertain state as a "poor person's crop", a "famine food" and even worse, a "bird seed". Consequently, in recent years this crop has started a threatening slide in production even in Africa

(National Research Council, 1996). In Kenya for example, a recent survey throughout the country by the National Genebank of Kenya in collaboration with the International Plant Genetic Resource Institute - Sub-Saharan Africa (Unpublished report, 1996), noted that, the crop is threatened with genetic erosion in most parts of the country where it is grown. The major causes reported are; introduction and preference of other crops including cash crops; changes in farming systems and; other socio-economic factors in the growing areas. Where its genetic diversity is still intact, it was noted that it is either inter-cropped with other crops (such as maize and sorghum) or grown under shift cultivation.

Given the current and potential uses of this versatile cereal crop in feeding the world's ever increasing human population, more research, recognition, and favourable policies should be put into it, in an effort to promote its conservation and utilization.

1.1.4.2 Amaranths

Amaranth, a little known crop of the family *Amaranthaceae,* is grown either as a grain crop or as a leafy vegetable. The genus *Amaranthus* is the most significant, with well over 60 species (Willis, 1973) and distributed widely throughout the tropical and sub-tropical regions of the world.

Amaranthus hybridus is a weedy amaranth and one of the most common leafy vegetables. It is a herb that grows up to 1.5m tall and said to have its origin in tropical America, and spread to tropical areas. Quite oftenly, *A. hybridus* grows wild on moist ground, in waste places or along roadside.

Like most amaranth leafy vegetables, *A. hybridus* is an excellent choice. It is fast growing, high yielding and less susceptible to soil-borne diseases, in addition to showing favourable reaction to fertilizer and organic manure (Grubben and van Stolen, 1981). In the local markets, bundles of leafy shoots as well as uprooted young plants are offered at cheap prices in many parts of Eastern and Central Africa (National Research Council, 1984).

Amaranth leaves are nutritionally similar and even in a few cases better than other leafy vegetables, such as spinach. They are a good source of carotene, iron, calcium, vitamins A, B and C, and also folic acid (Grubben and van Stolen, 1981; National Research Council, 1984; Chweya, 1994).

The amino-acid composition of *A. hybridus* leaf-protein has a high chemical score, comparable to that of spinach (National Research Council, 1984). High levels of nutritionally critical amino acids, like lysine and methionine, have been reported (Koch *et al.*, 1965; Imbamba, 1973) in leaves of 13 amaranth species including *A. hybridus*.

The genetic resource potential in *A. hybridus* is immense. Due to their weedy nature, *A. hybridus* types must have developed valuable competitive characters. They are thus worth serious attention as breeding material in other cultivated forms and especially the grain amaranths, through cross breeding. Grain amaranths do not carry the same potential for weediness. For example, *A. hybridus* is the wild progenitor of the present day cultivated *Amaranthus hypochodriacus* (a grain amaranth) and easily exchanges genes with it (National Research Council, 1984). Thus, *A. hybridus* is an invaluable source for such desirable characters as fast maturation, disease and pests resistance, and wider adaptability in cultivated forms of *A. hypochondriacus*.

Though not actually threatened with extinction, weedy and wild species of amaranth are facing the danger of genetic erosion. Few of these have been domesticated, while most grow as weeds and wild species in virgin, undisturbed or cultivated areas. With increasing pressure on agricultural land, their ecological niches are fast disappearing. Genetic erosion, hence, is bound to be rapid. To date, few of these species have been systematically collected, documented, characterised and evaluated. This is primarily because of the low priority and status accorded to these crops nationally and internationally. This is regrettable considering the significant contribution these local vegetables have played in the nutritional well being of many rural populations, especially in Africa. Furthermore, beyond Africa these vegetables have a significant role to play in widening the world's currently narrow food base.

It is important, therefore, that germplasm of these vegetable amaranth be systematically collected, conserved, characterised and documented for present and future use. It is unfortunate, however, that little is known of amaranth seed storage physiology. Such storage studies are, therefore,

important for effective management of amaranth seeds under long-term storage. This would minimise genetic erosion of germplasm arising as a result of long-term storage ageing processes.

1.1.5 The role of genebanks/ seed banks in plant germplasm conservation

There are two main options to germplasm conservation namely, *ex situ* and *in situ* conservation. As for the *in situ* (on-site) conservation, plant species are maintained within their natural habitats as natural populations and communities but in stable and protected environments. The establishment of natural or genetic reserves, recognises the long-term objectives and the need for continued evolution of the conserved species within their natural environments. *In situ* conservation is an option, applicable mainly to forest and pasture species, wild relatives of crops and primitive forms of crop plants. The *in situ* conservation of cultivated species is primarily concerned with the on-farm maintenance of traditional crop varieties (landraces) and with forage and agroforestry species (Hong *et al.*, 1996). The main disadvantage of *in situ* strategy for germplasm conservation lies in the fact that, the conserved materials are prone to natural calamities (such as droughts, floods, fires, landslides etc), destruction of natural habitats and changes in land use systems. The problem of accessibility and ease in exchange of germplasm, in addition to the cost of protecting the conservation sites and the opportunity cost of the land set aside are other additional disadvantages.

Ex situ conservation option on the other hand maintains plant genetic resources away from their natural areas of growth. Propagules and other plant parts may be collected from the field to a suitable form of *ex situ* storage facility. Orthodox seeds (seeds capable of tolerating desiccation and freeze storage) and pollen may be maintained in cold storage of different types. Whole plants and perennating organs may be maintained in a field collection, arboreta or botanical gardens and *in vitro* collections in the tissue culture laboratory under slow growth conditions or cryopreservation.

Thus, different species and situations will require complementary *in situ* and *ex situ* conservation strategies. *Ex situ* conservation is, however, a major option for crop genetic resources conservation. This is mainly due to reduced space requirements, especially for seed banks and *in vitro*

banks. Natural evolutionally process is, however, interrupted when materials are conserved *ex situ* in addition to selection due to differential survival in storage and during germplasm rejuvenation.

In recognition of the threat to its genetic heritage, the Government of Kenya with technical and financial assistance from the Federal Republic of Germany, established a crop genetic resources centre, the Genebank of Kenya, at Muguga. The genebank was fully operational by mid - 1988 as a service institution within the frame work of Kenya Agricultural Research Institute (KARI). Among the responsibilities of the Genebank of Kenya are; to explore and collect germplasm at National level; to exchange germplasm and relevant information; to multiply, rejuvenate, characterise and preliminarily evaluate germplasm; to co-ordinate crop plant genetic resources activities in Kenya and; lastly to undertake long-term conservation of germplasm (Seme, 1991). The Genebank of Kenya lays its priorities for crop collection on the economic and social importance of any crop, its genetic state of development and the degree of genetic erosion. Special attention is given to those crops with unique diversity, mainly the landraces of indigenous crops. Currently, the Genebank of Kenya is holding a total of more than 40,000 accessions of seeds in cold storage. Ever since its establishment, the genebank has distributed more than 2,000 accessions of seeds both locally and internationally to various users including plant breeders. In addition to the germplasm held under cold storage, about 400 accessions of recalcitrant seeded (seeds not able to tolerate desiccation and freeze storage) plants and vegetatively propagated crops are being held at various sites in Kenya as field genebanks.

Genetic resource centres (seed banks/Genebanks) may take responsibility for three types of collections, namely; base collections, active collections and duplicate collections (of base collections). Base collections are for long-term conservation (typically considered as 10 to 100 years or more). Samples will be drawn from base collections only when they can not be obtained from the active collection. They are therefore, considered to be collections for posterity . For security, samples of the base collections are often kept in different locations away from the corresponding collections, as duplicate collections. Active collections are for medium-term conservation (usually 3 to 5 years but at

times up to 20 years). For those genebanks with both active and base collections, it is common practice to have similar accessions in both types of collections. However, only the accessions stored in the active collections facilities may be drawn out for regeneration, multiplication and distribution, or for evaluation. Active collections are thus, likely to be larger than the base collections and the rate of depletion is likely to be more rapid.

Once a decision has been reached on how long it would be necessary to maintain germination capacity of the seed lots, the storage environment will be an important issue to consider. Ambient temperature and seed moisture content are the two most important factors that influence deterioration of seeds (Delouche, *et al.*, 1973; Harrington, 1970) and hence their life span in storage.

Genebank seed storage standards are classified as either acceptable or preferred (FAO/ IPGRI, 1994). While acceptable standards are minimal, but considered adequate (at least in short-term), preferred standards are higher and thus safer. Acceptable seed storage conditions for base collections (long-term storage) are, sub-zero temperatures (<0°C) with 3-7% seed moisture content (depending upon species), while -18°C or cooler temperatures with 3-7% seed moisture content is the preferred standard (FAO/ IPGRI, 1994). It is now recommended that active collections (medium-term storage) be kept under conditions which ensure a viability level of at least 65% for 10-20 years (FAO/ IPGRI, 1994). The precise storage regimes used to fulfil this objective vary and depend on the species stored, the prevailing ambient environment and the relative local costs of (principally) electricity. Cromartly *et al.* (1985) for example, classified active collections as those housed at temperatures between 0°C and 10°C.

Seed deterioration under the above recommended long-term and medium-term storage conditions will be slow, with the lowest rate of deterioration expected to occur under long-term storage conditions. Nevertheless, it is important to know the probable time taken for seed viability to fall sufficiently low so as to regenerate fresh seed stock. Viability monitoring tests, carried out periodically during storage, will determine when such regeneration is actually due. These monitoring tests are,

however, expensive in addition to depleting valuable seed samples. Great viability losses are on the other hand associated with genetic changes in surviving seeds (Robert, 1975; IBPGR, 1976), such that, regeneration of seeds, whose levels of viability are sufficiently low, is a very necessary genebank operation.

In order to reach a compromise between the frequency of viability monitoring tests and the actual regeneration period, a seed storage investigation is necessary. Information about the rates at which seeds deteriorate under various unfavourable storage temperature and moisture content conditions, will enable predictions to be made about longevity of seeds under optimal storage conditions. Using viability equations that were proposed earlier (Roberts, 1960; Ellis and Roberts, 1980a) it is possible to suggest suitable monitoring frequencies.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Seed storage physiology

Seeds of different species respond differently to the environment before and after storage. Accordingly, three main categories of seed storage behaviour are now recognised. Roberts (1973a) defined two categories of seed storage namely orthodox and recalcitrant. Seeds of species with orthodox storage behaviour can be dried to low moisture content (5 % or less) without damage and, over a wide range of environments, their longevity increases with decrease in storage moisture content and temperature in a quantifiable and predictable way (Roberts, 1973a). Recalcitrant seeds in contrast, are defined (Roberts, 1973a) as, those which cannot be dried below a relatively high moisture content without sub-cellular damage and hence, can only be stored for relatively short periods in a moist condition. Recently, a third seed storage behaviour intermediate between orthodox and recalcitrant categories, has been identified (Hong and Ellis, 1996). This was as a result of accumulated evidence, that the previous categories of seed storage behaviour as defined by Roberts (1973a), could not satisfactorily account for all observations on seed storage behaviour. For example, Teng and Hor (1976), showed that seeds of both starfruit (*Averrhoa carambola*) and paw paw (Carica Papaya) withstood desiccation to around 10-12% moisture content and could be stored successfully in hermetic containers at these levels of moisture content. In contrast to orthodox seeds, however, they lost viability much more rapidly in air-dry storage at 0°C than at warmer temperature of 12°-21°C. Tompsett (1984), made a further observation on seeds of *Araucaria columnaris* whose longevity could be increased in air-dry storage by reducing storage temperature and moisture content, but only within a limit. Further reduction in moisture content below 12% reduced the viability of *A. columnaris* with desiccation.

Hong and Ellis (1996), based work on seeds of arabica coffee (Ellis *et al.*, 1990; 1991a, Hong and Ellis, 1992) robusta coffee (Hong and Ellis, 1995), oil palm (Ellis *et al.*, 1991c), papaya (Ellis *et*

al., 1991b) and several citrus species (Hong and Ellis, 1995), to demonstrate and define the intermediate seed storage behaviour. They defined intermediate storage behaviour as one under which, mature whole seeds are able to tolerate desiccation to seed moisture content in equilibrium at 20°C with about 40-50% relative humidity. Further desiccation often reduces viability and always results in more rapid deterioration in subsequent hermetic storage. Thus, the essential feature of intermediate seed storage behaviour is that the negative relation between seed longevity in air-dry storage and storage moisture content is reversed at values below those in equilibrium (at 20°C) with about 40 - 50% relative humidity.

2.1.1 Quantification of orthodox seed deterioration

Deterioration is a normal ageing process in all organisms which ultimately ends in death. Deterioration in seeds may be defined as an increased probability of death of an individual seed per unit time as age increases, failure to germinate being indicative of seed death (Ellis and Roberts, 1981). Seed ageing, however, cannot be considered solely as a function of time, since the environmental factors during storage are important. It has generally long been known that, the greater the moisture content and storage temperature of orthodox seeds, either singly or in combination, the shorter is the period of seed survival (Roberts, 1973a). This qualitative statement, however, is only of limited use in designing and managing seed storage systems unless the relationship between longevity (period until seed death) and the environment is quantitatively described.

Changes in some quantifiable traits occur when seeds deteriorate, some of which have been used to estimate and quantify deterioration. Perhaps, the most widely accepted and useful index of seed deterioration is the reduction in viability. A viable seed will germinate and develop into a plant, given favourable conditions and provided any dormancy that may be present is removed. Viability of a seed or seed lot may be defined thus, as its ability to germinate under favourable conditions (Ellis, 1984). Consequently, the period until which a seed/seed lot is unable to germinate under favourable conditions, is its lifespan or longevity.

2.1.2 Seed survival curves

The lifespan of an individual seed cannot be investigated directly since its germination is an "all or nothing" response (i.e. when exposed to conditions suitable for germination a seed either germinates or not). Nevertheless, it is possible to infer what happens to individuals, by studying the distribution of lifespans from a sequence of germination tests sampled from the same population during storage. Roberts (1960), while working with seeds of wheat and other cereals, observed that when the number of seeds dying is plotted against time, the frequency of deaths follows a cumulative normal distribution. He further observed that, a plot of germination against time, gave a sigmoid curve of negative slope, the seed survival curve. The sigmoidal pattern of loss in percentage viability demonstrates that individual seeds within a homogenous population die after different periods in storage (Roberts, 1960; Ellis, 1984). The slope of the sigmoidal survival curve is a measure of the variation in the times at which individual seeds die (Ellis and Roberts, 1980a) such that, the steeper the survival curve the less the seed-to-seed variation (Ellis, 1984). From such sigmoid curve it is imperative, therefore, that only a few seeds die per unit time earlier on during storage, but as the storage period increases, the number of seeds dying per unit time increases. A maximum value is reached when half of the seeds have died. The number of seeds dying thereafter declines in a manner that mirrors the previous increase; that is, the frequency of deaths in time is symmetrical about the point in time where 50% of the population has died (Ellis, 1984).

Different studies have shown the sigmoidal pattern of seed survival curves to hold for different orthodox seeded species (e.g. Roberts, 1960; Ellis and Roberts, 1980b; Bennet-lartey, 1991; Lotito and Quagliotti, 1993; Bonner, 1994). Roberts (1972a), pointed out that the most convenient way of dealing with a cumulative normal distribution data such as the one in seed survival, is to plot percentage germination on a probability scale against time, or what amounts to the same thing, to plot the probit of percentage germination against time. When this is done a sigmoidal survival curve becomes a straight line of negative slope (Roberts, 1972a; Ellis and Roberts, 1980a; Ellis, 1984).

2.1.3 Viability equations

Many attempts have been made to quantify the relationship between orthodox seed storage environment and survival. Goodspeed (1911) and Groves (1917), are possibly the earliest investigators who quantified the relationship between high temperatures and the longevity of barley and wheat seed, respectively. Some thirty years later Hutchinson (1944), made a major advance by defining longevity periods (start of death and complete death) and also incorporating a term to describe the effect of moisture content on wheat seed longevity. Hutchinson's equations were, however, not widely applied, possibly because they had been determined at high temperatures (Ellis and Roberts, 1981). The most significant advance in quantifying orthodox seed storage environment and survival, was the development of three basic viability equations (Roberts, 1960). These equations in addition to quantifying the influence of both storage temperature and seed moisture content on a single criterion of longevity (50% survival period), broke new ground by also describing the complete seed survival curve and relating it to the environment. It thus became possible to relate combinations of time, temperature and moisture to any level of percentage viability. Roberts (1960) observed that, the frequency of deaths with time in a population of orthodox seeds, stored in constant conditions, could be described by the normal distribution:

$$Y = (1 / \sigma\sqrt{2\pi}) \exp[-(p - \bar{p})^2 / 2\sigma^2] \qquad [1]$$

Where Y is the relative frequency of deaths occurring at time p, $\bar{p}$ is the mean viability period (time in days for seeds to reach 50% viability) and σ is the standard deviation of the distribution of deaths with time. Roberts (1960), argued that equation [1] described a cumulative normal distribution of negative slope, and which in effect conformed to the sigmoidal pattern of seed survival curves. He also observed further that, the spread of the distribution (standard deviation, σ) is directly proportional to the mean viability period, such that, when several constant conditions are compared, the mean viability period and the standard deviation vary in the same proportion, that is:

$$\sigma = K_\sigma \bar{p} \qquad [2]$$

Where K_σ is a constant for each species.

Using results on wheat and other cereals stored at temperatures from 15°C - 25°C and moisture contents from 11% - 23%, Roberts (1960) presented an equation, to describe the time taken for seeds to reach 50% viability, while taking into account the effect of moisture and temperature as:

$$\text{Log } P_{50} = K_v - C_1 m - C_2 t \qquad [3]$$

where; P_{50} is the mean viability period (time in days for seeds to reach 50% viability), m is the percentage moisture content of the seed on a fresh weight basis, t is the storage temperature in °C, K_v a viability constant, C_1 a moisture content constant and C_2 a temperature constant

The viability constants have some real meaning in terms of viability behaviour (Roberts, 1960); the value K_v indicates the potential viability under ideal conditions; the value C_1 indicates the relative sensitivity of viability to moisture content, while C_2 indicates the relative sensitivity of viability to temperature. The above basic viability equations have since then undergone further modification (Ellis and Roberts, 1980a).

Ellis and Roberts (1980a) argued that, since seed survival curves conform to negative cumulative normal distributions [equation (1)], each curve may be quantified by only two attributes both of which may be measured in days; the mean viability period, $\bar{p}$, and the standard deviation of the distribution of deaths in time, σ. Consequently, by combining equation [2] and [3] they came up with a new equation [4], which describes the relationship between the standard deviation of the distribution of seed deaths in time and the storage environment as:

$$\text{Log } \sigma = K_L - C_1 m - C_2 t \qquad [4]$$

Where $K_L = \text{Log } K_\sigma + K_v$ [5]

Ellis and Roberts (1980a) further described the straight line seed survival curves (after plotting probit percentage viability against time), by an equation, as :

$$V = K_i - (1/\sigma)P \quad [6]$$

Where V is probit percentage viability, P is storage period (days) and K_i is probit percentage viability at the beginning of storage. The slope of the curve is in turn given by $(1/\sigma)$, which accordingly is negative. By substituting equation [4] into equation [6] Ellis and Roberts (1980a) came up with another equation:

$$V = K_i - P / 10^{K_L - C_1 m - C_2 t} \quad [7]$$

Equation [7] relates probit percentage viability V, at any time P, to any combination of moisture content, m and temperature, t. Ellis and Roberts (1980a), pointed out that, three of the constants K_L, C_1 and C_2 are specific to the species, but independent of genotype and initial seed quality. The constant, K_i, is specific to the seed lot and is a measure of initial quality. Its value is dependent on genotype, the pre-storage environment and their interaction (Ellis and Roberts, 1980a).

From storage experiments carried out under several combinations of temperature and moisture content, the species constants K_v, K_σ, K_L, C_1 and C_2 may be determined for any seed species with orthodox seed storage behaviour. What is more necessary, is to obtain several estimates of P_{50} values (equation 3) or several of σ (equation 4) and then solve the resultant simultaneous equations for the respective species constants. The constants may then be substituted into equation [7] so as to predict

the percentage viability to be expected after any given period, under different combinations of temperature and moisture conditions, at least within a range of medium-term storage (Ellis and Roberts, 1980a). It is also necessary to obtain the value of K_i for the seed lot in question which may be accurately determined from a rapid ageing test in constant conditions, or estimated roughly from a single germination test by transforming percentage initial viability to the equivalent probit value (Hong *et al.*, 1996).

Equation [7] allows accurate predictions to be made, of the percentage viability expected, after any given period under different combinations of storage temperature and moisture content, within a range of medium storage (Ellis and Roberts, 1980a). Different investigators (Tompsett, 1984; Dickie *et al.*, 1985; Kraak and Vos, 1987; Lotito and Quagliotti, 1993) have suggested the same. The equation has been found to be useful as a guide to the expected behaviour of stored seed lots. Ellis and Roberts (1980a) have, however, cautioned that at extreme storage conditions, equation [7] is no longer applicable. They cited three major limits to the application of equation [7] at extreme moisture content levels. There is a low moisture content limit below which a further reduction in moisture content no longer increases seed longevity. This limit varies substantially among species, with values of 2-3% moisture content being a rough indication of this limit (Ellis *et al.*, 1988). There is also an upper moisture content limit, termed critical (Ibrahim *et al.*, 1983), beyond which longevity of seeds increases with increase in their moisture content, but only if oxygen is present. For example, the upper moisture content limit is about 15% in lettuce (*Lactuca sativa*) (Ibrahim and Roberts, 1983), 18% in onion (*Allium cepa*) (Ellis and Roberts, 1977) and 24% to 28% in tef (*Eragrostis tef*) (Zewdie and Ellis 1991b). Lastly, a combination of very high moisture content and sub-zero temperature may lead to greater decline in viability than would be predicted by the equation [7]. Such decline being attributed to freezing injury (Roberts, 1972a).

As far as temperature is concerned, there is no abrupt limitation to equation [7] as there is for moisture content. Ellis and Roberts (1980a), have however, pointed out that there is strong evidence to

suggest that the relationship described between seed longevity and temperature may vary when dealing with a very wide range of temperatures.

Ellis and Roberts (1980a) have in turn proposed an improved equation, to predict viability after any period of time over a far wide range of storage conditions and which takes into account the initial seed quality. The improved equation is;

$$V = k_i - p/10^{K_E - C_W \log m - C_H t - C_Q t^2} \quad [8]$$

Equation [8] is now considered as a universal viability equation for orthodox seeds. K_E, C_W, C_H and C_Q are the viability constants of a particular species or variety. The constant C_W indicates the relative sensitivity of longevity to moisture content while C_H and C_Q indicate the relative sensitivity of longevity to temperature.

From the point of view of seed storage for genetic conservation the main objective is to maintain genetic integrity of stored accessions for as long a period as possible. As such, the viability of each seed accession should be maintained in storage above a minimum level (the regeneration level), below which seeds should be grown out to provide fresh stocks of seed.

It has long been argued previously that regeneration should be done before there has been very much loss of viability (Roberts, 1975; IBPGR, 1976), in order to avoid in surviving seeds, genetic changes associated with loss of viability. Abundant scientific evidence point to occurrence of genetic alterations in seeds during storage (e.g. Balton, 1961; Roberts, 1972b; 1973b; 1981; Roos, 1980; 1984; Roberts and Ellis, 1984; Stoyanova, 1992). Majority of these studies have investigated the induction of chromosomal aberrations during seed ageing. It is almost universally agreed that chromosomal aberrations are a function of seed deterioration (loss of viability). Apart from chromosomal aberrations, other genetic alterations (e.g. point mutations) have been implicated in aged seeds (Roberts, 1981; Roos 1984; Roberts and Ellis, 1984).

This genetic alteration of conserved germplasm material will significantly affect breeding programmes, aimed at improving crops. Roberts (1981), has pointed out that, although most genetic abnormalities are not manifested until subsequent generations when the mutations have had the opportunity to segregate, genetic changes which occur during ageing lead to increased variance and changes in mean values of the morphological and phenological characters in the progeny of aged seeds. Such changes would have an obvious infuluence on genetic evaluation and characterization programmes of the stored samples. At the same time, it is important to appreciate that some of the germplasm collected is rare, such that, its genetic erosion while in storage, could lead to irreversible loss of valuable genotypes. Long-term preservation of seeds is a method for preventing genetic changes in the conserved materials and forms the basis for the organization of genebanks. Proper genebank management practices, right from seed handling and processing to packaging and storage, are as such, of paramount importance. Once seeds are put into storage at recommended conditions, one of the most important practice in genebank operations, is occasional monitoring to check the level of viability.

The objective of viability monitoring tests is to investigate whether the regeneration level has been attained, and hence leads to recommendation on the appropriate period when regeneration should be undertaken. It has been recommended that regeneration, be undertaken when viability falls to 85% of the initial value (FAO/ IPGRI, 1994). Initial germination values should in turn, exceed 85% for most cereals and 75% for some vegetables and even lower for some wild or forest species with normally low germination levels (FAO/ IPGRI, 1994).

Viability monitoring test intervals, will vary among species and storage conditions. However, too frequent monitoring would not only deplete valuable seed accessions, but also add extra expenses to genebank operational costs. Moreover, regeneration done when the level of viability is still high enough, to warrant minimal genetic alterations, will be unnecessarily expensive and at the same time probably lead to genetic alterations in the regenerated seed stocks. Worse still, failure to identify the

actual time in storage when regeneration should be carried out could lead to irreversible genetic alterations on the conserved seed germplasm. This would, in addition to the loss in genetic diversity, lead to financial losses, since a lot of resources (labour, electricity) will already have been spent on the lost germplasm.

Genebank managers are, therefore, faced with the challenge of ensuring that materials being held within cold stores are maintained at a desirable level of viability and by extension of genetic integrity. Precise guide about decline in viability of conserved accessions is as such important, and will aid the managers in making decisions on the appropriate time to regenerate accessions whose level of viability is compromising. Such guide can be offered by the viability equations discussed in the preceding paragraphs. In essence, the principal function of viability equations is to provide to genebank managers, suitable viability monitoring frequencies for seed germplasm under cold storage conservation. Once the storage temperature and moisture content conditions are defined for a particular seed species, it is possible to predict the level of viability to be expected after a given storage period. This would ease the work of a genebank manager, and at the same time lead to a more sound and cost effective genebank operation.

Without prior determination of the species viability constants, however, viability equations are of limited use to genebank managers. In fact, despite the usefulness of viability equations in viability monitoring only a negligible 3% of all plant species (Hong *et al.*, 1996) have had their viability constants determined. This leaves a large majority of seed species held under genebank storage, to be managed on the basis of experience, and on studies done on a limited number of species.

2.1.4 Seed vigour concept

Many attempts have been made to rigorously define the term vigour as applied to seed. Isley (1957) made one of the first attempts to conceptualise and define seed vigour. He termed seed vigour as " the sum of all attributes which favour stand establishment under unfavourable conditions". Delouche and Caldwell (1960), pointed out that, Isley's definition was restrictive in the sense that it

was limited to stand establishment under unfavourable conditions. Following a series of several other definitions, (Woodstock, 1969; Perry, 1972; Heydecker, 1972; Pollock and Roos, 1972), it was evident that vigour was a concept that described several characteristics, which in turn were associated with various aspects of performance of germinating seed or subsequent seedling. A broadly based definition was thus adopted by the ISTA congress in 1977 (Perry,1978) as: "*the sum total of those properties of seed which determine the level of activity and performance of the seed or seedling emergence. Seeds which perform well are termed high vigour seeds and those which perform poorly are termed low vigour seeds*". The definition also specifies those aspects of performance reported to show variations associated with differences in vigour as; biochemical processes and reactions during germination, such as enzyme reactions and respiratory activities; rate and uniformity of seed germination and seedling growth; rate and uniformity of seedling emergence and growth in the field; and emergence ability of seeds under unfavourable conditions.

Loss in vigour like viability decline, is a consequence of deterioration of the seed at seed response level. This loss is usually manifested as a progressive reduction in those aspects of performance capable of showing vigour related variations. Seed vigour loss precedes viability decline, so that, although seed lots may have similar high germination values, they may differ in their physiological age (that is, the extent of deterioration) and so differ in vigour and ability to perform (ISTA, 1995). A viability test alone may as such be of limited ability in detecting physiological quality differences among seed lots. Roberts (1984), has pointed out that, a viability test is limited in detecting quality differences among high germinating seed lots. Ellis and Roberts (1980c), have also pointed out that, due to the nature of the normal distribution on which seed survival curves are based, a small difference in percentage germination represents a large difference in progress of deterioration. It is on the basis of such circumstances, therefore, that a more sensitive differentiation of potential seed performance is necessary (Hampton and Coolbear, 1990). Moreover, seed vigour testing could supplement a viability test with more information about the physiological quality of a seed lot.

Furthermore, although a seed may be viable, it may still fail to germinate under stressful conditions. Results of vigour tests could help identify when such stand failures are likely (Fay *et al.*, 1993).

Several vigour assessment techniques have been developed over the past years which have been classified broadly into direct and indirect tests (Perry, 1987). Direct tests impose stress factors likely to reduce emergence in the field, but under controlled conditions. Widely used direct tests include cold and Hiltner tests (ISTA, 1987). Direct vigour test methods, particularly those involving field soils, prove difficult to standardise between testing stations and tend to give more variable results (Perry, 1987). Indirect vigour test methods, are those in which a seed characteristic in the laboratory is related to performance in the field. Amongst the most widely used vigour evaluation indirect tests are; rate of germination (estimated by a first count in the germination test); rate of seedling growth; tetrazolium test; accelerated ageing test; and electrical conductivity test (ISTA, 1987). The rate of seedling growth is estimated by measuring seedlings after a specified period. In the tetrazolium test, seeds are immersed in tetrazolium chloride solution which reveals the presence of dead tissue, the location and extent of which are related to vigour level. Accelerated ageing test correlates the proportion of seeds surviving treatment at high temperature and moisture content which induces rapid deterioration. The electrical conductivity test serves as an indicator of cellular electrolyte and solute leakage. The quantity of electrolytes (measured as electrical conductivity) leached into water during a specified soak period is correlated with emergence.

2.1.5. Vigour and membrane integrity

Changes in the organization of cells occur during the development of seeds prior to physiological maturity, desiccation before harvest, and during imbibition prior to germination (Abdul-Baki, 1980). Cell membranes are the main components of this cell organization, and cell membrane integrity can be considered to be a fundamental cause of differences in seed vigour (Powell, 1988). Electrical conductivity test serves as an indicator of the integrity of cell membranes. The premise of this vigour test lies in the rapid reorganisation of cellular membranes in high vigour seed during

imbibition, thus preventing extensive electrolyte leakage (Fay *et al.*, 1993). As seed rehydrates during early imbibition, the ability of its cellular membranes to re-organise and repair any damage that may have occurred will influence the extent of electrolyte leakage (ISTA, 1995). The greater the speed with which the seed is able to re-establish its membrane integrity the lower the electrolyte leakage. Higher vigour seeds are able to re-organise their membranes more rapidly, and repair any damage to a greater extent than low vigour seeds. Consequently, conductivity readings are higher for low vigour seed lots than high vigour seed lots, as the seeds are rehydrated (AOSA, 1983; ISTA, 1995).

Hibbard and Miller (1928), first recognised the usefulness of the electrical conductivity test in providing electrolyte leakage measurements for seeds of several species. Mathews and Bradnock (1967), later developed it into a routine vigour test to predict field emergence of garden pea (*Pisum sativum L.*). Conductivity test is now used extensively for this species in Europe, Australia, New Zealand and North America (ISTA, 1995). The test has been extended to many other species, especially the large seeded legumes (ISTA, 1995). Conductivity of soak water in which bulk seed samples (25 - 50 seeds) have been soaked identifies seeds low in vigour.

Significant correlations between electrical conductivity readings of soak water and several vigour related qualities (such as field emergence, seedling growth rate) have been observed by different workers in different species. Mathews and Bradnock (1968), and Mathews and Whitbread (1968), observed significant correlations between seed exudation (as measured by electrical conductivity) and the emergence of wrinkle - seeded peas (*Pisum sativum* L.) and French beans (*Phaseoulus sp.*). Abdul-Baki and Anderson (1975), noted an increase in leaching of materials from embryonic axes of soybean (*Glycine max* L.) merr.] seeds, low in vigour. Similar trends of higher electrical conductivity readings being associated with low vigour have been observed in several other seed species, such as clover (*Trifolium incarnatum* L.) (Ching and Schollcraft, 1968) and ryegrass (*Lolium perrenne L.*) (Happ *et al.*, 1993) barley (*Hordeum Vulgare L.*) (Abdul-Baki and Anderson 1970), rice (*Oryza sativa*) (Agrawal, 1977), maize (*Zea mays*) (Gill and Delouche, 1973; Tao, 1980) and *Rudbeckia*

fulgida (Fay *et al.*, 1993).

Unlike most other vigour and viability assessment tests, electrical conductivity test is quick to undertake. Within 24 hours only, it is possible to asses and determine the level of deterioration in seed lots. In contrast, most of the other tests take as many as three weeks to determine the quality of seeds. Furthermore, with proper standardisation, only a limited number of seeds would be required to undertake the test, which would in turn ensure minimum depletion of stored samples. The test is also easy to undertake, such that, no special training is necessary to the personnel using it. It can therefore be easily adopted as a routine seed deterioration assessment test, for genebanks. Despite this usefulness and potentiality, however, electrical conductivity test has been adopted on routine basis for only a limited number of large seeded legumes. There is, therefore, a great need to develope and extend the test to other species, especially the small seeded species of plants, in which it has been given limited attention. Studies on the application of electrical conductivity test in determining the quality of seeds of many other species is the only sure way of ensuring its adoption on routine basis. It is against such background that electrical conductivity test has been used in this study to investigate the influence of storage temperature and moisture content on vigour of two small seeded species, *E. coracana* and *A. hybridus*.

The **objectives of the present study were:-**

1. To determine the progress and extent of seed deterioration (viability and vigour) at different temperature and moisture content regimes during storage for the two species, *Eleusine coracana* L. Var. KAT/FM-1 and *Amaranthus hybridus* L. Ssp. *Cruentus*
2. To quantify, using the simple viability equations (equations 1 to 6), the effect of storage regimes on the viability of the two species.
3. To determine and apply the simple viability constants in predicting seed longevity for the two species under various combinations of temperature and moisture content other than those used in the study.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Seed procurement and processing

In this study one seed lot of each species was used. *Amaranthus hybridus* seeds of the sub-species *cruentus* were collected locally from natural stands near the Genebank of Kenya, Muguga. Shooty heads of *Amaranthus hybridus* were collected and pre-dried at 20°C and 15% relative humidity, in a drying room for 48 hours. The dry heads were then machine-threshed and seeds obtained. A seed blower was used to remove loose debris materials and damaged seeds from the experimental seed lot.

One seed lot of finger millet (Var. KAT/FM-1) was acquired from Katumani Dryland Research Station - K.A.R.I, Machakos. The threshed seeds of finger millet which had been harvested approximately six months prior to acquisation, were only cleaned to remove any debris materials and any damaged or foreign seeds.

From each seed lot, a sample was randomly drawn for initial seed moisture content determination. Another sample was also drawn for the initial viability test. The remaining seed lot for each species was sub-divided into different lots for storage at different moisture content levels. The targeted moisture content levels were initially; 5%, 11% and 16% (fresh weight basis).

3.2 Seed moisture content determination and adjustment

To determine seed moisture content, both initially and subsequently, during adjustments, the high constant-temperature oven method (ISTA, 1985) in its modified form (Hanson, 1985) was used. Approximately, 1.5 g of seeds in two replicates contained in clean pre-weighed dishes were accurately (to 4 decimal places) weighed. The seed containing dishes were then transferred into a pre-heated oven at 130°C, where they remained for varying periods of time depending on the species. Finger millet seeds were exposed for 2 hours, while *Amaranthus hybridus* seeds were exposed for 1 hour only (ISTA, 1985). The different exposure periods depending on the species, ensure the removal of as much

moisture as possible whilst ensuring that oxidation, decomposition or the loss of other volatile substances is reduced (Cromarty *et al.*, 1985). At the end of each exposure period, seeds in the dishes were allowed to cool for about 30 - 45 minutes inside a desiccator before their weights were taken and seed moisture content expressed on a fresh weight basis as:

$$\% \text{ Seed moisture content} = \frac{\text{Initial seed weight (g)} - \text{seed weight after drying (g)}}{\text{Initial seed weight (g)}} \times 100$$

The average of the two replicates (Hanson, 1985) was taken in each case.

Seed moisture content was adjusted from the initial values, either by cool air drying (in a dehumidified room) at 20°C and 15% relative humidity or by humidification above water in a desiccator at 20°C. In the cool air drying method, sub-samples of seeds were put into porous cloth bags and placed on racks in a room whose temperature and relative humidity were maintained at 20°C and 15%, respectively. Samples were occasionally withdrawn from the porous bags into laminated aluminium foil packets, that were immediately sealed and placed in a room at 5°C for 48 hours. This was to allow moisture to equilibrate within and among seeds in each packet. Seed moisture content was then determined periodically until the targeted levels were achieved, for both species.

In the humidification method, sub-samples of seed on petri-dishes were placed in a desiccator whose bottom part was filled with water instead of silica gel, at 20°C. Using a psychrometer, the relative humidity inside the desiccator was determined to be $\geq$ 98 %. Samples were occasionally withdrawn from the desiccator and seeds sealed in laminated aluminium foil packets to allow moisture to equilibrate at 5°C, for 48 hours. Seed moisture content levels were determined periodically until the desired level(s) were obtained.

3.3 Seed storage

Once the desired moisture content levels were achieved, approximately 250 seeds at each level for each species, were hermetically sealed in laminated aluminium foil packets. At each moisture content level, enough packets, to last at least 4 months of storage, were placed in incubators at temperatures; 15°C, 25°C and 35°C. At an equal interval of 21 days, three packets of seeds at each treatment combination were for 126 days, withdrawn to test germination and electrical conductivity of seed leachate. After 126 days of storage, only two treatment combinations per species, had produced complete seed survival curves. Thus to obtain a minimum of three seed survival curves necessary in quantifying viability, one additional moisture content level (15.2%) was for *E. coracana* seeds combined with temperature 40°C. At the same time, sampling was continued for this species at treatment combinations with additional seeds in storage. As for *A. hybridus*, an additional moisture content (17.3%) was introduced with two temperatures (25°C and 35°C). Seeds used in the additional storage treatments were for both species obtained from a sample which (for each species) had been stored at the beginning of the experiment, with a moisture content of 5.3% at 5°C.

3.4 Germination tests

At the beginning of the study and subsequently after each sampling interval, 200 seeds of finger millet in eight replicates of 25 seeds each were randomly set on top of a Jacobsen germinator at 25°C. The completely separated seeds were arranged on round filter papers (70mm, Schleicher & Schuell type) and 0.2% potassium nitrate solution used to initially wet the papers on a Jacobsen germinator. The germination regime was obtained and modified from ISTA (1985).

For *A. hybridus*, 200 seeds in 4 replicates of 50 seeds each, were arranged on top of 4 filter papers (size 90mm, Whatman No.1) that had been wetted with 0.2% potassium nitrate solution in petri-dishes. These were then prechilled for 7 days at 5°C, before being transferred and randomly arranged in an incubator at 35°C ±0.5. Distilled water was occasionally used thereafter to moisten filter papers. This germination regime was modified from the ISTA prescription (ISTA, 1985). Both species have a

suspected physiological dormancy (ISTA, 1985) and as such 0.2 % potassium nitrate solution and the prechilling treatment for *A. hybridus* used above, were dormancy breaking techniques.

The speed at which seeds germinate is often evaluated as a measure of variations associated with differences in seed vigour(ISTA, 1987). Generally, seeds with high vigour germinate more rapidly compared to those with low vigour. In the current study, germination speed (vigour) has been evaluated on the basis of germination at first count. The test was held on the premise that, seeds high in vigour will produce more normal seedlings within the first four days of evaluating germination. This is because it is well established that low vigour seeds usually have slow and widely distributed germination rates compared to high vigour seeds (Roberts, 1973c; 1981; ISTA, 1987).

For both species, germinated normal seedlings were counted and removed on the fourth day of evaluating germination (first count). As for *E. coracana,* the final count was done on the tenth day of evaluating germination, while for *A. hybridus*, final count was on the fourteenth day. Germination at the first and the final count was then expressed as a percentage of the total number of seeds per replicate. A germinated normal seedling was for *E. coracana* considered to be one with a well formed shoot and root, and with the first leaf protruding above the coleoptile. As for *A. hybridus* a germinated normal seedling was taken to be the one with a well formed shoot and root and with its cotyledons well exposed from the testa. The criterion for normal and abnormal seedling evaluation was adopted from ISTA rules (ISTA, 1985).

3.5 *Electrical conductivity test*

For the first 126 days of storage, each time samples were withdrawn for germination test, electrical conductivity test was also performed. Four replicates of 100 seeds from each storage treatment combination were weighed to three decimal places before being soaked into 75 ml double distilled water in plastic bottles, at 20°C. Four control bottles containing double distilled water only were set up with each test run. All bottles were maintained in a room at 20°C for 24 hours. After the soak period, the solution and seeds in each bottle were gently swirled for 10 to 15 seconds, and

conductivity of the soak water measured by placing a dip cell, attached to a digital electrical conductivity meter (model: Jenway 4020). Several measurements were taken until a stable result was obtained. Between measurements the dip cell was rinsed twice in double distilled water and dried using clean dry paper towels. All measurements were conducted at 20°C. After subtracting the control bottle measurements (the mean of the readings) conductivity was expressed per gram of seed. The conductivity measurement was conducted according to recommendations by International Seed Testing Association (ISTA,1995).

3.6. *Statistical analysis*

Mean percentage normal seed germination data (both first and final count) was converted to corresponding probit values using Statistical Tables by Fisher and Yates (1953). The resultant probit values were then regressed on the corresponding storage period, to asses changes in germination (both first and final count) during storage at the various combinations of temperature and moisture content. Mean electrical conductivity values of soak water for seeds of each species stored at the various storage combinations of temperature and moisture content, were also regressed on their corresponding periods of storage. Final count normal germination percentage (viability) data was subjected to probit analysis to estimate p_{50}, σ and K_i values at treatment combinations whose complete survival curves were obtained. Genstat computer package (Genstat 5, release 3.2, 1995) was used. Values of σ were through a multiple regression analysis used to solve for the simple viability constants. Statworks computer statistical package (version 1.1, 1987) was used to perform all regression analyses.

CHAPTER FOUR

4.0 RESULTS

4.1 Seed moisture content determination and adjustment

The initial seed moisture contents were 11.3% and 15.8% for *E. coracana* and *A. hybridus*, respectively. Following moisture adjustment, *A. hybridus* seeds were set for storage at 5.3%, 11.2%, 16.4% and 17.3%, while *E. coracana* seeds were stored at 5.3%, 11.3%, 15.2% and 16.3% moisture levels.

4.2 Seed germination

Germination test results, at the beginning and during seed storage for the two species are summarised in tables 1 and 2. The mean germination percentages at the first count (a measure of vigour) are depicted in table 1, and those at final count (a measure of viability) are in table 2. Although the initial percentage germination (both first and final count) varies considerably for the two species, no definite trend with moisture content treatments was observed. Germination differences among moisture treatments for the two species were not significant ($p = 0.05$). This somewhat erratic initial germination, especially more pronounced for *A. hybridus* at first count (between 55 and 77 percent)(Table 1), was possibly due to difficulties in overcoming seed dormancy (Ellis *et al.*, 1985b). It has been observed that, almost without exception seeds of *Amaranthus* spp. show considerable innate (or primary dormancy) (Ellis *et al.*, 1985b). Such dormancy prevents seeds from germinating on the mother plant and also usually for some time after the ripe seed is shed or harvested. Thus, given that *A. hybridus* seeds used in this study were freshly harvested and immediately processed for storage, there was a high possibility of innate dormancy being expressed, at least in the initial germination test.

Complete loss in both vigour (as evaluated by germination at first count), and viability (as evaluated by germination at final count) during storage, was observed only at four treatment combinations. At each of these four storage treatment combinations for each species, results in tables 1

and 2 show that loss of vigour and viability, is faster the higher the temperature and/ or moisture content. Therefore, complete viability and vigour loss in *A. hybridus* seeds took place within 35 days at 17.3% m.c & 35°C, 63 days at both 17.3% m.c & 25°C and 16.3% m.c & 35°C, and 105 days at 16.3% m.c & 25°C. Results in table 1 also reveal some unusual pattern in the progress of percentage (first count) germination of *A. hybridus* seeds stored with a moisture content of 16.4% at 25°C and 35°C. The percentage (first count) germination increased for the first 21 days (at 35°C) and 42 days (at 25°C), before eventually declining to zero after different storage periods, depending on the temperature.

As for *E. coracana*, complete viability and vigour decline was only observed for seeds stored with a moisture content ≥ 11.3%, in combination with temperatures ≥ 25°C. The most rapid rate of viability and vigour decline occured at 15.2% moisture content and 40°C (seed death within 42 days), while the least was at 11.3% moisture content and 35°C (seed death within 252 days). Percentage (first count) germination (vigour) for *E. coracana* seeds, at all the four storage treatment (i.e. those conditions leading to complete viability and vigour loss), declined faster than the corresponding percentage (final count) germination. For instance, after storing seeds of this species for 42 days at 16.3% m.c & 25°C and 16.3% m.c & 35°C, percentage (first count) germination declined by 15.5% and 58.5%, respectively (Table 1). The corresponding decline in percentage (final count) germination after the same storage period is only 5.8% (at 16.3% m.c & 25°C) and 14.3% (at 16.3% m.c & 35°C) (Table 2).

Table 1. The first count *germination percentage of *E. coracana* and *A. hybridus* seeds stored under different combinations of temperature and moisture content for varying periods.

Species	Seed moisture %	Temp ^{0}C	Storage period in days															
			0	7	14	21	28	35	42	63	84	105	126	147	168	210	231	252
			% germination															
E. coracana																		
	5.3	15	84.5			82.5			85.0	87.0	88.0	84.5	73.5	81.5				
	5.3	25	84.5			85.0			81.0	83.0	87.5	81.0	85.5	87	89	92.5	90.5	91
	5.3	35	84.5			81.5			85.0	85.5	83.0	86.0	91.9	91	87.5	93	94	93
	11.3	15	92.5			92.0			79.0	79.0	89.0	88.0	76.5	84				
	11.3	25	92.5			86.5			84.5	87.0	90.0	91.0	83.5	87	86	91	87.5	86.5
	11.3	35	92.5			87.5			85.5	87.0	85.0	84.5	78.5	82	80.5	58	26	0
	15.2	40	93.0	86.5	79.5	80.5	35.5	8.5	0.0									
	16.3	15	89.5			92.0			80.5	82.5	84.5	81.5	80.5	85	89	87.5	90	88
	16.3	25	89.5			84.0			74.0	44.5	13.0	0.0						
	16.3	35	89.5			79.0			31.0	13.0	0.0							
A. hybridus																		
	5.3	15	68.5			54.5			37.5	64.0	72.0	69.0	62.5					
	5.3	25	68.5			67.5			64.0	93.5	70.5	78.5	94.0					
	5.3	35	68.5			56.0			73.5	90.0	87.5	91.0	90.5					
	11.2	15	77.0			85.5			68.0	86.5	90.0	55.5	56.5					
	11.2	25	77.0			83.0			69.0	81.5	92.0	72.0	70.5					
	11.2	35	77.0			66.5			66.5	85.0	85.0	75.5	89.0					
	16.4	15	55.0			74.5			73.5	73.0	71.0	84.5	74.5					
	16.4	25	55.0			64.0			69.5	46.0	38.5	0.0						
	16.4	35	55.0			71.0			21.5	0.0								
	17.3	25	73.0			55.5			28.5	0.0								
	17.3	35	73.0	55.0	46.0	25.0	10.5	0.0										

*Germination percentages are means of 8 and 4 replicates for *E. coracana* and *A. hybridus*, respectively

Table 2. The final count germination percentage of *E. coracana* and *A. hybridus* seeds stored under different combinations of temperature and moisture content for varying periods

Species	Seed moisture	Temp	Storage period in days															
	%	^{0}C	0	7	14	21	28	35	42	63	84	105	126	147	168	210	231	252
			% germination															
E. coracana																		
	5.3	15	94.0			95.0			93.0	94.5	92.5	90.5	92.5	93				
	5.3	25	94.0			93.0			95.0	94.5	94.5	91.0	92.5	93.5	93.0	93.5	92.0	92.5
	5.3	35	94.0			95.0			94.5	92	89.0	92.5	93.5	92.5	93.5	94.0	93.5	93.0
	11.3	15	97.5			98.0			95.5	92.5	93.0	94.0	95.5	96.5				
	11.3	25	97.5			94.0			95.5	96.0	93.0	95.0	90.5	93	93.0	96.0	91.5	94.5
	11.3	35	97.5			95.5			97.0	95.0	91.0	91.0	91.0	90.5	88.5	70.8	43.0	0.0
	15.2	40	93.3	90.0	92.0	89.5	76.0	26.3	0.0									
	16.3	15	94.3			96.5			95.0	91.0	92.5	86.5	89.0	92.5	92.5	93.0	93.5	93.0
	16.3	25	94.3			92.5			88.5	74.5	51.0	8.5	0.0					
	16.3	35	94.3			93.0			80.0	55.5	0.0							
A. hybridus																		
	5.3	15	98.5			93.5			91.0	90.0	92.5	88.5	90.0					
	5.3	25	98.5			95.0			91.5	96.5	92.5	95.0	95.5					
	5.3	35	98.5			95.0			90.0	91.5	87.5	93.0	93.5					
	11.2	15	97.0			97.5			86.5	93.5	95.5	89.0	94.5					
	11.2	25	97.0			96.0			82.5	91.5	93.5	88.5	91.0					
	11.2	35	97.0			91.5			92.5	89.0	86.0	86.0	93.0					
	16.4	15	96.5			96.0			85.5	96.0	90.5	92.0	91.0					
	16.4	25	96.5			79.0			75.0	56.5	51.5	0.0						
	16.4	35	96.5			89.5			53.0	0.0								
	17.3	25	95.0			65.5			38.0	0.0								
	17.3	35	95.0	83.0	56.5	31.0	14.0	0.0										

*Germination percentages are means of 8 and 4 replicates for *E. coracana* and *A. hybridus*, respectively

Mean germination percentages (both first and final count) at each storage treatment and period were transformed to probits (Appendix 1:1 - 1:4) and regressed on the corresponding storage periods. Results of the simple regression analysis are summarised for the two species in Appendix 2:1 - 2:4.

Significant negative regression between both first count (Appendix 2:1) and final count (Appendix 2:3) germination (probits) and the corresponding durations of storage was observed for *E. coracana* seeds at 35°C with moisture content levels 11.3% and 16.3%; 40°C with a moisture content of 15.2%; and 25°C with a moisture content of 16.3%. This is an implication that, both vigour (first count germination) and viability (final count germination) declined significantly ($p \leq 0.05$) with storage time at those conditions.

For *A. hybridus* seeds, the fitted negative regression is of relatively low significance, despite observation that, percentage (first count) germination declined to zero (Table 1) during storage at the following treatment combinations: 35°C with a moisture content of 16.3% ($r= 0.879$, $p= 0.121$); and 25°C with a moisture content of 16.3% ($r= 0.745$, $p= 0.088$) (Appendix 2:2). This could be attributed to the initial phase of increasing percentage (first count) germination observed at those storage conditions (Table 1). Thus for this species, negative regression between percentage (first count) germination (probits) and storage duration was only significant for seeds stored with a moisture content of 17.3%, in combination with temperature; 25°C ($r= 0.893$, $p= 0.042$) and 35°C ($r= 0.940$, $p= 0.005$). When percentage (final count) germination (probits) for *A. hybridus* were regressed on the corresponding storage period at each treatment (Appendix 2:4) only the following combinations showed a significant decline : 35°C with moisture content levels; 11.2% ($r= 0.888$, $p= 0.008$) and 17.3% ($r= 0.956$, $p=0.003$) and 25°C with a moisture content of 16.4% ($r= 0.876$, $p= 0.022$). Although percentage (final count) germination declined to zero during storage (Table 2), the fitted regression is of relatively lower significance at storage treatment combinations of; 35°C with a moisture content of 16.4% ($r= 0.939$, $p= 0.061$); and 25°C with a moisture content of 17.3% ($r= 0.941$, $p= 0.059$). This could be attributed to the rapid rate of decline in the percentage (final count) germination observed at

these conditions (Figures 2b and 3a, respectively) than initially expected. This resulted in small degrees of freedom (3 in each case) in the course of fitting the existing regression (Appendix 2:4).

There was a highly significant positive linear regression between first count germination (probits) and storage period for *E. coracana* seeds stored with a moisture content of 5.3 % at temperatures 25°C ($p = 0.001$, $r = 0.817$) and 35°C ($p = 0.000$, $r = 0.893$)(Appendix 2:1). There was a significant positive regression ($p = 0.011$, $r = 0.868$) between first count germination (probits) and the storage period in *A. hybridus* seeds stored at the same moisture content, at a temperature of 35°C (Appendix 2:1). This result suggests a significant increase in the speed at which seeds of both species germinated at the respective conditions during storage.

4.2.1 Seed survival curves

Complete survival curves (percentage final count normal germination, plotted against storage time) were obtained for *E. coracana* seeds stored with 11.3% m.c at 35° C; 16.3% m.c at 25° C and 35° C; and 15.2% m.c at 40 ° C (Figures 1a – 2a). For *A. hybridus*, complete survival curves were obtained only for seeds stored at 25 ° C and 35 ° C with moisture contents of 16.3 % and 17.3 % (Figures 2b – 3b). At the rest of the storage combinations for each species, viability did not decline below 90% at the time of terminating the experiment and thus their survival curves were incomplete. Such incomplete curves are only demonstrated for seeds of the two species, stored with a moisture content of about 16% at 15°C in Figures 1b (*E. coracana*) and 2b (*A. hybridus*). Insignificant viability loss and thus incomplete survival curves at the above storage conditions could be attributed to the short experimental duration used in this study.

It is evident from the survival curves that the higher the storage temperature and/ or moisture content for each species, the faster the rate of viability decline. The general pattern of viability decline is observed to be fairly sigmoidal at most of the treatment combinations. In this pattern, viability loss is initially slow then rapid, before eventually being slow towards seed death.

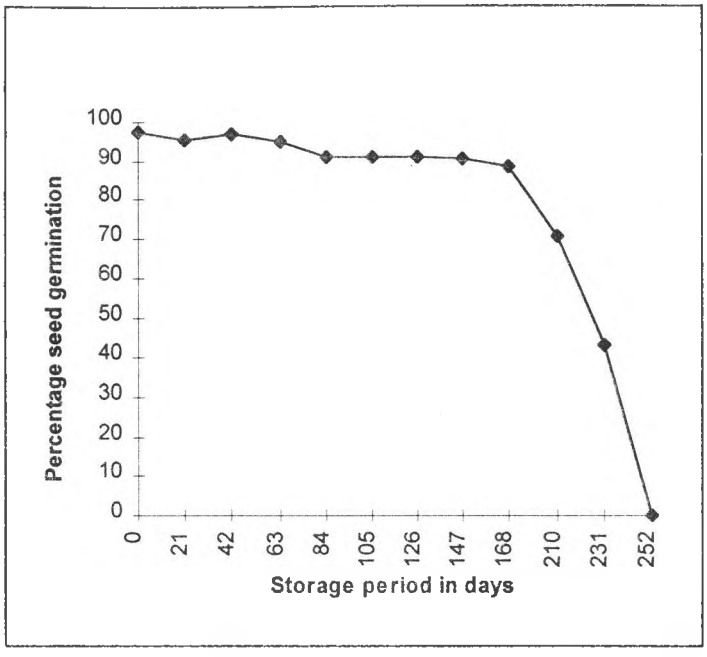

Figure 1a. A seed survival curve (percentage normal germination, plotted against storage period) for *E. coracana* stored with a moisture content of 11.3% at 35°C for 252 days.

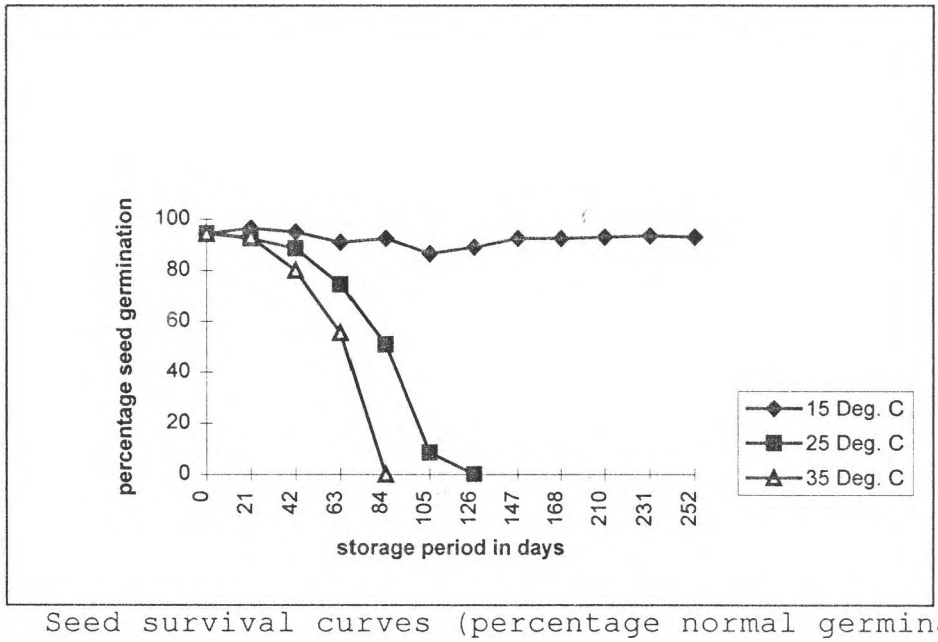

Figure 1b. Seed survival curves (percentage normal germination, plotted against storage period) for *E. coracana* stored with a moisture content of 16.3% at temperatures 15°C, 25°C, and 35°C for periods up to 252 days.

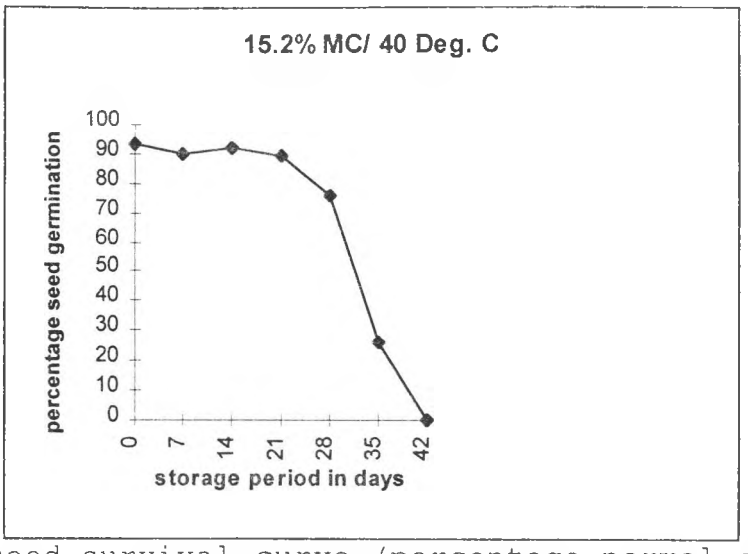

Figure 2a. A seed survival curve (percentage normal germination, plotted against storage period) for E. coracana stored with a moisture content of 15.2% at 40°C for 42 days.

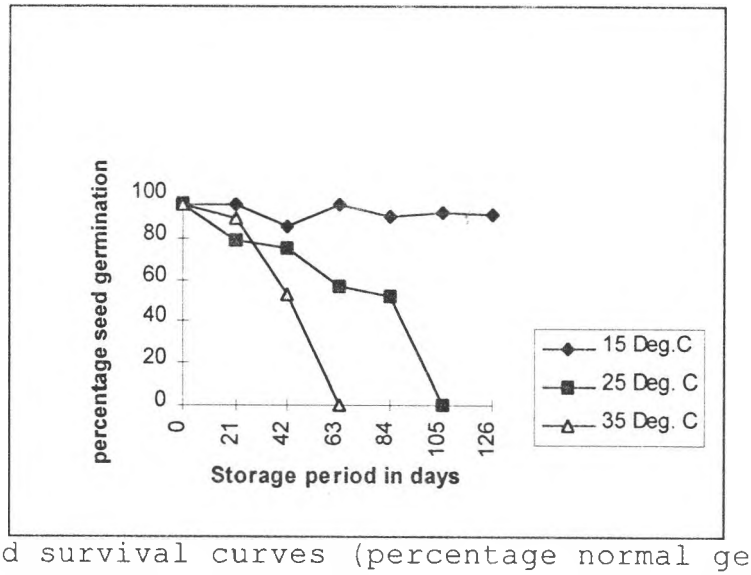

Figure 2b. Seed survival curves (percentage normal germination, plotted against storage period) for *A. hybridus* stored with a moisture content of 16.4% at temperatures 15°C, 25°C, and 35°C for periods up to 126 days.

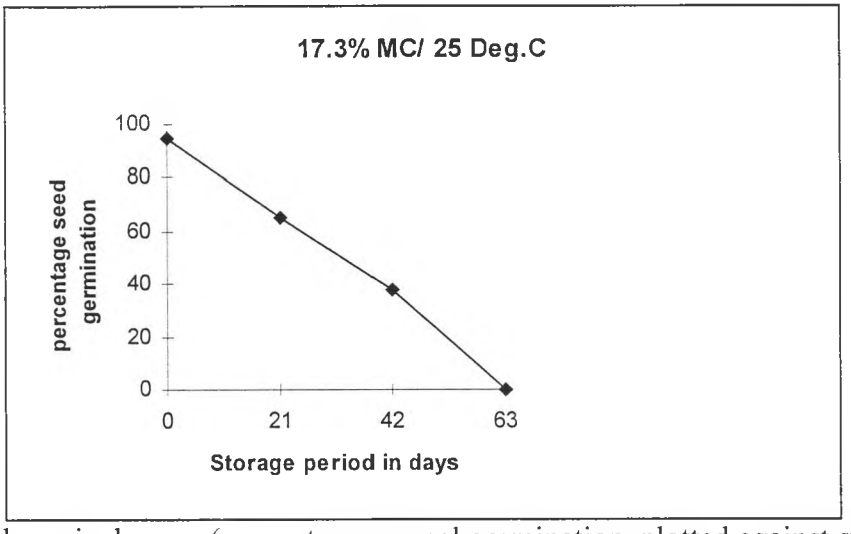

Figure 3a. A seed survival curve (percentage normal germination, plotted against storage period) for *A. hybridus* stored with a moisture content of 17.3% at 25°C for 63 days.

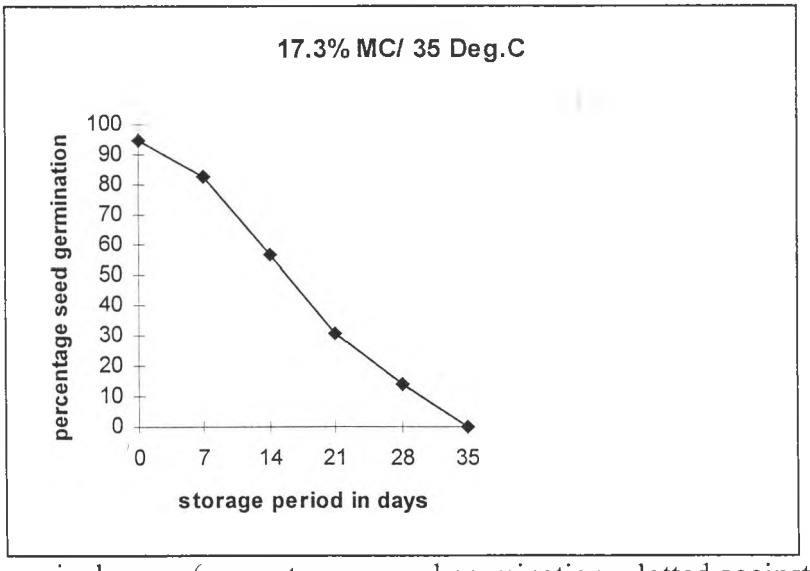

Figure 3b. A seed survival curve (percentage normal germination, plotted against storage period) for *A. hybridus* stored with a moisture content of 17.3% at 35°C for 35 days

Probit analysis (Finney, 1962) was applied to describe the complete survival curves by equation [6] (Chapter 2). This was done, by fitting the corresponding viability decline data by probit analysis to equation [6] (Ellis and Roberts, 1980a). It was thus possible to obtain estimates of K_i (the initial probit percentage germination), σ [the standard deviation of the distribution of seed deaths in time; which is the reciprocal of the slope of the seed survival curves when germination percentages are transformed to probit values (Roberts, 1972a)] and P_{50} (the time taken for viability to fall to 50%). The resultant estimates are shown in Table 3.

Table 3. Summary of fitting straight lines by probit analysis to viability decline of *E. coracana* and *A. hybridus* seeds stored at various moisture contents (m.c) and temperatures.

Species	Temp (°C)	Seed m.c (%)	K_i	slope (1/σ)	σ	P_{50} (s.e)*
E. coracana						
	35	11.3	7.629	-0.012	83.3	216.00(3.499)
	25	16.3	7.308	-0.031	32.3	75.09(1.431)
	35	16.3	7.412	-0.043	23.3	55.92(1.165)
	40	15.2	7.377	-0.082	12.2	29.13(0.5350)
			7.432**			
A. hybridus						
	25	16.4	6.844	-0.029	34.5	63.59(1.435)
	35	16.4	6.556	-0.066	15.2	38.89(0.9378)
	25	17.3	6.601	-0.054	18.5	29.48(0.8798)
	35	17.3	6.719	-0.107	9.3	16.09(0.4314)
			6.680**			

* Standard error of the estimation

** Average K_i value for each of the two species at four treatments combinations

For each of the two species in this study, the estimated K_i values are closer in magnitude at each of the storage treatment. This is an indication that the probit analysis curves, extrapolated to fairly equal estimates of the origin, K_i. The average of the K_i values for *E. coracana* seeds is 7.432 probits (equivalent to 99.3% germination) and 6.680 probits (equivalent to 95.4 % germination) for *A. hybridus* seeds. These extrapolated initial percentages of normal (final count) germination are no more than 4.5% greater or less than the observed initial germination (Table 2).

Estimated P_{50} values decrease with increased in storage temperature and/ or seed moisture content. Accompanying the decrease in the p_{50} values is an increase in the magnitude of the slopes (and hence a decrease in the value of σ) to the fitted curves. Therefore as storage conditions become more severe, reduction in seed longevity (P_{50}) was accompanied by a reduction in the seed-to-seed variation in the times at which individual seeds die (σ). For example, at storage temperature 35°C, an increase of seed moisture content for *E. coracana* from 11.3% to 16.3% results into a 3.9- fold decrease in the value of the value P_{50} and a 3.6- fold decrease in the value of σ (Table 3).

Estimates of σ (reciprocal of the slope) were through a multiple regression (Ellis and Roberts, 1980a) regressed on the corresponding storage temperature (t) and seed moisture content (m), in accordance with equation [4] (Chapter 2). The resultant viability constants for both species are compared to those of several other species worked out earlier in Table 4. It is evident that the simple viability constants obtained in this study fall in the same range as those for the other species. There appears to be rather more variability in the constants K_L, which reflects a species inherent longevity in storage.

Table 4. A comparison of the simple viability constants for *E. coracana* and *A. hybridus* (standard errors in parentheses) with those for other species

Species	cultivar	Viability constants*		
		K_L	C_1	C_2
E. coracana	KAT/FM-1	4.606 (1.076)	0.135 (0.048)	0.034 (0.018)
A. hybridus	cruentus	6.713 (0.569)	0.267 (0.033)	0.033 (0.003)
Lupinus polyphyllus	-	4.495	0.111	0.049
Capsicum annum	Quadrato d`Asti rosso	6.063	0.111	0.088
Oryza sativa	Norin	4.980	0.150	0.047
Triticum aestivum	Atle	4.611	0.108	0.050
Hordeum distichum	Proctor	4.130	0.098	0.050
Lactuca sativa		6.586	0.262	0.082

* Viability constants taken from Dickie *et al.*, (1985, Table 3) except *Capsicum*(Lotito and Quagliotti, 1992), *Lactuca*(Kraak and Vos, 1987), and *E. coracana* and *A. hybridus*(present study).

The viability constants obtained in the present study were substituted into the viability predictive model (Equation 7, Chapter 2), in order to calculate hypothetical times (in days) for seeds of the two species stored under various temperatures and moisture contents to decline from an initial level of 95% to 85% (the regeneration level). The resultant predictions are shown in Tables 5a and 5b for *E. coracana* and *A. hybridus* seeds, respectively.

Table 5a. Predicted time in days for *E. coracana* seed viability to decline from an initial viability of 95% to the final viability of 85% under various hypothetical combinations of seed moisture content and storage temperature

Storage Temp (°C)	**seed moisture content (%)**				
	5	7	8	10	12
			Days		
5	3509.6	1884.8	1381.2	741.7	398.3
10	2372.8	1274.2	933.8	501.5	269.3
15	1604.2	861.5	631.3	339.0	182.0
20	1084.6	582.5	426.8	229.2	123.1

Table 5b. Predicted time in days for *A. hybridus* seed viability to decline from an initial viability of 95% to the final viability of 85% under various hypothetical combinations of seed moisture content and storage temperature.

Storage Temp. (°C)	**Seed moisture content (%)**				
	5	7	8	10	12
			Days		
5	99371.2	29057.7	15713.1	4594.7	1343.6
10	67961.1	19872.9	10746.3	3142.4	918.9
15	46479.4	13591.2	7349.5	2149.1	628.4
20	31787.8	9295.2	5026.4	1469.8	429.8

According to the predictive results in tables 5a and 5b, the lower the storage temperature and/ or moisture content, the longer seeds of both species will take to reach the regeneration level (85%). At the lowest temperature and moisture content combination (5°C and 5%, respectively), therefore, the regeneration level is predicted to be attained after approximately 99,371 and 3,509 days for *A. hybridus* and *E. coracana*, respectively. On the other hand, at the highest temperature and moisture content combinations (20°C and 12%, respectively), the same regeneration level is predicted to be attained within approximately 430 and 123 days for *A. hybridus* and *E. coracana*, respectively. The prediction results are in agreement with observations in this study that for the two species, loss of vigour (first count germination, Table 1) and viability (final count germination, Table 2) is greatest at the highest temperature and moisture content conditions, either singly or in combination. Prediction results also reveal that, at similar storage conditions, the two species vary considerably in the periods taken for seed viability to decline from 95% to 85%. Extreme longevity is in fact predicted for *A. hybridus* (Table 5b) compared to *E. coracana* (Table 5a) at relatively low moisture content and temperature combinations. This result is quite expected, given that *A. hybridus* has a numerically higher value of the constant C_1 compared to *E. coracana* (Table 4). It implies therefore, that the relative effect of moisture content on longevity of the former will be greater compared to that of the latter. As a result, seeds of *A. hybridus* will be longer lived than seeds of *E. coracana*, especially at storage combinations with low moisture contents.

4.3 Electrical conductivity

Change in the mean electrical conductivity ($\mu S\ cm^{-1}\ g^{-1}$) of seed leachate (soak water), during storage of *E. coracana* and *A. hybridus* seeds at 9 combinations of temperature and moisture content, for periods up to 126 days are shown in tables 6 and 7. Regression coefficients of electrical conductivity readings on the corresponding storage periods are presented at the bottom of each table.

Table 6. Mean electrical conductivity values ($\mu Scm^{-1}g^{-1}$) and the corresponding regression coefficients for *E. coracana* seeds soaked in deionised water after storage at various temperature and moisture content treatments.

Storage period (days)	Storage treatment combinations								
	5.3% & 15°C	5.3% & 25°C	5.3% & 35°C	11.3% & 15°C	11.3% & 25°C	11.3% & 35°C	16.3% & 15°C	16.3% & 25°C	16.3% & 35°C
0	97.0	97.0	97.0	93.5	93.5	93.5	91.5	91.5	91.5
21	124.8	130.7	131.1	149.7	169.1	155.6	132.1	125.9	141.7
42	108.3	99.1	108.2	124.1	128.3	133.2	118.4	114.3	112.3
63	107.3	90.1	100.6	131.9	126.0	128.3	120.1	120.9	113.4
8 4	95.8	99.1	91.3	131.24	120.8	112.6	119.9	112.3	107.2
105	104.5	110.7	112.1	137.13	131.9	136.5	124.0	127.2	
126	110.2	106.2	103.5	138.5	119.3	133.3	128.7		
DF	6	6	6	6	6	6	6	5	4
Slope	-0.024	-0.022	-0.062	0.198	-0.011	0.101	0.164	0.196	0.010
r	0.111	0.075	0.215	0.505	0.021	0.233	0.561	0.582	0.018
p	0.812	0.873	0.644	0.221	0.964	0.615	0.179	0.213	0.977

* Mean electrical conductivity is regressed on each corresponding storage period

Table 7. Mean electrical conductivity values ($\mu Scm^{-1}g^{-1}$) and the corresponding regression* coefficients for *A. hybridus* seeds soaked in deionised water after storage at various temperature and moisture content treatments.

Storage period (days)	Storage treatment combinations								
	5.3% & 15°C	5.3% & 25°C	5.3% & 35°C	11.2% & 15°C	11.2% & 25°C	11.2% & 35°C	16.4% & 15°C	16.4% & 25°C	16.4% & 35°C
0	29.4	29.4	29.4	25.9	25.9	25.9	21.4	21.4	21.4
21	49.5	37.9	34.3	29.7	41.2	32.0	29.7	28.6	34.7
42	31.3	33.6	32.9	31.9	36.2	35.8	30.2	31.2	36.9
63	27.0	29.4	29.7	28.9	31.9	33.5	22.8	30.9	42.0
105	28.0	31.1	28.9	27.1	31.1	32.9	22.7	24.28	
126	31.4	35.7	31.1	37.6	36.7	35.58	35.58		
DF	5	5	5	5	5	5	5	4	3
Slope	-0.064	-0.008	-0.013	0.050	0.017	-0.008	0.046	0.015	0.308
r	0.368	0.112	0.304	0.583	0.155	0.020	0.389	0.143	0.938
p	0.473	0.833	0.559	0.212	0.770	0.971	0.447	0.819	0.062

* Mean electrical conductivity is regressed on each corresponding storage period

There was a general trend of increasing electrical conductivity readings with increasing storage period at most of the treatment combinations for both species. Regression analysis of the seed leachate conductivity at each storage treatment on the corresponding storage duration, however, revealed that increased leachate conductivity with storage duration was insignificant, implying that the amount of electrolyte leaked from seeds at the various treatments did not vary significantly during storage for the two species.

CHAPTER FIVE

5.0 DISCUSSION

5.1 *Effect of storage temperature and moisture content on seed germination*

The gradual seed death (severe seed deterioration quantified by slow germination and viability loss) and low seed deterioration (not quantifiable by either slow germination or viability loss) that were associated with storage temperatures $\geq 25°C$ in combination with moisture contents $\geq 11.3\%$, and the rest of the storage regimes, respectively (Tables 1 and 2), during the entire experimental period reveal that the finger millet and amaranth seeds responses vary with different levels of temperature and moisture content. This confirms the physiological influence of temperature and moisture content during seed storage (Delouche *et al.*, 1973; Harrington, 1970; Perry, 1981 and Villiers and Edgcumbe, 1975). Deterioration of orthodox seeds increases with increase in seed moisture and high temperature (Villiers and Edgcumbe, 1975). Gradual seed deterioration has been associated with a sequence of the following events (Roberts, 1973c); membrane damage; impaired biosynthesis; slower germination; slower and more uneven growth and development; greater susceptibility to environmental stress, thus; poor emergence potential; morphological aberrations and finally, inability to germinate (equated with death).

In the present study, percentage (first count) germination of *E. coracana* seeds declined earlier than the corresponding decline in the percentage (final count) germination, an indication that loss in germination speed preceded viability loss. This observation was in agreement with the aforementioned suggestion of Roberts (1973c) on the possible sequence of changes leading to viability loss. According to the sequence by Roberts (1973c), seeds lose their speed of germination prior to loosing their ability to germinate. Similar results have been observed by Pandey (1989a; 1989b) on seeds of French beans (*Phaseolus vulgaris* L.). In both cases, germination speed declined earlier during deterioration, compared to viability decline.

When the rate of the percentage (first count) germination decline was compared to corresponding decline in the percentage (final count) germination, the results indicated that decrease in the germination speed of *A. hybridus* seeds accompanied rather than preceded viability loss. This was possibly as a result of relative dormancy (Ellis *et al.*, 1985a) variations within the experimental seed lot. This was especially evident in seeds of this species stored with a moisture content of 16.4% at temperatures 25°C and 35°C. The initial phase of increasing percentage (first count) germination for seeds of *A. hybridus* sored at these conditions is an indication of a temporal increase in germination speed. Increased germination speed was probably caused by after-ripening dormancy loss in the seeds of this species. It is also postulated that, warm temperature after-ripening dormancy loss, was the cause of the observed significant increase in the percentage (first count) germination of *A. hybridus* seeds stored with a moisture content of 5.3% at temperature 35°C and *E. coracana* seeds stored with a moisture content of 5.3% at temperatures 25°C and 35°C. After-ripening dormancy loss in stored seeds has been observed in *A. retroflexus* (Omami *et al.*, 1992), wild oat (*Avena fatua*) (Foley, 1994) and Idaho fescue (*Festuca idahoensis*) (Goodwin *et al.*, 1995). Omami *et al.* (1992), observed that after subjecting seeds to a range of after-ripening temperatures for various durations, those stored at highest temperature of 36°C were least dormant. Goodwin *et al.* (1995), observed that storing seeds at warmer temperatures promoted rapid germination in seeds that had broken dormancy. In his investigation, Foley (1994), combined after-ripening temperature with different seed moisture contents and observed an inverse relationship between temperature and seed moisture content as measured by seed germination. He thus, concluded that, as the after-ripening temperature increases, the seed moisture content must decrease for maximum after-ripening (i.e. more rapid germination to occur).

Therefore, increase in the speed at which seeds germinate observed in this study (as indicated by significant increase in the percentage (first count) germination of *A. hybridus* and *E. coracana* during storage at 5.3% moisture content and temperatures of 25°C and 35°C, depending on the species), is in agreement with the above mentioned observation by Foley (1994). Seeds of *A. hybridus*

stored at 16.4% moisture content and temperatures 25°C and 35°C, in addition to showing rapid germination rates at the initial stages of storage, also exhibited deteriorative germination decline. The promotive effect of warmer temperatures, leading to rapid germination in seeds that had broken dormancy, was only limited since deteriorative changes due to the combined effect of high temperature and moisture content was also taking effect. Thus, seeds stored at 35°C declined earlier and faster than those stored at 25 °C at the same moisture content of 16.4 %.

There may be many causes of seed deterioration observed at different storage conditions used in this study. As postulated by many authors (e.g., Roberts, 1973b, 1981; Abdul-Baki and Anderson, 1972; Halmer and Bewley, 1984), these may include physiological, cytological and biochemical changes, particularly changes in enzymatic activity, respiratory and synthetic pathways, membranes, storage compounds and chromosomes. The primary causes of seed deterioration, the sites and nature and sequence of biochemical reactions involved are nevertheless still poorly understood.

5.1.1 Seed survival curves

The pattern of percentage viability loss observed in this study is in agreement with what is already known that, even in a genetically uniform sample of seeds stored under a constant environment, there is considerable variation in the longevity of individual seeds (Roberts, 1960; 1973b). Irrespective of the actual storage conditions, previous studies have shown that variation in longevity results from a normal distribution of seed deaths in time, and thus survival curves conform to negative cumulative normal distributions. This has been observed in the majority of orthodox seed such as; wheat (*Triticum aestivum*) (Roberts, 1961a); rice (*Oryza sativa*) (Roberts, 1961b); barley (*Hordeum distichum*) (Ellis and Roberts, 1980b); pepper (*Capsicum annum*) (Lotito and Quagliotti, 1993). Thus, under any given set of constant storage conditions, a seed lot has a particular mean viability period around which there is a random distribution of seed viability periods.

Modification to the typical seed survival curve distribution observed in this study at some of

storage treatments, is consistent with the suggestion of Roberts and Abdalla (1968), that under extreme storage conditions the distribution of deaths in time may become skewed. Consequently, the tail of the distribution tends to be lost due to rapid loss of viability (Roberts, 1972a).

Another possible explanation for the deviation observed in the survival curves is occurrence of some small percentage of seed which were non-viable for some other reasons than ageing (e.g. immaturity). Similar deviation has been observed in seeds of lettuce (*Lactuca sativa*) with some immature individuals within the lot during storage (Kraak and Vos, 1987). Alternatively, the presence of small percentage of seeds in which dormancy is not completely broken by the potassium nitrate and prechill treatments may account for the deviation in distribution of seed deaths in time.

It has been suggested previously that different storage conditions do not affect the K_i value for any given seed lot (e.g. Ellis and Roberts, 1980a). This was confirmed in this study by observation that, the estimated K_i values at each of the storage conditions were fairly equivalent for each of the two species. Since linear survival curves are described by only two variables (Equation 6, Chapter 2), it inevitably means that different storage conditions will only affect the value of σ, the seed-to-seed variation in longevity (Roberts and Ellis, 1984). This was confirmed in this study by the observation that the standard deviation of the distribution of seed deaths with time (σ) decreased with increase in storage temperature and/or seed moisture content. Consequent to the decrease in σ, the slope ($1/\sigma$) of the curves became steeper.

Roberts and Ellis (1984), have pointed out that in a storage environment which is more deleterious, (such as high temperature and/or seed moisture content) the longevity of the longest lived seed within a seed lot will be reduced in units of time much more than the short-lived seeds, leading to a reduction in the seed-to-seed variation and hence steeper survival curves. In this study thus, decrease in mean longevity period (P_{50}) was accompanied by steeper survival curves due to a reduction in the seed-to-seed variation in the times at which individual seed die (σ).

At comparable storage temperature and seed moisture content conditions, the mean longevity period (P_{50}), for *E. coracana* seeds was observed to be greater than that of *A. hybridus* seeds. This is a possible pointer to seed composition differences between the two species. It has long been known that species differ considerably in absolute seed longevity (Ewart, 1908) and that among species with orthodox seed, differences in seed longevity at the same temperature and seed moisture content are associated with seed composition (Cromartly *et al.*, 1985). However, when equilibrium moisture content levels are used in storing seeds, the absolute longevity differences are largely eliminated. For example, Zewdie and Ellis (1991a), observed an 11- to 12-fold greater seed longevity in the cereal tef (*Eragrotis tef*) than that of oil seed niger (*Guizotia abyssinica*), when both were stored at similar temperatures and moisture contents. However, when the two species were stored at the same temperature and similar relative equilibrium humidities, longevity in niger was no less than that of tef.

5.1.2 Seed viability constants and their application

Ellis and Roberts (1980a), have pointed out that, once the three viability constants K_L, C_1 and C_2 are determined, they should be applicable to all seed lots within a species. Thus using equation [7] (Chapter 2) alternative storage periods, P, for viability to decline to value, V, could be calculated for a given storage temperature (t) and seed moisture content (m) combination. It is only necessary to calculate the value K_i for each seed lot. The value of K_i may be estimated either roughly, by carrying out an initial germination test at the start of any storage period, or accurately through a rapid-ageing test. In such a test, a sample of seed is rapidly determined under adverse environment [typically at a seed moisture content of 15% in combination with a temperature of 40 or 45°C (Ellis and Roberts, 1980a)]. The resultant viability decline data would then be fitted by probit analysis to obtain the value of K_i as the intercept of the resultant straight line (equation 6, Chapter 2) at time zero.

Equation [7] (Chapter 2) has been used to provide estimates of the expected storage behaviour of several species. For example, Dickie *et al.*(1985), used a limited amount of data on the hermetic

storage of *Lupinus polyphyllus* in several environments, to obtain estimates of the seed viability constants. The estimated constants were subsequently substituted into equation [7] (Chapter 2) and used to estimate the viability of the same seed lot immediately after harvest (before its 44 months of storage). By re-arranging the equation to make K_i the subject, they computed a K_i probit value, equivalent to germination of 99.99 %. A test which had been carried out on 100 seeds of the same experimental seed lot soon after harvest gave 100% germination. The level of agreement between the calculated and the observed germination level confirmed the usefulness of the viability equation [7] (Chapter 2) and the constants for predicting seed viability in at least the short-term, over limited rages of seed moisture content and temperature (Dickie *et al.*, 1985). Reasonably accurate predictions of seed storage life under known conditions, especially in the short- and medium-term storage have also been reported in pepper (*Capsicum annum*) seeds (Lotito and Quagliotti, 1993) and Lettuce (*Lactuca sativa*) seeds (Kraak and Vos, 1987).

Thus in the present study, the respective estimated constants; K_L, C_1 and C_2 , for the two species, were applied to predict the storage behaviour for their seeds at various hypothetical storage temperatures (between 5°C and 20°C) and moisture contents (between 5% and 12%). The storage conditions included those within the recommendation (Cromartly *et al.*, 1985) for short- and/ or medium-term active collections for genebanks. Because such collections are often accessed frequently, they are often maintained above the regeneration level (usually 85% viability) for 3 to 4 years at temperatures between 0°C and 10°C. Though regeneration levels of as low as 65% have been recommended (FAO/ IPGRI, 1994), 85% was chosen in the present study as a precaution against possible inaccuracies in the predictive model. This was bearing in mind that, only limited ranges of storage temperature and moisture content were used in obtaining the species constants and that the storage periods in each case were relatively short.

It has generally long been known that, the greater the moisture content and storage temperature of orthodox seeds, either singly or in combination, the shorter the period of seed survival (Roberts,

1973a). This was confirmed in this study by observation that, the predicted periods in days for viability of *E. coracana* and *A. hybridus* to fall from an initial level of 95% to 85%, were shorter the greater the moisture content and/ or storage temperature. This observation in effect highlights the importance of lowering the moisture content and storage temperature in order to prolong for some time, the lifespan of orthodox seeds. It has been pointed out previously that, the precise regimes used to fulfil short- and medium-term (active collections) storage conditions for seeds in genebanks will vary depending on the species, the prevailing ambient environment and the relative cost of electricity (FAO/ IPGRI, 1994). This is because different combinations of moisture content and temperature, are capable of achieving a similar goal of ensuring that the viability of stored accessions remains above the regeneration level for a desired period.

On the basis of the longevity predictions in this study, therefore, the following sets of storage conditions could be chosen for storage of *E. coracana* seed germplasm, which would fulfil the active collection requirement to ensure at least a 3 to 4-years viability of materials above regeneration level (85% viability): 5% moisture content and temperatures ≤ 20°C; 7% moisture content and temperatures ≤ 10°C, and 8% moisture content and a temperature of 5°C. As for *A. hybridus*, the predictions suggest that only seeds stored with a seed moisture content of 12% and temperatures above or equal to 10°C will be unable to meet at least the active collection storage requirements. In fact the model predicts that, seeds stored with a moisture content of 5% and temperatures below or equal to 15°C, are capable of meeting base collection (long-term storage) requirement of ensuring a 100-year viability of materials above the regeneration level (FAO/ IPGRI, 1994).

5.2 Electrical conductivity

A loss in the integrity of plasma membrane has been demonstrated in deteriorated seeds by the extent of leakage of cytoplasmic components to the external medium (Mathews and Bradnock, 1968; Gill and Delouche, 1973; Happ *et al.*,1993 and Fay *et al.*, 1993). The cause of this imbibition leakage

is not completely understood, but it is likely that the selectively permeable membranes of the plasmalemma that normally retains solutes within cells lose their integrity upon drying of the mature seed (Halmer and Bewley, 1984). During the initial stages of imbibition, membrane integrity is incomplete for at least several minutes (Simon, 1974). This situation is reversed with time, with the membranes either physically reverting to their most stable configuration or else being repaired by some yet unidentified mechanism. In deteriorated seeds such repair mechanism might be absent or inefficient, or membrane disruption might be so extensive as to make repair impossible (Halmer and Bewley, 1984; ISTA, 1995). Electrical conductivity measurement of soak water in which bulk sample seeds has been soaked identifies such deteriorated seed lots. Such seed lots have high electrolyte leakage and are classified as low vigour, while those with low leakage are considered high vigour (ISTA, 1995).

In the present study, results of the electrical conductivity measurement do not reveal significant changes in the amount of electrolyte leaked from seeds of the two species during storage at various temperature and seed moisture content treatments. This was quite unexpected, at least at the treatment combinations, where for both species significant deterioration was observed through declining seed germination speed and viability loss. The amount of electrolyte leaked to the surrounding liquid is most likely influenced by the length of time that leakage occurs. The length of time that leakage occurs varies greatly between seeds, but is probably related to the size of the seed, the intactness of the structures surrounding the embryo and the time taken for the outermost layers of the embryo to become fully hydrated (Halmer and Bewely, 1984). Any or a combination of these factors influencing the amount of electrolytes leaked from a seed into the surrounding liquid could have led to the inability of the leakage test to detect seed deterioration in the present study.

5.3 Conclusion and further work

Based on the results of this study, it may be concluded that as *E. coracana* seeds loose viability (ability to germinate), and take longer to germinate. Therefore, assessment of first count germination

in this species may be a valuable vigour decline index, especially in supplementing a viability test with more information on the physiological quality of accessions before and during storage.

The study has also established that the viability decline pattern in seeds of both *E. coracana* and *A. hybridus* is effectively described by the basic viability equations (Ellis and Roberts, 1980a). It was therefore appropriate to use the viability constants obtained from the study, to predict the expected longevity for the two species, at several non-extreme storage treatment combinations. Based on the predictions, *A. hybridus* is expected to store longer than *E. coracana*, especially so, at relatively low seed moisture content and temperature levels. In addition, the viability constants may be of great value to genebank managers in providing a rough guide on the expected viability levels for the two species in the study, stored as active collections (i.e. within short- and medium- term storage conditions). Care should however, be exercised in application of the constants because the predictive equation and species constants were derived from results based on limited conditions and storage periods. For example, extreme storage conditions such as those recommended for base collections should not be used. Consequently, in order to come up with widely applicable constants, further work on the two species is recommended. This would require longer storage periods and wider ranges of temperature and moisture content levels, in order to be able to use the now universally accepted viability decline equation (equation [8], Chapter 2).

Based on observation that the bulk electrical conductivity test was unable to detect seed deterioration in the present study, further work is recommended on application of individual seed conductivity test. Commercial instruments are now available which monitor leachate from individual seeds (Steere et al., 1981). Studies on several species, including small seeded vegetable crops have demonstrated that the single seed instrument is sensitive in identifying seed vigour of many crops.

REFERENCES

Abdalla, F.H. and Roberts, E.H., 1968. Effect of temperature, moisture content and oxygen on the introduction of chromosome damage in seeds of barley, broadbeans and peas during storage. *Ann. Bot.* 32:119 - 136.

Abdul-Baki, A.A. and Anderson, J.D., 1970. Viability and leaching of sugars from germinating barley. *Crop science* 10: 31-34.

Abdul-Baki, A.A. and Anderson, J.D., 1972. Physiological and biochemical deterioration of seeds . In: Seed Biology vol. iii. Kozlowski, T.T.(ed.) Academic press, New York. (pp. 283 - 309).

Abdul-Baki, A.A. and Anderson, J.D., 1975. Vigour determination in soybean seed by multiple criteria. *Crop science* 13: 630 - 633.

Abdul-Baki, A.A., 1980. Biochemical aspects of seed vigour. *Hortscience* 15: 765 - 771.

Agrawal, P.K. and Anderson, J.D., 1975. Standardisation of the tetrazolium for ragi (*E. coracana*) seeds. *Seed Science and Technology* 3: 565 - 568.

Agrawal, P.K., 1977. Germination, fatty acidity and leaching of sugars from five cultivars of paddy rice (*Oryza sativa*) seeds during storage. *Seed Science and Technology* 5: 489 - 498.

Agrawal, P.K., 1986. Seed storage and packaging. In: Quality seed production. A.J.G. van Gastel and J. Kerley (eds.), ICARDA, Aleppo.(pp. 55-72).

AOSA, 1983. Seed vigour testing handbook. Contribution no. 32 to the handbook on seed testing Association of Official Seed Analyst, NE, USA.

Balton, L.V., 1961. Seed preservation and longevity. Intersciences, New York.

Bennet-Lartey, S.O., 1991. The longevity of peas, sunflower and groundnut seeds under controlled temperature and moisture content conditions. *Tropical Science* 31: 9 - 19.

Bewley, J.D., 1982. Protein and nucleic acid synthesis during seed germination and early seedling growth. In: Encyclopedia of plant physiology vol. 14A. D. Boulten and B. Parthier (eds.),

Newseries. (pp. 559 - 591).

Berjark, P. and Villiers, T.A., 1972. Age-induced damage and its repair during early germination. *New Phytologist* 71: 135 - 144.

Bonner F.T., 1994. Predicting seed longevity for four forest tree species with orthodox seeds. *Seed Science and Technology* 22: 361 -370.

Chin, H.F., 1994. Seed banks: conserving the past for the future. *Seed Science and Technology* 22: 385 - 400.

Ching, T.M., 1973. Adenosine triphosphate content and vigour. *Plant physiology* 51: 400 - 402.

Ching, T.M. and Schollcraft I., 1968. Physiological and chemical difference in aged seeds. *Crop science* 8: 407 - 409.

Chweya, J., 1994. Potential for agronomic improvement of indigenous vegetables in Kenya. In: Safeguarding the genetic basis of Africa's traditional crops. Putter, A. (ed.). CTA, The Netherlands, Rome. (pp. 105 - 114).

Cromartly, A.S., Ellis, R.H., and Roberts, E.H., 1985. Design of seed storage facilities for genetic conservation. Handbooks for genebanks no. 1, IBPGR, Rome.

Delouche, J.C. and Caldwell W.P., 1960. Seed vigour and vigour tests. *Proceedings of the Association of Official Seed Analyst* 50: 124 - 129.

Delouche, J.C and Baskin, C.C., 1973. Accelerated ageing techniques for predicting the relative storability of seed lots. *Seed Science and Technology* 1: 427 - 452.

Delouche, J.C. Mathews, R.K. Dougherty, G.M. and Boyd, A.H., 1973. Storage of seeds in the sub-tropical and tropical regions. *Seed Science and Technology* 1: 671 - 700.

Dickie, J.B., McGrath, S. and Linington S.H., 1985. Estimation of provisional seed viability constants for *Lupinus polyphyllus* Lindley. *Ann. Bot.* 55: 147 - 151.

Ellis, R.H., 1984. The meaning of viability. In: Seed management techniques for genebanks. J.B. Dickie, S. Linington and J.T. Williams (eds.). IBPGR, Rome. (pp. 146 - 181).

Ellis, R.H., 1988. The viability equation, seed viability equation nomographs and practical advice on seed storage. *Seed Science and Technology* 16: 29 - 50.

Ellis, R.H., Hong, T.D. and Roberts, E.H., 1985a. Handbook of seed technology for genebanks vol. i. Principles and methodology. IBPGR, Rome.

Ellis, R.H., Hong, T.D. and Roberts, E.H., 1985b. Handbook of seed technology for genebanks vol. ii. Compendium of specific germination information and test recommendations. IBPGR, Rome. (pp. 241 - 247).

Ellis, R.H., Hong, T.D. and Roberts, E.H., 1986. The logarithmic relationship between moisture content and longevity in sesame seeds. *Ann. Bot.* 57: 449 - 503.

Ellis, R.H., Hong, T.D. and Roberts, E.H., 1988. A moisture content limit to logarithmic relations between seed moisture content and longevity. *Ann. Bot.* 61: 405 - 408.

Ellis, R.H., Hong, T.D. and Roberts, E.H., 1990. An intermediate category of seed storage behaviour? I. Coffee. *J. Exp. Bot.* 41: 1167 - 1174.

Ellis, R.H., Hong, T.D. and Roberts, E.H., 1991a. An intermediate category of seed storage behaviour? II. Effects of provenance, immaturity and imbibition on desiccation tolerance in coffee. *J.Exp. Bot.* 42: 653 - 657.

Ellis, R.H., Hong, T.D. and Roberts, E.H., 1991b. Effects of storage temperature and moisture content on germination of papaya seeds. *Seed Science and Research* 1: 99 - 104.

Ellis, R.H., Hong, T.D., Roberts, E.H., and Soetisna, U., 1991c. Seed storage behaviour in *Elaeis guineensis*. *Seed Science Research* 1: 99 - 104.

Ellis, R.H., Osei-Bonsu, K. and Roberts, R.H., 1982. The influence of genotype, temperature and moisture content on seed longevity in chickpea, cowpea and soybean. *Ann. Bot.* 50: 69 - 82.

Ellis, R.H. and Roberts, E.H., 1977. A revised viability nomograph of onion. *Seed Research* 5: 93 - 103.

Ellis, R.H. and Roberts, E.H., 1980a. Improved equations for prediction of seed longevity. *Ann. Bot.*

45: 13 - 80.

Ellis, R.H. and Roberts, E.H., 1980b. The influence of temperature and moisture content on seed viability period in barley (*Hordeum distichum* L.). *Ann. Bot.* 45: 31 - 37.

Ellis, R.H and Roberts, E.H., 1980c. Towards a rational basis for testing seed quality. In: Seed production. P.P., Hebblethwaite (ed.). Butterworths, London. (pp. 605 - 645).

Ellis, R.H. and Roberts, E.H., 1981. The quantification of ageing and survival in orthodox seeds. *Seed Science and Technology* 9: 373- 409.

Ewart, A.J., 1908. On the longevity of seeds. *Proc. of the Royal Society, Victoria* 21: 1 -210.

FAO/ IPGRI, 1994. Genebank standards. Food and Agriculture Organization of theUnited Nations and International Plant Genetic Resources Institute, Rome.

Fay, A.M., McDonald, M.B. and Still, S.M., 1993. Vigour testing of *Rudbeckia fulgida* seeds. *Seed Science and Technology* 21:453-462.

Finney, D.J., 1962. Probit analysis, 2nd edition, Cambridge University press, London.

Fisher, R.A. and Yates, F., 1957. Statistical tables. Oliver and Boyd, Edinburgh. (pp. 60 - 62).

Foley, E.M., 1994. Temperature and water status of seed affect after-ripening in wild oat (*Avena fatua*). *Weed Science* 42: 200 - 204.

Gill, N.S. and Delouche, J.C., 1973. Determination of corn during storage. *Proceedings of the Association of Official Seed Analyst.* 63: 33 - 50.

Goodspeed, T.D., 1911. The temperature co-efficient of the duration of life of barley grains. *Bot. Gaz.* 51: 220 - 224.

Goodwin, R.J., Doescher, P.S. and Eddleman, L.E., 1995. After-ripening in *Festuca idahoensis* seeds: Adaptive dormancy and implications for restoration. *Restoration Ecology* 3(2): 137 -142.

Groves, J.F., 1917. Temperature and life duration of seeds. *Bot. Gaz.* 63: 169 - 189.

Grubben G.J.H. and van Sloten, O.H., 1981. Genetic resources of amaranths - a global plan of action, *IBPGR. Rome.*

Halmer, P. and Bewley, J.D., 1984. A physiological perspective on seed vigour testing. *Seed Science and Technology* 12: 561 - 575.

Hampton, J.G. and Coolbear, P., 1990. Potential versus actual seed performance - can vigour testing provide an answer?. *Seed Science and Technology.* 18: 215-228.

Hanson, J., 1985. Practical manuals for genebanks. No.1. Procedure for handling seed in genebank. IBPGR, Rome.

Happ, K., McDonald, M.B. and Danneberger, T.K., 1993. Vigour testing in perrenial rye grass (*Lolium perrenne* L.) seed. *Seed Science and Technology* 21: 375-383.

Harrington, J.F., 1970. Seed and pollen storage for conservation of plant genetic resources. In: Genetic resources in plants - their exploitation and conservation. Frankel, O.H. and Bennet, E. (eds.). Oxford and Edinburgh, Blackwell. (pp. 501-512).

Heydecker, W., 1972. Vigour. In: Viability of seeds. E.H., Roberts (ed.). Syracause University Press, Syracause, New York. (pp. 209-252).

Hibbard, R.P. and Miller, E.V., 1928. Biochemical studies on seed viability. I. Measurement of conductance and reduction. *Plant physiology* 3: 335-352.

Hong, T.D. and Ellis, R.H., 1992. Optimum air-dry seed storage environment for arabica coffee. *Seed Science and Technology* 20:547-560.

Hong, T.D. and Ellis, R.H., 1995. Interspecific variation in seed storage within two genera - *Coffea* and *Citrus*. *Seed Science and Technology* 26: 165-181.

Hong, T.D. and Ellis, R.H., 1996. A protocol to determine seed storage behaviour. IPGRI Technical Bulletin No.1. J.M.M. Engels and J. Toll (eds.). IPGRI, Rome.

Hong, T.D., Linigton, S. and Ellis, R.H., 1996. Seed storage behaviour; a compendium. Handbooks for genebanks: No.4. IPGRI, Rome.

Hutchinson, B.J., 1944. The drying of wheat. III. The effect of temperature on germination capacity. *J. Soc. Chem. Ind.* 63: 104 -107

IBPGR, 1976. Report of IBPGR working group on engineering, design and cost aspects of long-term seed storage facilities. IBPGR, Rome.

Ibrahim, A.E. and Roberts, E.H., 1983. Viability of lettuce seeds. I. Survival in hermetic storage. *J. Exp. Bot.* 34: 620-630.

Ibrahim, A. E., Roberts, E.H., Murdoch, A.J., 1983. Viability of lettuce seeds. II. Survival and oxygen uptake in osmotically controlled storage. J. Exp. Bot. 34: 631 - 640.

Imbamba, S.K., 1973. Lysine content of leaves of some Kenyan vegetables. *E. Afr. Agric. For. J.* 38: 246.

Isley, D., 1957. Vigour tests. *Proceedings of the Association of Official Seed Analyst* 47: 176-182.

ISTA , 1985. International rules for seed testing. *Seed Science and Technology* 13: 356-513.

ISTA, 1987. Handbook of vigour test methods. The International Seed Testing Association, Zurich.

ISTA, 1995. Handbook of vigour test methods (3rd edition). J.G., Hampton and D.M. Tekrony (eds.). ISTA, Zurich.

Jackson, M.T. and Ford-Lloyd B.V., 1990. Plant genetic resources - a perspective. In: Climatic changes and plant genetic resources. M.T. Jackson, B.V. Ford-Lloyd, M.L. Penny (eds.). Belhaven Press. London. (pp. 1-17).

Kempanna, C., 1975. Viability pattern and its impact on the structure yield in Ragi. University Agricultural Sciences, Bangalore.

Koch, B., Kota, M. and Horvath, I.M., 1965. Fodder crops as leaf protein. *Agrobotanika* 7:19 - 28.

Kraak, H.L. and Vos, J., 1987. Seed viability constants for lettuce. *Ann. Bot.*, 59: 343-349.

Lotito S. and Quagliotti L., 1993. The influence of storage temperature and moisture content on seed viability in pepper (*Capsicum annum* L.). *Agronomie* 13: 231-234.

Mathews, S. and Bradnock, W.T., 1967. The detection of seed samples of wrinkle-seeded peas (*Pisum sativum* L.) of potentially low planting value. *Proceedings of the International Seed Testing Association* 32:553-563.

Mathews, S. and Bradnock, W.T., 1968. Relationship between seed exudation and field emergence in peas and French beans. *Horticultural Research* 8: 89-98.

Mathews, S. and Whitbread, R., 1968. An association between seed exudates and the incidence of pre-emergence mortality in wrinkle seeded peas. *Plant Pathology* 17: 11-17.

National Research Council, 1984. Amaranth: modern prospects for an ancient crop. National Academy Press, Washington, D.C.

National Research Council, 1996. Lost crops of Africa vol.1. Grains. Board of Science and Technology for International Development, National Academy Press, Washington, D.C. (pp. 42 - 57).

Omami E.N., Medd, R.W. and Haigh, A., 1992. Germination and after-ripening responses in *Amaranthus retroflexus* seed. *Proceedings of the 1st International Weed Control Congress.* vol. 2: 372-374.

Okigbo, B.N., 1983. Agriculture and agroforestry: roles in natural resources development in tropical Africa. Special report to the United Nation University, Tokyo.

Okigbo, B.N., 1994. Conservation and use of plant germplasm in African traditional agriculture and land use systems. In: safeguarding the genetic basis of Africa's traditional crops. Putter, A. (ed.). CTA, the Netherlands, Rome. (pp. 15-38).

Pandey, D.K., 1989a. Ageing of French bean seeds at ambient temperature in relation to vigour and viability. *Seed Science and Technology* 17: 41-47.

Pandey, D.K., 1989b. Short duration accelerated ageing of French bean seeds in hot water. *Seed Science and Technology.* 17: 107-114.

Perry, D.A., 1972. Seed vigour and field establishment. *Hort. Abstracts* 42: 334-342.

Perry, D.A., 1978. Report of the vigour testing committee 1974 -1977. *Seed Science and Technology* 6: 159.

Perry, D.A., 1981. Introduction. In: Handbook of vigour testing methods. ISTA, Zurich. (pp. 3-7).

Perry, D.A., 1987. Methodology and application of vigour test method. In: Handbook of vigour testing methods. ISTA, Zurich. (pp. 3-9).

Pollock, B.M. and Roos E.R., 1972. Seed and seedling vigour. In: Seed biology. T.T. Kozlowski (ed.) Vol.1. Academic Press, New York. (pp. 313-387).

Powell, A.A., 1988. Seed vigour and field establishment. *Advances in research and technology of seeds* 11: 29-80.

Population Reference Bureau, Inc., 1997. Washington, D.C.

Roberts, E.H., 1960. The viability of cereal seed in relation to temperature and moisture. *Ann. Bot.* 24: 12-31.

Roberts, E.H., 1961a. Viability of cereal seed for brief and extended periods. *Ann. Bot.* 25: 373-380.

Roberts, E.H., 1961b. The viability of rice seed in relation to temperature, moisture content and gaseous environment. *Ann. Bot.* 25: 380-390.

Roberts, E.H., 1972a. Storage environment and the control of viability. In: Viability of seed. E.H. Roberts (ed.) Chapman and Hall, London. (pp. 14-58).

Roberts, E.H., 1972b. Cytological, genetical and metabolic changes associated with loss of viability. In: Viability of seeds. E.H. Roberts (ed.) Chapman and Hall, London. (pp. 253-306).

Roberts, E.H., 1973a. Predicting the storage life of seeds. *Seed Science and Technology* 1: 499-514.

Roberts, E.H., 1973b. Loss of viability: Chromosomal and genetical aspects. *Seed Science and Technology* 1: 515-527.

Roberts, E.H., 1973c. Loss of viability: Ultrastructural and physiological aspects. *Seed Science and Technology.* 1: 529- 545.

Roberts, E.H., 1975. Problems of long-term storage of seed and pollen for genetic resources conservation. In: Crop genetic resources for today and tomorrow. Frankel, O.H. and Hawkes, J.G. (eds.). Cambridge University Press, Cambridge. (pp. 269-296).

Roberts, E.H., 1981. Physiology of ageing and its application to drying and storage. *Seed Science and*

Technology 9: 359-372.

Roberts, E.H., 1984. The control of seed quality and its relationship to crop productivity. *Proceedings of the Australian Seed Research Conferences* 11-25.

Roberts, E.H. and Abdalla, F.H., 1968. The influence of temperature, moisture and oxygen on period of viability in barley, broadbeans and peas. *Ann. Bot.* 25: 380-390.

Roberts, E.H. and Ellis, R.H., 1984. The implication of deterioration of orthodox seeds during storage for genetic resources conservation. In: Crop genetic resources: conservation and evaluations. J.H.W. Holden and J.T. Williams (eds.). George Allen and Unwin, London. (pp. 19-37).

Roberts, B.F. and Osborne, D.J., 1973. Protein synthesis and loss of viability in rye embryos. The lability of transferase enzymes during senescence. *Biochemical Journal, London.* 135: 405-410.

Roos, E.E., 1980. Physiological, biochemical and genetic changes in seed quality during storage. *Hortscience* 15: 781-784.

Roos, E.E., 1984. Report of the storage committee working group on effects of storage on genetic integrity 1980-1983. *Seed Science and Technology* 12: 255-260.

Seme, E.N., 1991. The role of the genebank of Kenya. In: Crop genetic resources of Africa Vol. II. N.Q. Ng, P. Perrino, F. Attere and H. Zedan (eds.) IITA, IBPGR, UNEP. The Trinity Press, U.K. (pp. 287- 290).

Simon, E.W., 1974. Phospholipids and plant membrane permeability. *New Phytologist* 73: 377-420.

Steere, W.C., Levengoo, W.C. and Bondie, J.M., 1981. An electronic analyzer for evaluating germination and vigour. *Seed Science and Technology* 9: 567-576.

Stoyanova, S.D., 1992. Effect of ageing and regeneration on the genetic composition of wheat. *Seed Science and Technology* 20: 489-496.

Tao, K.J., 1980. Vigour referee test for soybean and corn seed. *Association of Official Seed Analyst Newsletter* 54: 40-68.

Teng, V.T. and Hor V.L., 1976. Storage of tropical fruit seeds. In: Seed technology in the tropics. H.F. Chin, I.C., Enoch and R.M., Raja Harun (eds.). Universiti Pertanian Malaysia, Malaysia.(pp. 49-53).

Tompsett, P.B., 1984. The effect of moisture content and temperature on the seed storage life of *Araucaria columnaris*. *Seed Science and Technology* 12: 801-816.

Villers, T.A., 1975. Genetic maintenance of seeds in imbibed storage In: Crop genetic resources for today and tomorrow. O.H. Frankel and J.G. Hawkes (eds.). Cambridge University press, Cambridge. (pp. 297-315).

Villers, T.A. and Edgcumbe, D.J., 1975. On the causes of seed deterioration in dry storage. *Seed Science and Technology* 3:761-774.

Wilson, E.O., 1988. The current state of biological diversity. In: Biodiversity. Wilson, E.D. and Francis, M.P. (eds.). National Academy Press, Washington D.C. (pp. 3-18).

Wills, J.C., 1973. A dictionary of the flowering plants and ferns. Cambridge University Press. Cambridge.

Woodstock, L.W., 1969. Seedling growth as a measure of seed vigour. *Prod. Int. Seed Test Assoc.* 34: 273-280.

Zewdie, M. and Ellis, R.H., 1991a. Survival of tef and niger seed following exposure to sub zero temperatures at various moisture content. *Seed Science and Technology* 19: 309-317.

Zewdie, M. and Ellis R.H., 1991b. The upper moisture content limit to negative relations between seed longevity and moisture in niger and tef. *Seed Science and Technology* 19: 295-302.

Appendix 1:1 Probit values corresponding to percentage (first count) germination of E. coracana seeds stored at various temperature(°C) and moisture contents(%).

Storage period (days)	Storage treatment combinations									
	5.3%& 15°C	5.3%& 25°C	5.3%& 35°C	11.3%& 15°C	11.3%& 25°C	11.3%& 35°C	15.2%& 40°C	16.3%& 15°C	16.3%& 25°C	16.3%& 35°C
0	6.01	6.01	6.01	6.44	6.44	6.44	6.48	6.25	6.25	6.25
7	-	-	-	-	-	-	6.10	-	-	-
14	-	-	-	-	-	-	5.82	-	-	-
21	5.93	6.04	5.90	6.41	6.10	6.15	5.86	6.41	5.99	5.81
28	-	-	-	-	-	-	4.63	-	-	-
35	-	-	-	-	-	-	3.63	-	-	-
42	6.04	5.88	6.04	5.81	6.01	6.06	1.91	5.86	5.64	4.50
63	6.13	5.95	6.06	5.81	6.13	6.13		5.93	4.86	3.87
84	6.18	6.15	5.95	6.23	6.28	6.04		6.02	3.87	1.91
105	6.01	5.88	6.08	6.18	6.34	6.01		5.90	1.91	
126	5.63	6.06	6.37	5.72	5.97	5.79		5.86		
147	5.90	6.13	6.34	5.99	6.13	5.92		6.04		
168		6.23	6.15		6.08	5.86		6.23		
210		6.44	6.51		6.34	5.20		6.15		
231		6.31	6.55		6.15	4.36		6.28		
252		6.34	6.48		6.10	1.91		6.18		

Appendix 1:2 Probit values corresponding to percentage (first count) germination of A. hybridus seeds stored at various temperatures(°C) and moisture contents(%).

Storage period (days)	Storage treatment combinations										
	5.3% &15° C	5.3% &25° C	5.3% &35°C	11.2% & 15°C	11.2% & 25°C	11.2% &35°C	16.4% &15°C	16.4% &25°C	16.4% &35°C	17.3% & 25°C	17.3% &35°C
0	5.48	5.48	5.48	5.74	5.74	5.74	5.13	5.13	5.13	5.61	5.61
7	-	-	-	-	-	-	-	-	-	-	5.13
14	-	-	-	-	-	-	-	-	-	-	4.90
21	5.11	5.45	5.15	6.06	5.95	5.43	5.66	5.36	5.55	5.14	4.33
28	-	-	-	-	-	-	-	-	-	-	3.75
35	-	-	-	-	-	-	-	-	-	-	1.91
42	4.67	5.36	5.63	5.47	5.50	5.43	5.63	5.51	4.21	4.43	
63	5.36	6.51	6.28	6.10	5.90	6.04	5.61	4.90	1.91	1.91	
84	5.58	5.54	6.15	6.28	6.41	6.04	5.55	4.71			
105	5.50	5.77	6.34	5.14	5.58	5.69	6.02	1.91			
126	5.32	6.55	6.31	5.16	5.54	6.23	5.66				

Appendix 1:3 Probit values corresponding to percentage(final count) germination of E. coracana seeds stored at various temperatures(°C) and moisture contents(%).

Storage period (days)	Storage treatment combinations									
	5.3%& 15°C	5.3%& 25°C	5.3%& 35°C	11.3%& 15°C	11.3%& 25°C	11.3%& 35°C	15.2%& 40°C	16.3%& 15°C	16.3%& 25°C	16.3%& 35°C
0	6.55	6.55	6.55	6.96	6.96	6.96	6.51	6.58	6.58	6.58
7	-	-	-	-	-	-	6.28	-	-	-
14	-	-	-	-	-	-	6.41	-	-	-
21	6.64	6.48	6.64	7.05	6.55	6.70	6.25	6.81	6.44	6.48
28	-	-	-	-	-	-	5.71	-	-	-
35	-	-	-	-	-	-	4.37	-	-	-
42	6.48	6.64	6.60	6.70	6.70	6.88	1.91	6.64	6.20	5.58
63	6.60	6.41	6.41	6.44	6.75	6.64		6.34	5.66	5.14
84	6.44	6.60	6.23	6.48	6.48	6.34		6.44	5.03	1.91
105	6.31	6.34	6.44	6.55	6.64	6.34		6.10	1.91	
126	6.44	6.44	6.51	6.70	6.31	6.34		6.23		
147	6.48	6.51	6.44	6.81	6.48	6.20		6.41		
168		6.48	6.51		6.48	5.55		6.44		
210		6.51	6.55		6.75	4.82		6.48		
231		6.41	6.51		6.37	1.91		6.41		
252		6.44	6.48		6.60			6.44		

Appendix 1:4 Probit values corresponding to percentage (final count) germination of A. hybridus seeds stored at various temperatures(°C) and moisture contents(%).

Storage period (days)	Storage treatment combinations										
	5.3% &15° C	5.3%& 25°C	5.3%& 35°C	11.2% & 15°C	11.2% & 25°C	11.2% &35°C	16.4% &15°C	16.4% &25°C	16.4% &35°C	17.3%& 25°C	17.3%& 35°C
0	7.17	7.17	7.17	6.88	6.88	6.88	6.81	6.81	6.81	6.64	6.64
7	-	-	-	-	-	-	-	-	-	-	5.95
14	-	-	-	-	-	-	-	-	-	-	5.16
21	6.51	6.64	6.64	6.96	6.75	6.37	6.75	5.81	6.25	5.40	4.50
28	-	-	-	-	-	-	-	-	-	-	3.92
35	-	-	-	-	-	-	-	-	-	-	1.91
42	6.34	6.37	6.28	6.10	5.93	6.44	6.06	5.67	5.08	4.69	
63	6.28	6.75	6.37	6.51	6.37	6.23	6.75	5.16	1.91	1.91	
84	6.44	6.44	6.15	6.70	6.51	6.08	6.31	5.04			
105	6.20	6.64	6.48	6.23	6.20	6.08	6.41	1.91			
126	6.28	6.70	6.51	6.60	6.34	6.06	6.34				

Appendix 2:1 A summary of the regression analysis of first count germination (probits) on storage period for E. coracana seeds stored at different temperature(Temp) and moisture content (m.c) treatments.

seed m.c (%)	Temp (°C)	DF	Slope	r(s.e)*	F-ratio	P
5.3	15	7	-0.001	0.40(0.167)	1.145	0.236
5.3	25	11	0.002	0.817(0.110)	20.105	0.001
5.3	35	11	0.003	0.893(0.110)	32.224	0.000
11.3	15	7	-0.003	0.524(0.259)	2.270	0.169
11.3	25	11	0.000	0.150(0.150)	0.229	0.643
11.3	35	11	-0.012	0.770(0.837)	14.581	0.003
15.2	40	6	-0.100	0.925(0.682)	29.637	0.003
16.3	15	11	0.000	0.144(0.190)	0.211	0.656
16.3	25	6	-0.040	0.942(0.614)	31.679	0.005
16.3	35	4	-0.051	0.976(0.434)	59.945	0.004

* Standard error in parentheses

Appendix 2:2 A summary of the regression analysis of first count germination (probits) on storage period for A. hybridus seeds stored at different temperature(Temp) and moisture content (m.c) treatments.

seed m.c (%)	Temp (°C)	DF	Slope	r(s.e)*	F-ratio	P
5.3	15	6	0.002	0.312(0.327)	0.503	0.510
5.3	25	6	0.007	0.612(0.441)	2.992	0.139
5.3	35	6	0.002	0.868(0.261)	15.306	0.011
11.3	15	6	-0.005	0.464(0.449)	1.371	0.246
11.3	25	6	-0.001	0.105(0.349)	0.056	0.823
11.3	35	6	0.004	0.642(0.263)	3.514	0.117
16.3	15	6	0.004	0.657(0.215)	3.793	0.107
16.3	25	5	-0.026	0.745(1.001)	5.003	0.088
16.3	35	3	-0.053	0.879(0.950)	6.785	0.121
17.3	25	4	-0.056	0.893(0.760)	11.756	0.042
17.3	35	5	-0.097	0.940(0.506)	30.331	0.005

* Standard error in parentheses

Appendix 2:3 A summary of the regression analysis of final count germination (probits) on storage period for E. coracana seeds stored at different temperature(Temp) and moisture content (m.c) treatments.

seed m.c (%)	Temp (°C)	DF	Slope	r(s.e)*	F-ratio	P
5.3	15	7	-0.001	0.606(0.089)	3.489	0.108
5.3	25	11	-0.001	0.373(0.083)	1.616	0.181
5.3	35	11	0.000	0.103(0.110)	0.106	0.751
11.3	15	7	-0.002	0.423(0.216)	1.311	0.232
11.3	25	11	-0.001	0.439(0.172)	2.383	0.137
11.3	35	11	-0.013	0.784(0.906)	15.988	0.003
15.2	40	6	-0.103	0.932(0.657)	32.809	0.002
16.3	15	11	-0.001	0.358(0.179)	1.472	0.187
16.3	25	6	-0.036	0.932(0.684)	33.107	0.002
16.3	35	4	-0.052	0.890(1.003)	11.404	0.043

* Standard error in parentheses

Appendix 2:4 A summary of the regression analysis of final count germination (probits) on storage period for A. hybridus seeds stored at different temperature(Temp) and moisture content (m.c) treatments.

seed m.c (%)	Temp (°C)	DF	Slope	r(s.e)*	F-Ratio	P
5.3	15	6	-0.005	0.741(0.243)	6.104	0.057
5.3	25	6	-0.002	0.394(0.261)	0.921	0.381
5.3	35	6	-0.004	0.563(0.399)	2.318	0.177
11.3	15	6	-0.003	0.411(0.317)	1.017	0.256
11.3	25	6	-0.004	0.509(0.305)	1.745	0.218
11.3	35	6	-0.006	0.888(0.148)	18.728	0.008
16.3	15	6	-0.003	0.498(0.270)	1.652	0.226
16.3	25	5	-0.037	0.876(0.902)	13.133	0.022
16.3	35	3	-0.077	0.939(0.935)	14.815	0.061
17.3	25	4	-0.065	0.941(0.768)	15.591	0.059
17.3	35	5	-0.115	0.956(0.515)	42.638	0.003

* Standard error in parentheses