INFLUENCE OF SEED STORAGE PRACTICES ON GERMINATION, GROWTH AND YIELD OF BAMBARA GROUNDNUT

[*Vigna subterranea* (L.) Verdc.]

BY

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ABSTRACT

Two experiments were conducted at Sokoine University of Agriculture to assess the influence of storage containers, seed shells and priming treatment on germination, growth and final seed yield of bambara groundnut [*Vigna subterranea* (L.) Verdc.]. Good quality seeds with germination of 95% were stored unshelled and shelled using seven different storage containers for a period of six months. After sixth months, germination test was conducted as per ISTA methods after priming seeds stored in different containers by soaking for 24 hours, wetting prior to germination with dry germination as the control. The results indicated that germination percentage was decreased in seeds stored in various containers and primed by different treatments. Storing seeds unshelled gave the higher germination of 10.07% compared to those stored after shelling which gave germination of 6.34%. Significant differences ($P \leq 0.05$) between storing in different containers were observed. Shelled seeds stored in deep freezer were the best in germination (27%) followed by unshelled seeds stored in basket and hanged over the fireplace (21.94%). Seeds stored in plastic bucket were the least with only 1.45% germination. Also container differences in maintaining 100 seed weight, seed moisture content and proportion of carbohydrate in food reserve was observed. Germination percentage was positively correlated to 100 seed weight ($r = 0.88^{**}$) and to proportion of carbohydrate in seed food reserve ($r = 0.51^{ns}$) but correlated negatively to seed moisture content after storage ($r = -0.47^{ns}$). Strong negative correlation was recorded between seed moisture content and proportion of carbohydrate in seed food reserve ($r = -0.93^{**}$). This study has shown that differences

in seed quality induced by different storage containers have major effects only on germination and mean relative growth rate at early growth stages. No significant differences in mean relative growth rate at later growth stages and partitioning coefficients of plants from surviving seeds were observed. Soaking bambara groundnut seeds for in water 24 hours significantly accelerated germination and early seedling growth. No significant differences in yield were observed between priming treatments tested. Based on the results in this study, storing of unshelled bambara groundnut seeds in jute bags or woven basket and hanged over the fireplace is recommended. However, further study is recommended to assess the role of pod drying methods in seed deterioration during storage.

DECLARATION

I, SEIF OMARI MKWACHU, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work and that it has neither been submitted nor concurrently being submitted for a degree in any other University.

Signed.....

Date.....30/10/2003

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DEDICATION

To my parents, Omari Mohamedi Mkwachu and Mwashamba Seif Sobo (Nyasobo) whose priority in educating the family is second to none.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABREVIATIONS AND SYMBOLS

%	percent
*	significant at 5% level
**	significant at 1% level
#L	number of leaves
^0C	degree celsius
AOAC	Association of Official Analytical Chemist
BAMNET	Bambara groundnut network
CHO	carbohydrate
cm	centimeter
cm^2	centimeter squire
CO_2	carbon dioxide
CRD	complete randomized design
CV	coefficient of variation
DAS	days after sowing
DMRT	Duncan's Multiple Range Test
DodR	Dodoma red
E	east
FAO	Food and Agriculture Organization
Fig.	figure
g	grams
H	plant height

ha	hectare
HI	harvest index
i.e.	that is
ISTA	International Seed Testing Association
Kg	kilograms
K_i	initial seed viability value
LA	leaf area
LAPC	leaf area partitioning coefficient
LDM	leaf dry matter
LPC	leaf partitioning coefficient
m	meter
m. a. s. l.	meter above see level
MC	moisture content
Mt	megatons
NA	not available.
Nod.	nodule
ns	not significant
O_2	oxygen
ODI	Overseas Development Institute
P	phosphorous
$P \leq 0.01$	significant at less or equal to 1% level
$P \leq 0.05$	significant at less or equal to 5% level

P_2O_5	phosphorous pentaoxide
PDM	pod dry matter
PVC	polyvinyl chloride
r	simple correlation coefficient
RCBD	randomized complete block design
RDM	root dry matter
RGR	relative growth rate
RH	relative humidity
S	south
s.e	standard error
SADC	Southern African Development Community
SDM	stem dry matter
SPC	shoot partitioning coefficient
SUA	Sokoine University of Agriculture
T	time
TOSCA	Tanzania Official Seed Certifying Agency

CHAPTER ONE

1.0 INTRODUCTION

Food security, improved nutrition and economic welfare of human beings in developing countries can be achieved through sustainable and increased economic production of food and industrial raw materials. This can be attained by developing and utilizing the untapped biological diversity of underutilized crops like bambara groundnut.

Seed is one of the basic inputs of any plant production activity. For example, Weisz and Spears (2000) reported that sixty percent of small grain crop's yield potential is determined at planting time, and one of the most important factor in yield potential is the quality of seed being planted. One way to be sure that seed is of the highest quality and purity is to purchase high quality certified seed from public or private seed sector. Unfortunately, bambara groundnut producers in Tanzania depend on landraces maintained by farmers. There is no seed supply available from formal seed sources such as Tanzania Seed Company (TANSEED) (currently not existing) or other private companies. Consequently, farmers use seed bought from neighbors or the local markets or seed saved from a previous crop. In the latter case, farmers usually store the seed from one harvesting season to the next planting season. Lauwaars *et al.* (1998) noted that during storage, seeds are highly vulnerable to reduction in quantity and quality due to pests, diseases, and physiological deterioration depending on the storage

conditions. Seed subjected to potential hazards such as high temperature, high moisture and attack by pests, can lose viability.

The use of poor quality seed can result in poor crop stand and poor crop performance. Field surveys of farmers' saved seed conducted in Ghana, Malawi and Tanzania by Wright and Tyler (1994) and Wright *et al.* (1995) as cited in Walker and Tripp (998) showed that seed management by smallholder farmers is a neglected area. Walker and Tripp (1998) showed that farmers are either unaware about the contribution of seed quality on poor seed germination and/or often attribute this problem to lack of soil moisture and field pests. The lack of a seed supply system from the formal seed sector is one of the constraints facing bambara groundnut producers in Tanzania

Due to the warm weather conditions prevailing in tropical countries the ideal seed storage condition is very difficult to attain at farm level. Hence farmers usually store grains intended for use as seed in the normal manner and conditions as they do for food.

It has been reported that groundnut (*Arachis hypogaea* L.) can lose more than 50% of its viability in 4 -5 months of storage (Nautiyal et al., 1990), similarly, bambara groundnut producers in Tanzania experience difficulties in maintaining seed viability during storage (personal observation). The actual cause of this loss of viability during storage is yet to be established. However, the storage facilities used are presumed

inadequate in maintaining proper seed storage environment. Due to this fact it is of interest to determine how best the seed can be stored using resources available to farmers to ensure that good quality seed for planting is made available at farm level.

The main objective of this work was to evaluate the influence of various storage practices on viability of bambara groundnut seed and subsequent growth and yield.

The specific objectives were:

(i) Assess the effect of seed shelling on germination.

(ii) Determine the effect of different storage structures on seed viability.

(iii) Evaluate the effect of seed priming on germination, growth and yield of bambara groundnut.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Bambara groundnut

2.1.1 Morphology and anatomy of bambara groundnut

Bambara groundnut [*Vigna subterranea* (L.) Verdc., syn. *Voandzeia subterranea* (L) Verdc.] is a leguminous crop which belongs to the subfamily papilionoidae (Linnemann, 1994). The plant is grown for its underground seeds. The entire plant is similar to the common groundnut although botanically they are not closely related, being a low, flat annual with compound leaves of three leaflets (Stephens, 1994). Bambara groundnut is an indeterminate, annual herb up to 30 cm in height with creeping, much branched, leafy lateral stems. A remarkable feature of leaves is the distribution of large bundle sheath cells around small even spaced vascular bundle. Although the plant is a C_3 species, its leaf structure resembles that of classical C_4 anatomy (Linnemann and Azam-Ali, 1993).

Like the groundnut, it forms pods and seeds just below the soil. To achieve this, the flower stalk elongates and penetrates the soil. The bulbous tip creates a tunnel through which the fertilized flower, attached just behind the tip, is drawn into the soil (Stanton, 1966; Stephens, 1994; Brink, 1998). The pods are round, or wrinkled. Each pod contains one or more seeds that are round, smooth, and very hard when dry. The seeds may be cream, brown, red, or black. The coloration is uniform, mottled, blotched or striped; some have an eye with a dark color around the white hilum, in total ,eight eye

patterns were identified in International Institute of Tropical Agriculture (IITA) bambara groundnut descriptor (Goli *et al.*, 1997). Seed color, size and hardness characterize farmers' local cultivars along with the plant form: spreading, bunched or semi bunched (Linnemann and Azam-Ali, 1993).

The plant has a well-developed taproot with lateral roots on the lower part. There are numerous nitrogen-fixing nodules on the roots. (Stanton, 1966; Stephens, 1994). Lateral branches may develop from the axils of the cotyledons, which remain underground (hypogeal germination). Differences in the length of internodes results in bunched, intermediate and spreading types. Plants in a bunch conformation have leaves in a cluster as a result of short internodes, plants in in semi bunch conformation have internodes moderately elongated while plants with spreading growth habit have internodes usually long (Goli *et al.*, 1997). Cultivated forms usually have a bunched or intermidiate growth habit in which leaves are pinately trifoliate, the petiolules of the first two to three leaves are very short but from the third to fourth leaf the petiolule of the terminal leaflet is longer than those of the lateral leaflet. The racemes consist one to three (usually two) whitish to yellow flowers on a short axillary peduncle (Linnemann and Azam-Ali, 1993). The anatomical and morphological features of bambara groundnut is shown in Figure 1.

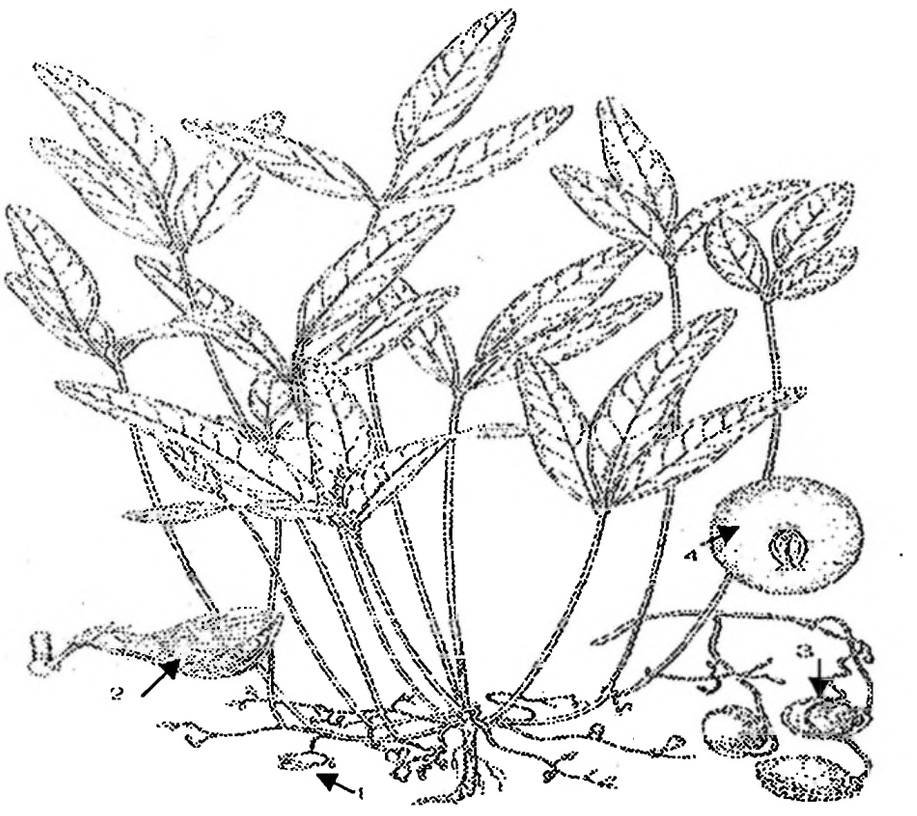

Fig.1: Bambara groundnut morphological and anatomical features.

1.habit of flowering plant; 2.flower; 3.fruit; 4.seed (Source: Linnemann, 1994)

2.1.2. Origin and ecological adaptation

Bambara groundnut is an indigenous African crop. Centre of origin has been a matter of dispute for many years (Linnemann and Azam-Ali, 1993). According to Ecoport Entity records (2000), bambara groundnut inhabits environments between latitude 20° North to 30° South and from 200-1400 meters above sea level (masl). It is adapted to semi arid Africa (Brink, 1998; Nkumba *et al.*, 1997). Bambara groundnut is reported to give reasonable yield in areas where soils are too poor and rainfall is too low for other legumes (Stephens, 1994; Brink; 1998; Salm, 1999). Major producers are Nigeria, Niger, Ghana, Upper Volter and Cote d'Ivoire, but it is widely grown in eastern Africa and Madagascar (Linnemann and Azam-Ali, 1993). Bambara groundnut has advantages of legumes in general; it is able to fix atmospheric nitrogen through symbiosis with Rhizobium bacteria (Brink, 1998). In addition, it can be inter-cropped with other crops such as cereals (millet, sorghum, or maize), root or tuber crops such as cassava, other legumes (groundnut or cowpea) or vegetables (okra or pumpkin) (Stanton, 1966; Linnemann and Azam-Ali, 1993).

Despite of all these features, bambara groundnut is still among the neglected and under utilized crop species (Roger, 1997; Padulosi *et al.*, 1999; Salm, 1999). In Tanzania, it is grown mostly by women farmers in the drier parts of the country (Collinson *et al.*, 2000), even though it ranks as the third most important legume after groundnut (*Arachis hypogeae* L.) and cow pea (*Vigna unguiculata* L.) in most semi arid parts of Africa. Bambara groundnut production areas in Tanzania are shown in Figure 2.

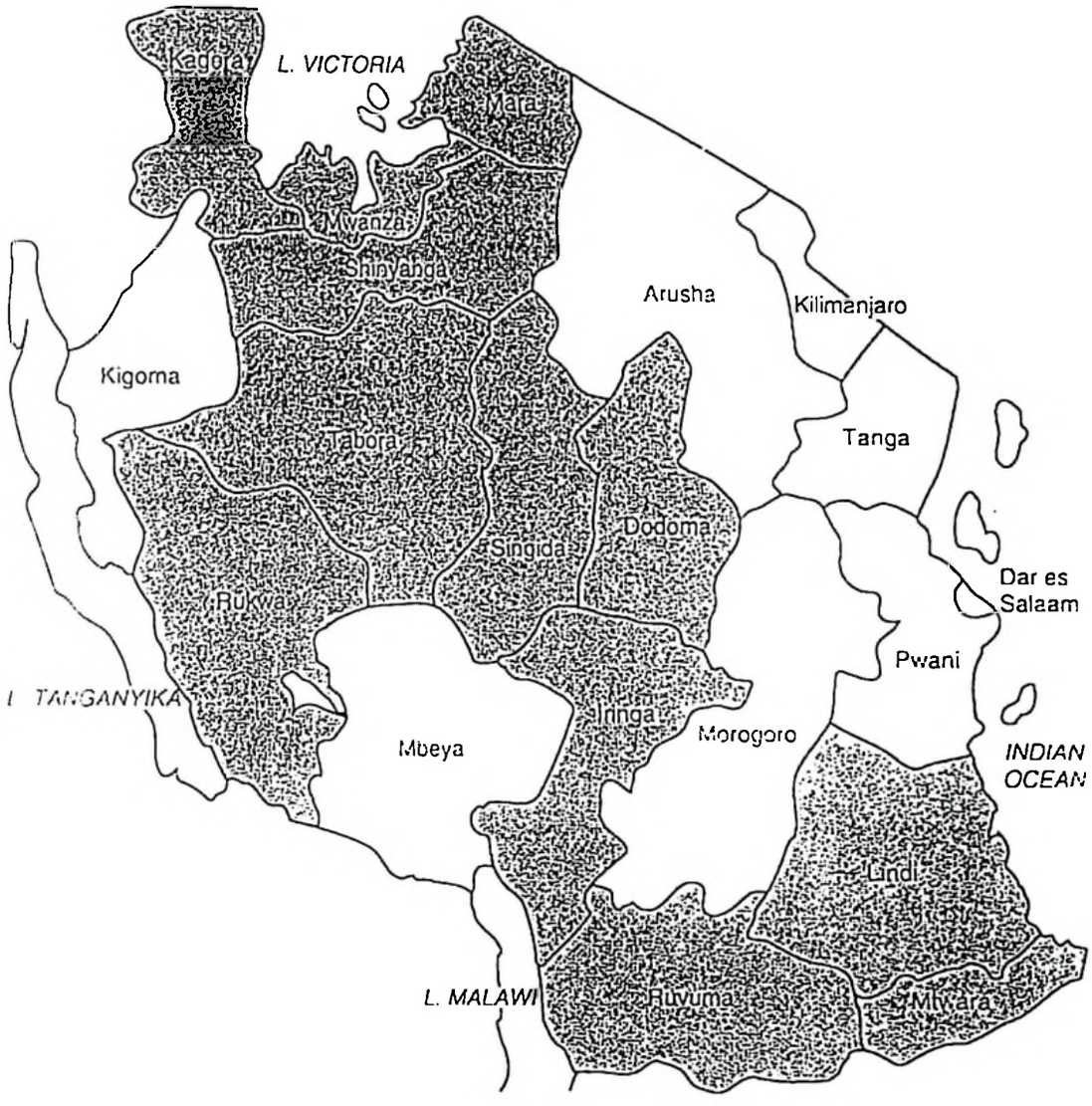

Figure 2. Map of Tanzania. Shaded area indicates major bambara groundnut production areas in Tanzania. (Source: Ntundu, 1997)

2.1.3.Economic importance of bambara groundnut

Bambara groundnut is highly appreciated as a food by native populations of sub Saharan Africa, but the areas cropped with bambara groundnut are still small (Bamfood, 1999). Since the crop is grown for home consumption, production figures are difficult to obtain. Available data indicates that average seed yield in Tanzania is about 650kg/ha (Collinson *et al.*, 2000). However, the highest recorded seed yields in field conditions are close to 4000kg/ha which was recorded in Zimbabwe (Linnemann and Azam-Ali, 1993). Estimated annual production trends for the past five years in Africa South of the Sahara are shown in Table 1.The average yield/ha has been stable since1998.

Table 1. Bambara groundnut production trends in Africa South of Sahara.

Year	1996	1997	1998	1999 (Estimated)	2000 (Estimated)
Area cultivated (ha)	57 884	41 577	47 577	40 000	40 000
Total seed production (Mt)	43 281	32 265	49 160	425 000.0	425 000
Average seed yield (kg/ha)	448.4	784.3	1 033.3	1 062.5	1 062.5

Source: FAOSTAT records, FAO (2000)

Bambara groundnut is predominantly grown for human consumption and can be consumed in many ways for example seeds can be boiled in the immature, green state either shelled or unshelled until soft or alternatively is to roast, boil, crush and eat the seeds as a relish with other food e.g. maize meal porridge. However, production, consumption and sale of bambara groundnut are mostly for local requirements (Linnemann and Azam-Ali, 1993). Bambara groundnut seed contain 14-24% protein, 60% carbohydrate, and 6-12% oil (Stephens, 1994).

Duke et al., (1977) reported oil extraction from bambara groundnut by the Azande of the Congo and large scale canning of bambara groundnut has been reported in Zimbabwe and Ghana (Johnson, 1968; Lartey, 1976). Oluyem et al. (1976) and Doku and Karikari, (1971) as cited by Linnemann and Azam-Ali (1993) reported that bambara groundnut seed can also be used as a feed for pigs and poultry and the haulms as fodder.

Although bambara groundnut is an important secondary food crop in rain fed agriculture in drier parts of Africa, it may also provide additional income as any excess production can easily be sold in local markets (Linnemann and Azam-Ali, 1993). For example in Morogoro, Eastern Tanzania, the average market price of bambara groundnut lies well above the price of other pulses such as pigeon pea and cowpeas.

2.2 Seed in crop production

2.2.1 The seed

A true seed is a fertilized mature ovule that possesses an embryonic plant, stored food material, and a protective coat or coats. The embryo is made up of one or more cotyledons, a plumule (embryonic bud), hypocotyle (stem portion), and a radicle (rudimentary root) (Kozlowski, 1972). Heydeker (1973) described the seed as an end and a beginning; it is the bearer of the essential of inheritance. It symbolizes multiplication and dispersal, continuation and innovation survival, renewal and birth.

Apart from the normal chemical constituents found in all plant tissue, seeds contain stored food reserves to accommodate germination (Owen, 1957; Copeland, 1988). Due to its chemical nature, seeds serve the followings function in daily human life. First, seeds are basic source of food for both human and animals, secondly, seeds are an important source of medicine and drugs, and thirdly, seeds contain reserve food supplies and growth substances that influence seed germination and seedling vigor, seed storage and longevity as well as industrial and other agricultural uses (Kozlowski, 1972; Copeland, 1988).

For seed production, bambara groundnut is harvested when foliage turns yellow and withers after which the plant is uprooted. Dry weather is best for harvest. The pods then sun dried and then shelled and husks winnowed off (FAO, 1961). Shelling maybe done by pounding the dried pods in a pestle and mortar, but it is time consuming and

sometime difficult job and care has to be taken to avoid damaging the beans (BAMNET, 2000). The seed can be stored in gunny bags, straw receptacles, earthenware jars and in granaries with ashes sometime added to control storage pests (FAO, 1961).

The testa of bambara groundnut seed consists of an outer epidermis covered by a cuticle, a hypodermal layer of hourglass-shaped pillar cells with a distinct girdle of thickening in the central region of the radicle walls and thin walled parenchyma with some vascular tissue (Rowson, 1952). Palisade cells with bottle-shaped lamina form the epidermis. In colored seeds, pigment is found in the cavities of the epidermal cells. The parenchyma cells of the cotyledons contain oil drops minute aleurone grains, and starch grains (Vaughan, 1970, as cited by Linnemann and Azam-Ali, 1993).

2.2.2 Seed supply systems

Seed of Agricultural crops is distributed in various ways such as by government agencies, co-operative societies and private seed companies. Seed is also made available through direct distribution from farmer to farmer as seed exchange, barter or as gifts. Louwaars *et al.* (1998) identified three major groups of seed supply systems as follows: -

Local seed supply systems, which cover methods of local seed selection, production and diffusion. These systems are described as traditional and informal operating

mainly at community level through exchange mechanisms and involving limited quantities per transaction. This system is also refereed to as "traditional seed sector" by Camargo *et al.* (1989) and Cromwell *et al.* (1992). Formal seed supply system, which covers seed production and supply mechanism that are ruled by defined methodologies and controlled stages of multiplication, and are backed by national legislation and international standardization of methodologies. It involves cash transaction and large uniform quantities. This is otherwise known as the "conventional seed sector" or "institutional seed sector"(Camargo *et al.*, 1989). Integrated seed supply systems, which cover methodologies that aim at improving the local supply systems by borrowing technologies and improvements from the formal seed sector and using informal channels. This is also known as "non –conventional seed supply system" (Camargo *et al.*, 1989)

Farmer to farmer seed exchange and local seed markets function throughout Africa but are not adequately linked to systems for improved seed (World Bank, 1999). Certified seed use in the Southern African Development Community (SADC) region is largely restricted to maize and cotton (Muliokela, 1997). Muliokola (1997) further noted that for other crops, the majority of small holder farmers use farm saved seed and thus, the use of improved variety is correspondingly limited. One of the reasons leading to this situation is lack of policy attention to minor crops. In Tanzania performance of national seed industry for many years has been poor, only less than 10% of the total national seed requirement per year have ever been made available to farmers (United

Republic of Tanzania, 1997). Mtenga (1999) found that in Tanzania, most of seed sown by farmers is farm saved seed and seed provision between farmers who are relatives or neighbors in very informal system.

2.2.3 Seed supply as constraint to bambara groundnut production

Being among the neglected underutilized species, bambara groundnut production in Tanzania is faced with many constraints. Among the constraints, is lack of good quality seed from formal seed sectors. Consequently, farmers rely on own saved seed stored from their crop harvest to next planting season using local/traditional facilities /technology within their capability. However, these traditional storage systems have limitations in maintaining proper moisture content of the seed, reduction of storage temperature and modification of storage atmosphere (Vanek *et al.*, 1988). Such practices are not able to slow down respiration and other metabolic processes without injuring the embryo, therefore, the storage practices used are not able to provide storage conditions that maintain seed viability hence result in poor quality seed. Earlier investigation by Sreeramulu (1983a) indicated that during storage in gunny bags at room temperature (25-35^{0}C) composition of bambara groundnut seed changes and such changes associated with loss of viability.

2.2.4. Seed storage

2.2.4.1 General principle

According to Thomson (1979) the aim of seed storage is to maintain the germination capacity of the seed. This often requires more stringent conditions than conservation of nutritional or industrial quality. Seed is a living organism whose activities increase with temperature and moisture. Seed viability is highest at the time of physiological maturity and gradually declines with time and its longevity depends on the environmental conditions to which the seed is exposed (Copeland, 1988; Lynn, 2000). Therefore, good storage practices are those that will provide storage conditions that maintain seed viability. Such practices should be able to slow down respiration and other metabolic processes without injuring the embryo (Hertmann and Kester, 1959, Justice and Bass, 1979; Lynn, 2000). The most important conditions are; maintaining proper moisture content of the seed, reduction of storage temperature and modification of storage atmosphere i.e. CO_2/O_2 ratio (Hertmann and Kester, 1959; Lynn, 2000). It does seem that in case of CO_2/O_2 ratio, an increase in partial pressure of oxygen decreases the viability of seeds (Duffus, 1980).

Depending on their storage characteristics, seed can be classified into two categories, either orthodox or recalcitrant. For orthodox seed, viability can be preserved by lowering seed temperature and moisture (at least down to the 5 to 10% range). With orthodox seed, there is a linear relationship between storage temperature or moisture and logarithm of longevity. This category includes seed of a diverse array of species of

world major crops such as cereals, pulses and oil seeds. On other hand, recalcitrant seeds are those that are sensitive to desiccation and /or chilling, have short lifetime under common storage conditions and therefore, they are difficult to store. This group is commonly encountered in a number of plantation crops, forests and in few temperate species (Basu, 1994; Iowa State University, 1994). Bambara groundnut seed belongs to the orthodox type, hence its storage life depends on the ability of the storage medium to maintain appropriate temperature and seed moisture content.

2.2.4.2 Types of seed storage

Throughout the world, various storage methods have been developed locally for individual crops. According to Louwaars *et al.* (1998), often unthreshed heads of sorghum, ears of wheat, panicles of rice and cobs of maize and unshelled pods of groundnuts and beans store better than do the same seeds, when threshed and cleaned.

Farmers' storage practices are adapted to the local climate of the area concerned. In dry tropics, seed is kept in woven sacks or in heaps with protection against animals and perhaps shading against the sun. In temperate regions, seed is stored in woven sacks or on barn floors, but always under cover. In wet tropics, more care is taken, seed is often stored in earthenware bins sealed with clay and baskets are sometimes used, being hung in the kitchen out of reach of rodents and in a dry smoky atmosphere (Thomson, 1979). Walker and Tripp, (1998) found that in Ghana and Zambia a wide variety of containers are used for seed storage, these include pots, tins and baskets, but the most

common is jute or polypropylene sacks. In Tanzania, similar storage facilities are also widely used (Mallya, 1988). Harrington (1972) classified these storage practices as "carryover" storage since it involves only storage from harvesting to next planting season, and it accounts for about 20% of world need for seed storage.

For large quantities of seed, purpose-built stores are used. Within the building the seed may be contained in bins, boxes or bags (Thomson, 1979). Harrington (1972) termed this system as commercial seed storage and it accounts for about 80% of world need. The building should be of good construction providing protection against rodent, rain/ moisture and must not be a heat trap (Harrington, 1972). Aeration system is sometimes incorporated which can be used for initial drying of the seed after harvest or to cool the seed when the ambient air is of relatively low humidity and temperature (Thomson, 1979). For long term storage of seed, refrigeration and dehumidification systems are necessary, but these systems are expensive. However, on a medium scale, insulated rooms fitted with a domestic air conditioner can be used (Thomson, 1979). For example in germplasm collection, where seeds are kept for many years, cold temperature is economically possible (maintained in range of 5 to –10 °C) and seed moisture in equilibrium with 20 to 25% relative humidity are basic requirements (Harrington, 1972).

2.2.5 Seed deterioration in storage

Poor storage conditions certainly affect the process of quality deterioration during the storage period where changes in physiological and chemical properties of stored grain such as change in appearance and reduction in nutrient contents commonly occur (Mulyo, 1998). When the viability of homogeneous seed population does decline, it follows a negative sigmoid pattern, reflecting the normal distribution of seed death with time (Coolbear, 1994).

The deteriorative changes which become evident well before any detectable loss in viability includes; lose of vigor (manifested by remarked decreases in rate of germination), an increase in the number of abnormal seedlings appearing in the germination test, color changes of seed coat and increase in free fatty acid levels within the seeds (Coolbear, 1994). The two widely accepted causes for the loss of seed viability are reduced physiological efficiency and damage by pathogens (West, 1986). Theories on why seeds lose their ability to germinate after deterioration during storage can be summarized into the following physiological events; depletion of food reserves, alteration of chemical composition, membrane alteration, enzyme and protein changes, genetic damage, hormonal changes and microorganism activities (Roos, 1986; Coolbear, 1994).

2.2.6 Influence of seed viability on crop performance

The deterioration leading to loss of viability in seeds can affect the yield of a crop in two ways. First the decreased germination can lead to a sub-optimal population of plants per unit area, secondly, the deterioration of which seed viability may be an index may result in a poor performance by the surviving plants (Roberts, 1972).

The problem of reduced population on yield is particularly obvious in species that are unable to compensate for a reduced density (for example by tillering) and thus have a population- yield curve that shows a relatively sharp yield peak at a particular population density (Roberts, 1972). Roberts (1972) suggested that percentage viability is an excellent index of the loss of yield potential of the surviving seeds. It was further, suggested that a simple germination test can act as an indicator of the potential yield of the surviving seeds for a given species. The same opinion was suggested by Wu *et al.* (1990) who noted that seed vigor, which is defined as the sum total of those properties of the seed that determine the level of activity and performance of seed or seed lots during germination and seedling emergence, is an important criterion to determine the field planting value of the seed. However, TeKrany and Egli (1991) as cited by Finch-Savage (1994) provided evidence which indicates that in the absence of density effects, there is a great importance of seed vigor in crops harvested during vegetative growth (e.g., lettuce and carrot) or early reproductive growth (e.g., tomato and green peas) than in crops harvested at full reproductive maturity (e.g., soy bean and wheat). They argue that at commercial densities, seed vigor affects vegetative growth, but

there are usually no effects of seed vigor at full maturity because dry seed yield is not closely associated with vegetative growth. According to Lynn (2000) the best way to test seeds for viability after storage is to conduct germination test. Good germination rate is 70% to 80%, and lower than 70% will need either thicker planting or saved batch of seed for re-planting

2.2.7 Seed germination process

One of the physiological aspects of seed quality is the ability to germinate at the desired time and to assure an adequate level of initial growth (vigor) of all essential parts of the seedling. This condition is one of the prerequisites for the development of a productive plant (Lauwaars *et al.*, 1998). Thomson (1979) defined essential structures of normal seedling as a well developed root system, a well developed and intact hypocotyl or epicotyl and normal plumule (or in cereals and grasses, a well developed first leaf) and one or two cotyledons in seedling of dicotyledonous plants in other (non cereal) plants.

Seed germination is the resumption of active growth of the embryo that results in the rupture of the seed coat and the emergence of the young plant (Copeland, 1988). Morphologically, germination is the transformation of an embryo into seedling. Physiologically, germination is the resumption of the metabolism. Biochemically, germination is the sequential differentiation of oxidative and synthetic pathways (Jann *et al.*, 1977). Duffus (1980) and Copeland (1988) described germination as a process

that involves the following steps. Water is absorbed through natural openings in the seed coat and diffuses through the seed tissue. The water causes the cell to become turgid, the entire seed grows in volume, and the seed coat becomes more permeable to oxygen and carbon dioxide. As the swelling occurs, the seed coat often ruptures, facilitating both water and gas uptake and resulting in emergence of the growing points.

Water absorbed in the seed tissue activates the various enzyme systems, which serve to break down stored tissue, aid in the transfer of nutrients from storage areas in the cotyledons or endosperm to the growing point, and trigger enzyme activities which use breakdown products in synthesis of new material. Following enzyme activation, new materials begin to be synthesized, reflected by an increase in the size of the root- shoot axis (epicotyl, hypocotyl, and radicle). Depending on specie, this initial growth may be by cell division or by elongation. During the imbibition stage, swelling of the seed may rupture the seed coat. However, the rupture is usually caused by internal pressure from enlarging root-shoot axis. Ordinarily, the primary root is the first structure to emerge, for example in bambara groundnut, however in some species the shoot emerges first. Initially, the established seedling is dependent to some extent, on food breakdown from reserve storage tissue. But as the seedling becomes firmly established in the soil, water uptake begins and the seedling manufactures its own food, gradually becoming independent of the storage tissue. At this point, the process of germination is complete.

2.2.8 Seed priming

Priming is a hydration treatment of seed to improve the sowing value of seed (Msuya and Tarimo, 1996). Priming involves hydration treatment of the seed to initiate the process of germination but not to the level of radical emergence (Copeland, 1988). There are various methods of seed priming that can be categorized as either, osmotic priming or hydropriming. Osmotic priming or simply osmopriming involves hydrating the seeds using osmotic substances to regulate water uptake (Alverado *et al.*, 1987) these substances include inorganic salts such as those of potassium, magnesium and sodium and organic salts notably polyethylene glycols. Hydropriming involves hydrating the seeds using water alone with no osmotic substances. It is considered to be a simple and inexpensive method of priming (Fujikola *et al.*, 1993)

Capron *et al.* (2000) have shown recent studies in optimizing priming treatment and its importance in molecular and biochemical processes involved in germination of seed. In their review, Msuya and Tarimo (1996) noted that little work has been done on aspects of pre-sowing hardening in many drought stricken areas of Tanzania although it is important practice as a means of combating drought. Msuya and Iringo (1998) showed that all primed seeds of common beans, maize and rice germinated faster than unprimed seeds, however, long priming time in maize seems to cause a reduction in vigor of the seed.

2.3 Germination and growth studies in bambara groundnuts

Germination, yield stability and grain yield of bambara groundnut vary widely (Bamfood, 1999). Kocabas *et al.* (1996) studied the effect of temperature on germination and reported that germination took 7-15 days and maximum fractional germination occurred between 22°C and 36°C. Massawe *et al.* (1999) studied the effects of hydration on germination and early seedling growth of bambara groundnuts and reported that for landrace Dodoma red (DodR) the optimum soaking time was 24 hours and accelerated growth was more pronounced in soaked seeds compared to unsoaked seed. However, these workers suggested the need for further investigations to determine whether the effects of such accelerated growth would be reflected in seed yield.

Sreeramulu (1983a) studied bambara groundnut seed following different length of storage time in gunny bags under laboratory conditions (25-35°C) and reported that seed deterioration during storage was manifested by delayed germination, reduced germinability and increased number of stunted seedlings. Stanton (1966), Stephens (1994) and Brink (1998) reported that it takes between 90-180 days for seeds to mature.

2.4 Influence of day length on bamabra groundnut

Apparently, bambara groundnut has photo regulation of its development that provides the plant with a flexible mechanism to adopt to circumstances that create seasonal

fluctuation in length of the growing period (Linnemann, 1991). Nishitani *et al.* (1988) tested the photoperiod response of 29 bambara groundnut varieties to photoperiod by exposing to 8 or 24 hours of day length. Out of the 29 varieties, 19 could initiate flowers in both long day and in short day photoperiods, but only seven varieties could set fruits under both photoperiods. However, there were fewer matured pods per plants in long day than in short day conditions. Linnemann (1991) distinguished between two groups of bambara groundnut based on their photoperiod response. Day neutral for flowering, with fruit set delayed by long photoperiods (14hours), and day neutral for flowering with fruit set inhibited by long photoperiods. Photoperiod also indirectly influences dry matter partitioning in bambara groundnut through its effects on development. Linnemann (1994) pointed out that as for source-sink relations, under longer photoperiods (14 and 16 hours) the source (leaf area) was at least as large as under the shorter photoperiods (10 and 12 hours), but plants under 14 and 16 hours had extensive potential sinks that consisted of a very large number of fertilized, but undeveloped ovaries. Based on these studies, Linnemann (1994) concluded that photoperiod has significant effects on the sink strength of the ovaries. Muraki and Nishitani (1991) found that maximum photosynthetic potential of leaves of bambara groundnut under shading decreased with decreasing light intensity. Kajuna (1999) reported that cream and red seeded landraces grown under ambient light produced high dry seed weight compared to when grown under shade due to the fact that under ambient light, a higher number of leaves were produced hence increasing light interception.

2.5 Growth and yield variation in bambara groundnut

In their review, Linnemann and Azam-Ali (1993) indicated that the crop has poor field establishment largely due to poor germination and emergence. As one of the measures to improve bambara groundnut production, Doku (1996) suggested the need to understand the growth and development of bambara groundnut, particularly the partitioning of photosynthates to the various plant organs, especially the grain. Doku (1996) further proposed that in the absence of sophisticated equipments, the growth analysis procedures such as measurement of leaf area and plant dry weights (whole plant, leaves, stems and roots) taken at regular interval during the growth of the plant until maturity are quite adequate for this purpose. These procedures had been well explained earlier by Hunt (1978) and Hay *et al.* (1989).

Partitioning of resources between plant organs depends on developmental stage and the level of environmental stresses. On the other hand, the rate of development to flowering and then to podding depends on day length and temperature (Azam-Ali, 1999). Phosphorous supply is among the environmental condition, which determine growth and partitioning of dry matter in bambara groundnut. Plants with high phosphorous supply continue to grow vegetatively for longer than plants with low phosphorous, and in contrast, plants with lower phosphorous level invest relatively more biomass in their root system (Azam-Ali, 1999; Ramolemana, 1999).

In bambara groundnut, day length control of podding appears to influence the balance between vegetative and reproductive growth rather than the rate of dry matter production. Therefore, continued leaf production under longer day length is associated with corresponding decline in the fraction of total dry matter allocated to the pod structure (Azam-Ali, 1999).

Elia and Mwandemele (1986) reported that both bambara groundnut vegetative and reproductive growth were influenced by soil moisture level. Plant height and dry weight were reduced as water deficit became more severe. Similarly, the number of flowers, branches and pods per plant were significantly reduced by decreased water availability in the soil (Elia and Mwandemele, 1986). However, it has been reported that drought stress increases the relative allocation of dry matter to roots (Azam-Ali, 1999). Compared to groundnut, pronounced proportion of accumulated dry matter per unit of transpired water is allocated to reproductive yield. For example, in most drought-stressed treatment, groundnut failed to produce any pods wheres bambara groundnut produced a seed yield of 300kgha^{-1} (Nyamudeza (1989) as cited by Linnemann and Azam-Ali, 1993). Collinson *et al.* (2000) showed that under rain fed conditions in Tanzania, bambara groundnut landrace DodR from Tanzania produced harvest index ranging from 0.04 to 0.39. Karikari (2000) found that early maturing bambara groundnut landraces (maturity period = 120 days) were high yielding because they emerged rapidly, flowered within 40 days and had enough time for pod maturity before the advent of unfavorable weather like low moisture and decreased temperature.

In the broader sense seed yield depends on size, duration and activity of the source and sink capacity (Hamid *et al.*, 1989). Begemann (1988) reported significant variations in bambara groundnut grain yield due to differences in the number of pods per plant and seed sizes. Nkumba *et al.* (1997) analyzed the relationship between grain yield and various components of yield (viz. plant height, pod/plant, branch/plant and 100 seed weight), and reported that all parameters were significantly correlated with grain yield. Moreover, Mkandawire (1996) noted that the number of pods and dry matter of pod and kernels are the most important factors that determine the productivity of the crop. Therefore, any factor that affects these yield components will affect the final yield of the crop. Mkandawire (1996) also found that increasing crop's plant, decreased plant growth characteristics such as flowers, pegs, pods, shoot, root dry matter and leaf area per plant, and these effects were reflected in decreasing the final grain yield. Karikari (2000) reported that in bambara groundnut, yield is a complex terminal outcome of growth and interrelated developmental tracks. Although many characters were correlated with yield, the characters had a more complex relationship with each other that could not be explained linearly (Karikari, 2000).

Though the crop can be found in vastly different environments in Africa there are indications that individual cultivars are not themselves very adaptable. High yielding types from one location may fail when grown elsewhere. For example, Tanzanian cultivars have yielded poorly in Zambia (National Research Council, 1979) and Botswana (Karikari, 2000).

In the literature reviewed there is a great deal of evidence that the percentage viability of seed decreases with age and it is obvious that one way in which yield could be affected is through failure of seed lot germination capacity to meet crop population density required to optimize yield. The influence of seed vigor on field crop performance for many species has been critically reviewed and it has been shown that storage period had important influence on loss of viability in bambara groundnut. However, there is limited information on the influence of storage facilities/containers used on growth and yield of bambara groundnut. Therefore this study was undertaken with main objective of evaluating the influence of seed storage practices on germination, growth and yield of Bambara groundnut.

The specific objectives were: -

i. Assess the effects of seed shelling on germination.

ii. Determine the effect of different storage containers on seed viability.

iii. Evaluate the effect of seed priming on germination.

CHAPTER THREE

3.0 MATERIALS AND METHODS

The study comprised of one laboratory experiment and two field experiments. The field experiments were conducted during the 2001 short rain (*vuli*) season and 2002 long rain (*Masika*) season. A red seeded bambara groundnut landrace code-named Dodoma Red (DodR) was used in both experiments. This landrace is commonly grown in Dodoma, central Tanzania. Seed for the experiment was obtained from Sokoine University of Agriculture (SUA) bambara groundnut germplasm. The seed used was previously grown and harvested during the 2000 cropping season.

3.1 Laboratory Experiment

THE EFFECT OF STORAGE METHODS ON GERMINATION.

A laboratory experiment was set up in which the germination of bambara groundnut was assessed using shelled (a_1) and unshelled (a_2) seeds stored for six months using six storage containers viz.; earth pots (b_1) plastic buckets (PVC material)(b_2), plastic bags (commonly used for grocery shopping)(b_3), jute bags (b_4), interwoven baskets (b_5) and plastic bags (polypropylene material)(b_6). Storage in a deep freezer (b_7) was included as a control. Seed stored in earth pots, plastic buckets, plastic sacks and jute bags and in deep freezer were kept in a Department of Crop Science and Production laboratory at (SUA) at normal room conditions (Average temperature was 30°C and 70-80% RH), while seed stored in baskets was hanged over a fire place in a farmer's house in Mzinngwi village (also known as Falkland) adjacent to SUA campus.

Before storage, 100 seed weight was determined and seed food reserve was determined by proximate analysis. The analysis was done at the Department of Animal Science and Production laboratory at SUA. Seed moisture content was determined and germination test was done as specified by the International Seed Testing Association (ISTA), (ISTA, 1993). Moisture determination and germination test was conducted at the National Seed Testing Laboratory located in Morogoro. Details of all these measurements are given in section 3.1.1, 3.1.2 and 3.1.4

At the end of storage period (six months), a seed sample from each storage container was again subjected to 100 seed weight determination, food reserve determination and seed moisture content determination. The seed was then subjected to a germination test. Before the germination test was conducted seed was subjected to hydro priming treatment, i.e. soaking for 24hours(c_1) as recommended by Masawe *et al.* (1999), wetting immediately before sowing (c_2) and no hydro-priming (c_3) (Contol)

Seed moisture content, 100 seed weight and proportion of food reserve were evaluated in a split -split plot design in which shelled or unshelled seeds were the main plots and storage containers were the sub plots. In germination test the three experimental factors were organized in a split-split plot arrangement in which shelled and unshelled seeds were the main plots, storage containers were the sub plots and pre sowing hydro-priming as sub-subplot factor in a complete randomized design (CRD) layout in four replications. Details of the measurements conducted are described below.

3.1.1 Proximate analysis

Two sub samples from each treatment were analyzed using standard proximate method as described by the Association of Official Analytical Chemists (AOAC) (1990) to determine crude protein (%), acid detergent fiber (%), ether extractable fat (%) and ashes (%)

3.1.2 Determination of seed moisture content

A sample of 10 seeds were taken from each seed lot and grounded, then a sub sample of 10 g was taken and placed in a container of a specific weight M_1 (g) and then weight of container and sample taken and recorded as M_2 (g). The container and grounded seed was then placed in an oven kept at 130-133°C for a period of four hours. (ISTA, 1993). At the end of this period the container was placed in a desiccator for cooling for 45 minutes.

After cooling, the container and its content were weighed and their dry weight recorded as M_3 (g). The moisture content (MC) then calculated as;

$$MC\% = \frac{M_2 - M_3}{M_2 - M_1} * 100\%$$

3.1.3 Priming treatment

(i) Treatment C_1 (Soaking for 24 hrs)

Four hundred seeds from each storage container were soaked in tap water (fully immersed) for 24 hours prior to germination test.

(ii) Treatment C_2 (Wetting immediately before sowing)

Also 400 seeds from each storage container were immersed in tap water in a container for five minutes and then immediately taken out and placed in a germination medium, (sand).

(iii) Treatment C_3 (No priming)

This was a control treatment; seeds were placed in a germination medium directly from the storage container without soaking.

3.1.4 Germination test

Sand kept in aluminum containers was used as substrate. Four hundred seeds from each storage container in four replicates of 100 seeds each were used. In order to achieve adequate spacing each replication was divided into sub replicates of 50 seeds each. The seed was planted on a level layer of moist sand and covered with 10 to 20mm of uncompressed sand. The substrate was watered to keep it moist and placed on a laboratory bench. The temperature range in the laboratory was 27-35°C during the night and day time respectively and relative humidity was 70 (at night) and 80%(during daytime). Seedling evaluation was done at the end of 14 days after

sowing. During evaluation observation were made and data recorded on the following variables:

3.1.4.1 Percentage of normal seedlings

All seedlings, which were either

- Which consist of well developed, complete and healthily root system, shoot axis, cotyledons and terminal bud (intact seedlings), or
- Showed certain slight defects of their root system, shoot axis, cotyledons and terminal bud due to secondary infections were classified as normal seedling. This category was also referred to as percentage germination.

3.1.4.2 Abnormal seedling percentage

All seedlings, with one or more of the following characteristics were classified as abnormal seedling

- Missing any of the root system, shoot axis, cotyledons or/and terminal bud,
- Badly damaged or diseased as a result of infection from parent seed,
- Weakly developed root system, shoot axis, cotyledons and terminal bud

3.1.4.3 Ungerminated seeds

Seeds that had not germinated at the end of 15 days were recorded and classified into;

(a) Hard seeds: Seeds that remained hard because they had not absorbed water.

(b) Dead seeds: Seeds that at the end of the test period were neither hard nor fresh but had not produced any part of the seedling.

3.1.5 Vigor index

This was done following procedures described by Kim *et al.* (1994). Vigor levels for each treatment were calculated by multiplying percent normal germination by the average plumule length of the seedlings after the 14 days of application of the germination test.

3.1.6 Data analysis

Since raw data for normal germination count and vigor index score in the laboratory experiment did not followed the linear additive model and there was a wide range of sample means (hence positive correlation between means and variances), the raw data for these two were first transformed by square root method as described by Little and Hills (1978). For the rest of variables, data was not transformed.

All data were subjected to analysis of variance using MSTAT-C computer statistical package. (Michigan State University, 1993). Treatment means was separated using Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$. As suggested by Little and Hills (1978) weighted means for germination percentage and vigor index were used.

Analysis model for split-split plot analysis

$$X_{ijkm}=\pi+R_i+A_j+\alpha_{ij}+B_k+AB_{jk}+\beta_{ijk}+C_m+AC_{jm}+BC_{km}+ABC_{jkm}+\in_{ijkm} \quad \ldots\ldots\ldots\ldots(1)$$

Where X=Response. π=General effect, R=Replication, A=main plot effect, B=sub plot effect, α= main plot error, AB_{jk} main plot and subplot interaction effect, β=Sub plot error, C=Sub-Sub-plot effect, AC = main plot and sub-sub plot interaction, BC = sub plot and sub-sub plot interaction, and ABC =main plot, sub plot and sub -sub plot interactions effects, and $\in$= Sub-sub plot error effect.

3.2 Field Experiment One

EFFECTS OF STORAGE ON FIELD GERMINATION, GROWTH AND YIELD OF BAMBARA GROUNDNUT.

Objective: Evaluate the influence of storage containers and pre-sowing hydration on field germination, subsequent growth and yield.

3.2.1 Location and layout of the experiment

A field experiment was conducted at SUA farm, Morogoro (located at latitude 6°49' S; longitude 37°40E and 525 m.a.s.l). SUA is in a typical rain shadow area and receives mean annual rainfall of between 600 and 800mm (Masseri and Jana, 1979). Morogoro region in general experiences a bimodal rainfall pattern with short intermittent rain falling between October and January and long rains between March and May. The experiment was conducted during the 2001-02 short rains season (November - February). Due to irregular nature of the short rains supplemental irrigation was used to sustain crop growth during the experimentation period. Monthly weather data for the duration of experiment was recorded (see Appendix I)

The land was disc-ploughed and harrowed to form a fine seedbed. Prior to sowing, triple super phosphate (TSP) was broadcast over all plots at the rate of 22kg P_2O_5 per hectare and incorporated into the soil by use of hoes. Physical and chemical characteristics of the soil at the site were determined and are indicated in Appendix II.

The treatments were arranged in a split plot and laid out on a randomized complete block design (RCBD) with four replications. From the results of laboratory germination test, unshelled seeds were used. Storage structures were the main plots and priming was the subplots. The main plots consisted of seed stored in deep freezer, basket hanged over fireplace and storage in a jute bag, while subplots were soaking for 24 hours, wetting prior to sowing and no priming. Each plot was 2.80 m by 2.10 m with rows at 0. 30 m apart. Each plot consisted of eight rows; two outer guard rows, one sampling row on either side, and one inner guard row on either side enclosing the two harvesting rows in the middle of the plot. Sowing was done on 1/December /2001. Two seeds per hill was sown at 15 cm interval within the row and thinned to one at 15 days after sowing (DAS) to give a plant population of 130 plants /plot which is equivalent to 22 plants/m^2 (or 222,222 plants /ha). Plots were maintained weed free by periodic hand weeding at three-week intervals. Plots were earthen up at 48 DAS, i.e.10 days after the first flowers were observed.

3.2.2 Crop growth variable determination

Two randomly picked plants were uprooted from each plot and used to determine different growth variables as indicated bellow. This growth analyses were taken at 30 days interval starting at 30 days after sowing (DAS) (i.e.30, 60,90 and 120 DAS). Each plant was partitioned into roots, stem, leaves and pods (when available), and each part was oven dried at 80°C for 72 hours before weighing.

3.2.2.1 Number of days to first flowering

These were the number of days from emergence to the appearance of the first flower in each plot. This was determined by visual observation.

3.2.2.2 Number of flowers

Flowers were removed from the peduncles of plants used for growth analysis and were counted. Flower counts started at 60 DAS.

3.2.2.3 Plant height

This was measured by ruler from the crown to the tip of the longest leaf (identified by extending /stretching all leaf petioles on a flat table) and recorded in cm. This was done on plants used for growth analysis (i.e. at 30, 60, 90 and 120 DAS).

3.2.2.4 Number of leaves per plant

The numbers of fully opened trifoliate leaves were counted at each sampling time on plants used for growth analysis. (i.e. at 30, 60, 90 and 120 DAS).

3.2.2.5 Leaf area

Leaf area was estimated by using a graph paper method as outlined by Rhoads and Blooduorr (1964). A sub sample of four plants was randomly selected from a sample of 8 uprooted plants for each treatment at each sampling time. Each fully opened trifoliate leaf from the selected plant sample was traced on graph paper and the area

determined by the leaf weighing method as outlined in Appendix III. Leaf area was recorded as cm^2/plant.

3.2.2.6 Leaf dry matter

All leaf blades of fresh living leaves were removed from the two uprooted plants from each plot. These leaves were then oven dried to constant weight at 80°C for 72 hours. Leaves were then weighed and recorded as g/plant.

3.2.2.7 Stem dry matter

Prostrate stems and the crown were all considered as part of the stem. These were removed from the two uprooted plants and then oven dried to constant weight at 80 °C for 72 hours and then weighted and their weight recorded as g/plant.

3.2.2.8 Presence or absence of nodules

Determined by observation of presence or absence of small round regular swellings on the roots and root hairs and was recorded as number of nodules per plant. This was done on plants used for growth analysis at DAS.

3.2.2.9 Number and dry weight of pods

All pods from the two uprooted plants were removed from the stems and counted. Pod count per plant started at 60 DAS. The pods were then oven dried at 80°C for 72 hours and then weighed and recorded as g/plant.

3.2.2.10 Root dry matter

At each sampling time root dry matter was determined from parts of the plant below the crown. Roots were removed from the soil using a root core of 10cm diameter by 15cm depth. Roots were separated from soil by rinsing in water and oven dried to constant weight at 80°C for 72 hours then weighed and recorded as g/plant

3.2.3 Growth rate and dry matter partitioning

3.2.3.1 Relative growth rate (RGR)

Mean relative growth rate (RGR) between two growth measurement periods (T_1 and T_2) was calculated based on the following equation as given by Wynne *et al.* (1982).

$$RGR_{T1\text{-}2} = \frac{\text{Log}_e W_2 - \text{Log}_e W_1}{T_2 - T_1} \; (\text{g.g}^{-1}\text{day}^{-1}) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where T is time (days) and W is plant total weight (in grams).

3.2.3.2 Partitioning coefficients

Mean dry matter partition coefficients were calculated on each 30 days growth period starting on 30 DAS following the method described by Tollenaar (1989). Leaf area partition coefficient (LAPC) was calculated as increase in leaf area per unit increase in total dry weight, shoot partition coefficient (SPC) was calculated as proportion of increase in total dry weight that was allocated to shoot (Δshoot weight (g)/Δtotal weight (g)) and leaf partition coefficient (LPC) was calculated as proportion of increase in shoot weight that was allocated to leaf blades (Δleaves weight/ Δ shoot weight)

3.2.4 Yield and yield components determination

At harvest time (120 DAS), plant population taken from two harvesting rows (0.84 m^2) in each plot were counted and then uprooted. Number of leaves, leaf dry matter (only intact living leaves), leaf area (only intact living leaves), number of pods, nodules, root dry weight, stem dry weight and pod dry weight were taken as described in sections 3.2.2.1 through 3.2.2.12. In addition, the following measurements were also taken:

3.2.4.1 Grain yield

Harvested pods were shelled and kernels were oven dried to constant weight at 80°C and then recorded as g/plant. Grain yield was expressed at 9 %MC (fresh weight basis)

3.2.4.2 Shelling percentage

Shelling percentage was calculated as kernel weight divided by total weight of pods multiplied by 100; i.e.

$$\text{Shelling \%} = \frac{\text{Kernel weight(g)}}{\text{Pod weight (g)}} * 100 \quad \text{.............................(3)}$$

3.2.4.3 Harvest index (HI)

Harvest index (HI) was computed by dividing the grain yield by the total biological yield and results expressed as a ratio.

$$\text{HI} = \frac{\text{Economic yield (grain yield) kg/ha.}}{\text{Total biological yield, kg/ha.}} \quad \text{..........................(4)}$$

Economical yield was made up by grain weight while the biological yield was made up of all above ground parts.

3.2.4.4 One hundred-seed weight (seed size)

One hundred dry grains were weighed using electronic balance and expressed in grams.

This measurement was used as an indirect measurement of seed size.

3.2.5 Data analysis

All data were subjected to analysis of variance (ANOVA) using MSTAT C computer statistical package (Michigan State University, 1993). Treatment means was separated

using Least Significance Difference test (LSD). Correlation analysis between yield components and grain yield was performed.

The analysis model was

$$\mathbf{X_{ijk} = \pi + R_i + A_j + \alpha_{ij} + B_k + AB_{jk} + \in_{ijk}} \quad \ldots\ldots(5)$$

Where X= Response. π=General effect, R= Replication, A= main plot effect, B= sub plot effect, $\propto$= main plot error, AB_{jk} main plot and subplot interaction effect and $\in$= Sub plot error effect.

3.3 Field Experiment Two

EFFECTS OF PRIMING TREATMENT ON GROWTH AND YIELD OF BAMBARA GROUNDNUT

Objective: Evaluate the influence of pre-sowing seed priming on growth and yield of bambara groundnut.

3.3.1 Location and design of the experiment

Location and land preparation were as outlined in section 3.2.1. This experiment was conducted during 2002 long rain (*masika*) season (March - July).

Randomized complete block design (RCBD) with four replications was used. Treatments were the three priming conditions namely soaking for 24 hours, wetting immediately before sowing and no hydration treatment. The seeds were sown on 12th March 2002. Plot sizes and management were as outlined in section 3.2.1.

3.3.2 Sequential growth measurement

Dry matter production was assessed from four sequential growth analyses taken at 30 days interval starting from 30 days after sowing (DAS) up to 120 DAS. On each occasion, four plants were harvested from sampling rows on each plot to make a total of 16 plants for each treatment. Plants from each plot were placed in separate paper bags. Then each plant was separated into roots, stem, leaves and pods, and oven dried at 80°C for 72 hours before weighing.

Growth and yield variable data was recorded as; days to 50% emergence, days to first flower, days to 50% flowering, number of flowers, plant height, number of leaves per plant, leaf area (cm^2/plant), dry weights of leaves, stems (g/plant), presence or absence of nodule, number and dry weight of pods and at final harvest grain yield (g/plant), shelling percentage, harvest index and 100 seed weight were determined Details of measurements are outlined below.

3.3.2.1 Days to 50% emergence

This was the number of days from one DAS to the time when half of the total number of seeds planted on each plot had emerged. A seed was considered to have emerged if the plumule broke through the surface of the soil.

3.3.2.2 Number of days to first flowering

As in section 3.2.2.1.

3.3.2.3 Number of days to 50% flowering

This was the number of days from one DAS to the time when half of the total number of plants in each plot had flowered. This was determined by physical count.

3.3.2.4 Number of flowers plant height and number of leaves per plant

As in section 3.2.2.2 through 3.2.2.4

3.3.2.5 Leaf area

This was done as described under section 3.2.2.5 but four plants per plot were used.

3.3.2.6 Other growth variables

As in section 3.2.2.6 through 3.2.2.10

3.3.3 Growth rate and dry matter partitioning

3.2.3.1 Relative growth rate (RGR)

Mean relative growth rate (RGR) between two growth measurement periods (T_1 and T_2) was calculated as described in section 3.2.3.1

3. 2 3 2 Specific leaf area (SLA)

SLA = Leaf area (cm^2) /Leaf weight (g)

3.2.3.3 Fraction of weight partitioned to above ground parts (f_{ag})

$$f_{ag} = \frac{\text{Leaf weight (g)} + \text{Stem weight (g)} + \text{Pod weight (g)}}{\text{Total weight (TDM) (g)}} \quad \ldots\ldots(6)$$

(European Union (EU), 1998)

3.2.3.4 Fraction of above ground weight partitioned to pod (f_p)

$$(f_p) = \frac{\text{Weight of pod (g)}}{\text{TDM} * f_{ab}} \quad \ldots\ldots(7)$$

(EU, 1998)

3.3.4 Final harvest

At harvest time (120 DAS), all procedures as described above in sections 3.2.4 through 3.2.4.4 were taken.

3.3.5 Data analysis

This was done as already explained under section 3.2.5 but treatment means was separated using LSD.and the analysis model was

$\mathbf{X}_{ij}=\pi+\mathbf{R}_I+\mathbf{A}_j+\in_{ij}$ ....(8)

Where X= Response. π = General effect, R= Replication, A= Treatment effect and $\in_{ij}$ Random error

CHAPTER FOUR

4.0 RESULTS

4.1 Influence of Storage Practices

4.1.1 Laboratory experiment

4.1.1.1 Seed characteristics

Bambara groundnut seeds used in this experiment were of good quality with germination percentage of 95% and good seedling vigor index (15.8) prior to storage. The characteristics of the seed used are summarized in table 2.

Table 2. Seed composition of Bambara groundnut before storage

Moisture content%	100 Seed weight (g)	CHO content (%)	Protein content (%)	Fat content(%)
9.4	55.7	57.2	22.9	7.1

Table 3 shows a summary of the analysis of variance for seed quality indices after storage. The data indicates that after six months of storage in two different shell status (shelled and unshelled) using seven different storage containers; significant differences ($P \leq 0.05$) were recorded for 100 seed weight, and grain nutrient composition (carbohydrates, protein and fat content).

Table 3. Summary of analysis of variance for seed quality indices after storage for six months

		Treatment mean square				
Source of variation	df	100-seed weight (g)	Carbohydrate (%)	Protein (%)	Moisture (%)	Fat (%)
Shell status $_{(m)}$	1	251.40^{**}	239^{**}	2.74^{*}	133.32^{**}	0.51^{ns}
Error (a)	2					
Storage container $_{(s)}$	6	110.00^{**}	59.84^{**}	5.00^{**}	31.03^{**}	2.55^{ns}
Shell status × storage container $_{(ms)}$	6	44.47^{**}	392.52^{**}	7.58^{**}	14.08^{**}	5.25^{ns}
Error (b)	12					
Total	27					
CV%		2.83	0.02	0.53	3.25	0.01

Note: * * = Highly significant ($P \leq 0.01$), * = significant ($P \leq 0.05$), df = Degree of freedom, CV = coefficient of variation, ns = not significant, m = main plot treatment and s = sub plot treatment.

Since both shell status, (main plot), storage container (sub plot) and their interactions were significant ($p \leq 0.05$), only the interaction effects are highlighted here and means for main plot and sub plot effects are shown in Appendix 4 and 5 respectively.

After six months of storage, results (Table 4) indicated that shelled seed stored in plastic bucket maintained significantly high proportion of seed moisture while unshelled seed stored in woven basket hanged over the fireplace had significantly low seed moisture content. However, shelled seed stored in earth pot and in deep freezer did not differ significantly in seed moisture content. Shelled seeds stored in earth pot, plastic bucket, polyethylene sacks and polypropylene bag did not differ significantly in 100 seed weight to each other. Unshelled seeds stored in woven basket hanged over the fireplace maintained significantly high proportion of carbohydrate food reserves. Meanwhile, all seed stored after being shelled with exception of the seed stored in deep freezer and woven basket over the fireplace, were heavily attacked by bruchid beetles (*Callosobruchus maculatus* and *C.subinnotatuts*)

Table 4. Means for interaction effects of shell status and storage containers on seed composition after six months

Storage method	MC(%)	100-seed weight (gm)	CHO content (%)	Protein content (%)	Fats content (%)
Shelled × Earth port	11.3^{e}	38.2^{f}	50.9^{k}	16.3^{hi}	8.1^{a}
Shelled × Plastic bucket	15.9^{a}	40.0^{f}	50.5^{l}	17.5^{c}	4.5^{e}
Shelled × Polyethylene sacks	12.1^{b}	40.6^{f}	49.1^{m}	23.2^{a}	3.9^{k}
Shelled × Jute bag	9.3^{e}	46.1^{de}	58.7^{e}	16.2^{i}	4.1^{i}
Shelled × Basket	5.1^{g}	46.3^{de}	63.8^{b}	17.2^{d}	4.5^{f}
Shelled × Polypropylene bag	11.5^{bc}	40.8^{f}	54.9^{j}	16.9^{ef}	4.2^{h}
Shelled × Deep freezer	11.2^{c}	49.8^{bc}	55.3^{i}	17.1^{de}	4.4^{g}
Unshelled × Earth port	9.3^{e}	51.2^{b}	57.6^{g}	16.6^{g}	3.9^{k}
Unshelled × Plastic bucket	10.1^{d}	48.0^{cd}	57.1^{f}	16.8^{fg}	4.1^{j}
Unshelled × Polyethylene sacks	6.1^{f}	44.7^{e}	62.7^{c}	16.4^{h}	4.1^{i}
Unshelled × Jute bag	5.2^{g}	46.2^{de}	62.7^{c}	16.9^{fg}	4.5^{ef}
Unshelled × Basket	3.1^{i}	47.6^{cde}	65.8^{a}	17.2^{d}	5.1^{d}
Unshelled × Polypropylene bag	4.0^{h}	50.1^{bc}	61.1^{d}	17.9^{b}	6.9^{c}
Unshelled × Deep freezer	9.0^{e}	55.7^{a}	56.4^{h}	18.1^{b}	7.0^{b}
Mean	8.8	46.1	57.7	17.5	4.9
Se ±	02	0.9	0.01	0.06	0.01
CV %	3.3	2.8	0.01	0.53	0.01

Means in the same column followed by the same letter(s) do not differ significantly at ($p \leq 0.05$) according to DMRT.
Se = Standard error and CV = Coefficient of variation

4.1.1 2 Seed germination

Analysis of variance among different treatment tested is summarized in Table 5 bellow. There was a significant influence ($P \leq 0.05$) on the mean germination percentage due to the shell status and storage container interactions.

Table 5. Summary of analysis of variance of germination percentage and seedling vigor index

Source of variation	df	Germination %	Vigor index
Replication	3		
Shell status	1	*	*
Error (a)	3		
Storage	6	*	*
Shell status X Storage	6	*	*
Error (b)	36		
Priming	2	*	*
Shell status X Priming	2	ns	ns
Storage X Priming	12	ns	ns
Shell status X Storage X Priming	12	ns	ns
Error (c)	84		
Total	167		
CV %		30.23	22.18

Note: * = significant ($P \leq 0.05$) and ns = not significant.

The effects of shell status and storage container interactions on standard germination are summarized in Table 6. In most of storage containers with exception of deep freezer, shelled seed did not store well. Shelled seed stored in deep freezer germinated with significantly high percentage (27.2%) followed by unshelled seed stored in woven basket and hanged over the fireplace (21.9%). Shelled seed stored in plastic bucket did not germinate at all while shelled seed stored in polyethylene bags and polypropylene bags germinated with mean germination percentages of 0.1 and 0.2 respectively. Vigor indices followed the same trend.

Table 6. Mean germination percentages

	Shell status		
Storage container/Facility	Shelled	Unshelled	Mean
Earth port	1.3	3.9	2.5
Plastic bucket	0.0	2.5	1.2
Polyethylene sacks	0.1	4.2	2.2
Jute bag	3.0	12.9	8.0
Basket	10.3	21.9	16.1
Polypropylene bag	0.2	6.5	3.3
Deep freezer (Control)	27.2	12.6	19.9
Mean	6.0	9.2	7.6
LSD (5%)	0.4	0.4	

4.1.1.3 Effect of seed priming

Regardless of the storage container used, seed priming or pre-hydration treatments significantly ($P \leq 0.05$) improved the mean germination percentage and vigor indices compared to untreated seeds (Table 7). However, interactions of priming treatments with shell status and storage containers did not show significant effect at $P \leq 0.05$ as indicated in Table 7.

Table 7. Mean germination and seedling vigor indices for priming treatments

Priming treatment	GERMINATION (%)	VIGOR INDEX
Soaking for 24 hours	7.37[a]	1.46[a]
Wetting prior to germination	6.87[b]	1.31[b]
No priming (Control)	3.21[c]	0.63[c]
Mean	5.80	1.13
s.e ±	0.10	0.04
CV (%)	30.23	22.18

Means in the same column followed by the same letter(s) do not differ significantly ($P \leq 0.05$) according to DMRT.

4.1.1.4 Correlation between various seed quality attributes

Correlation analysis between various seed quality indices tested showed that germination percentage and seedling vigor had perfect correlation ($r = 1^{**}$). Correlation analysis also showed that there is strong positive correlation between mean seed size (100 seed weight) and mean germination percentage ($r = 0.9^{**}$). Strong negative correlation was indicated between seed moisture content after storage and carbohydrate content ($r = -0.9^{**}$). Correlation matrix for various seed quality indices tested for seven different storage facilities are shown in Table 8.

Table 8. Simple correlation matrix between various seed quality indices for seed stored for six months using seven different storage containers

Quality index	Seedling vigor	Germination %	Fat %	Protein %	CHO %	MC %	Seed size
Seed size	0.9^{**}	0.9^{**}	0.5^{ns}	-0.3^{ns}	0.2^{ns}	-0.1^{ns}	
MC %	-0.5^{ns}	-0.5^{ns}	0.4ns	0.1^{ns}	-0.9^{**}		
CHO %	0.5^{ns}	0.5^{ns}	-0.2^{ns}	-0.2^{ns}			
Protein %	-0.1^{ns}	-0.1^{ns}	-0.4^{ns}				
Fat %	0.3^{ns}	0.5^{ns}					
Germination %	1^{**}						
Seedling vigor							

Note:

CHO = Carbohydrate, MC = Moisture content, ns = not significant and ** = highly significant ($p \leq 0.01$).

4.1.2 Field experiment one

4.1.2 1 Field germination

Analysis of variance (Appendix 6) of germination test under field conditions, for seeds stored unshelled in deep freezer, woven basket and jute bag, showed that there were no significant differences between the three storage containers. However, priming resulted in highly significant differences ($p \leq 0.05$) in germination percentages. Interaction effects were not significant at $p \leq 0.05$. Means of germination percentage for the three priming treatments tested over three storage facilities are summarized in Table 9. The data indicated that under field conditions, dry planted seeds had significantly ($p \leq 0.05$) higher germination percentage and seeds that were soaked for 24 hours before sowing had significantly low germination percentage.

Table 9. Percentage germination under field conditions

	Type of Storage Container			
Priming treatment	Deep freezer	Basket	Jute bag	Mean
Soaking	1	6	5	4
Wetting	7	13	13	11
Control	11	15	19	15
Mean	6.3	11.3	12.3	10
LSD (5%)	3.4	3.4	3.4	3.4

4.1.2.2 Days to first germination and to first flowering

Data on days to first germination and days to first flower indicated that all plots had their first plant germinating at 15 DAS. Seed stored in deep freezer produced their first flower on 38 DAS while seed stored in basket and jute bag had first flower at 39 DAS. Seeds soaked for 24 hours had its first flower at 38 DAS while wetted seeds and dry planted seeds produced first flower one day later. Since less than 50% of sown seed germinated, data on days to 50% germination and 50% flowering were not recorded.

4.1.2.3.Growth variables

Data for dry matter partitioning between roots, leaves, stem and pods did not differ significantly at 30, 60 and 90 DAS. At 120 DAS seed stored in jute bag and in deep freezer significantly ($P \leq 0.05$) allocated more dry matter (weight) to roots (0.46g) compared to seed stored in basket that averaged 0.26g (Table 10). A similar trend was recorded for SDM (Table 11). Pod dry matter (PDM) accumulation was not significantly different between storage containers. The patterns of growth variables over time for different containers are summarized in Figure.3.

No significant differences were observed, between wetting and dry planting, in plant height, number of leaves per plant, leaf area per plant and number of nodes at each measurement. Similarly, no significant differences were observed in dry matter partitioning to roots, leaves, stem and pods between wetting and dry planting. The

patterns of growth variables trend over time are summarized in Figure.4. Interaction between storage containers and priming treatment was not significant.

Table 10. Root dry matter weight (g/plant) at 120 DAS

Storage	Priming treatment		Mean
	Wetting	Dry planting	
Deep freezer	0.37	0.25	0.31
Basket	0.22	0.30	0.26
Jute bag	0.45	0.47	0.46
Mean	0.34	0.34	0.34
LSD (5%)	0.18	0.18	0.18

Table 11. Stem dry matter (g/plant) at 120 DAS.

Storage	Priming treatment		Mean
	Wetting	Dry planting	
Deep freezer	19.95	11.62	15.78
Basket	8.23	8.47	8.4
Jute bag	14.10	15.05	14.57
Mean	14.09	11.71	12.91
LSD (5%)	5.83	5.83	5.83

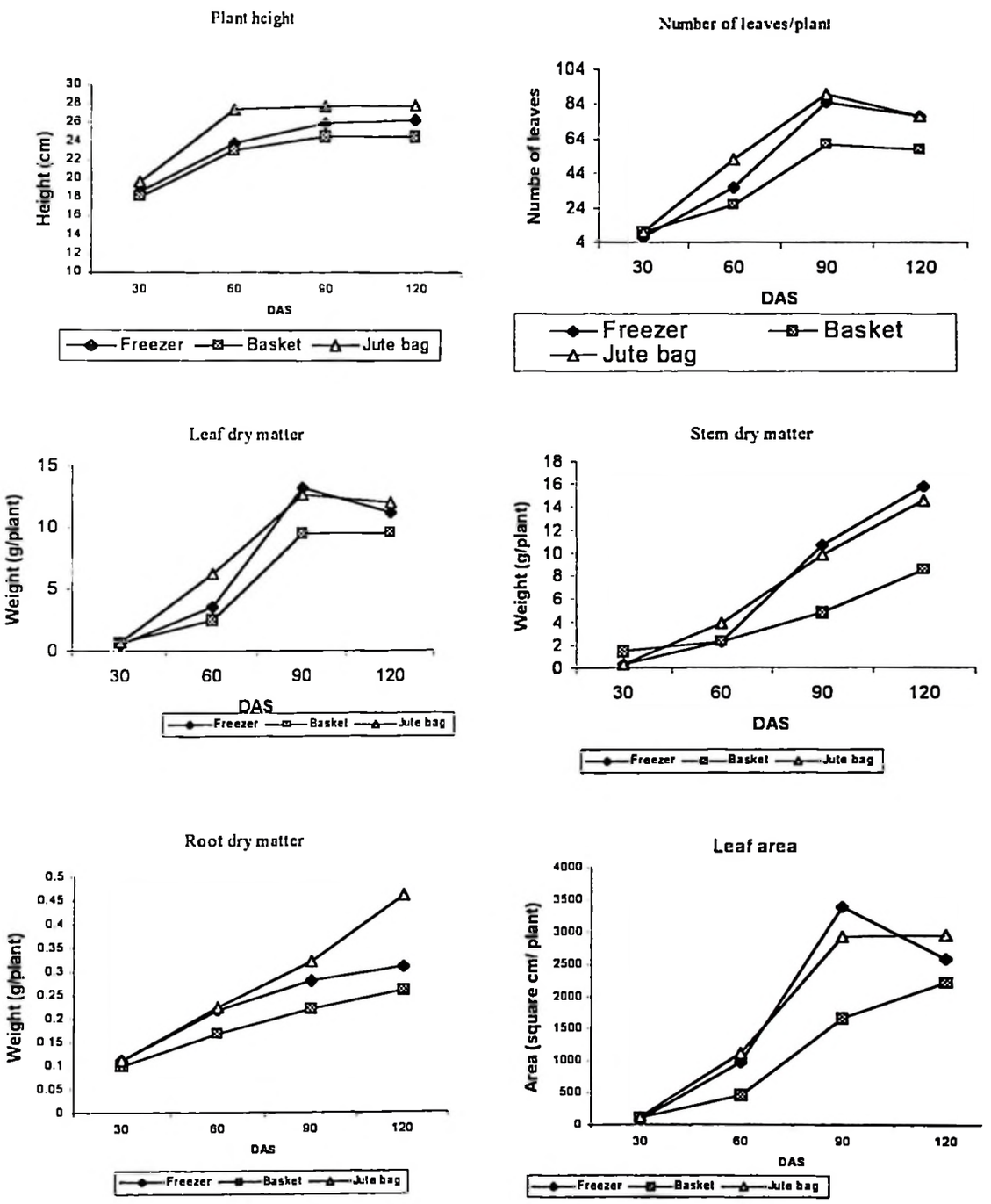

Figure 3. Effect of storage containers on growth variables.

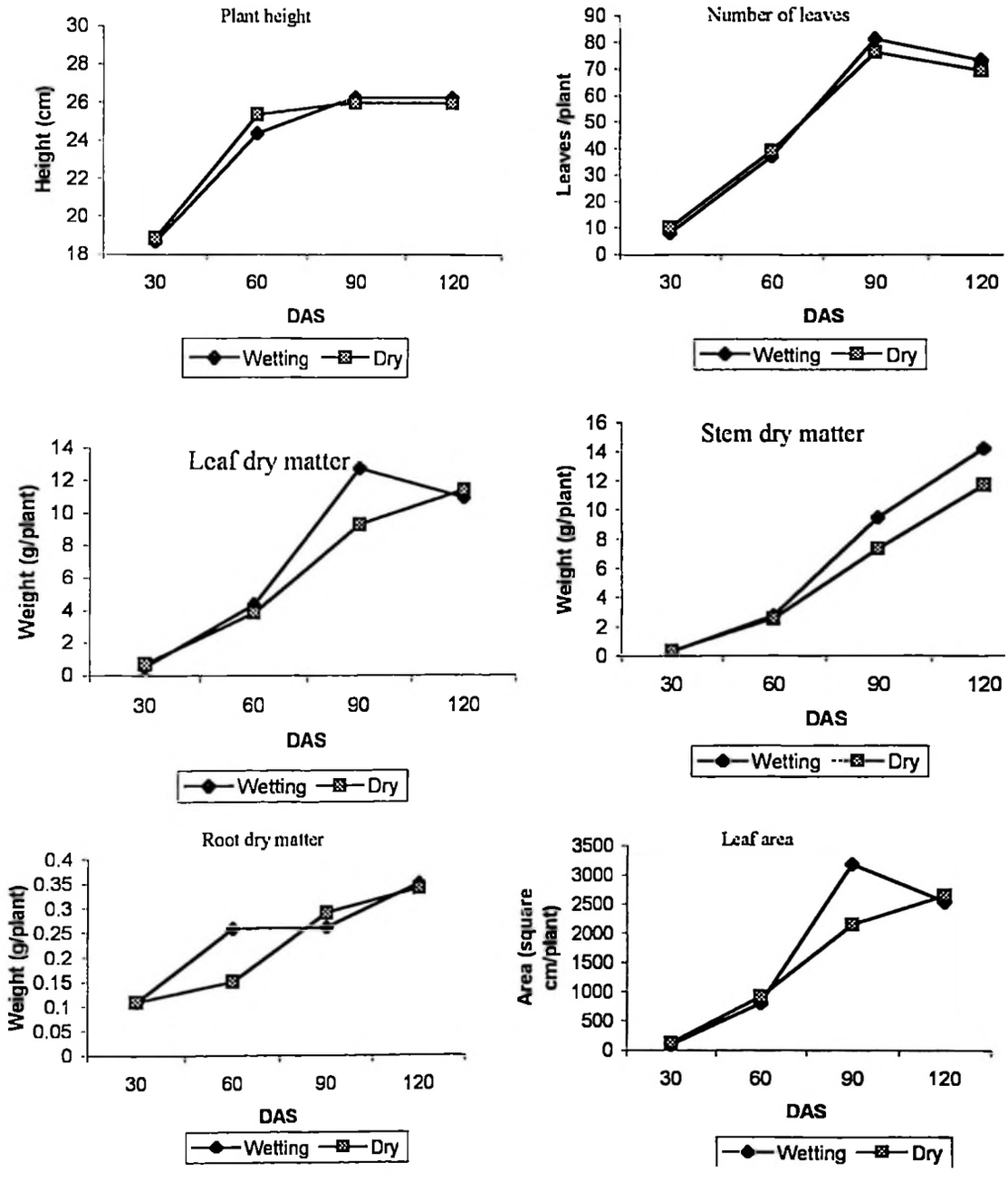

Figure 4. Effect of priming treatments on growth variables.

4.1.2.4 Relative growth rates and partitioning coefficients.

Effects of storage containers and priming treatment on relative growth rate (RGR) and partitioning coefficients are summarized in Table 12. No significant difference at $P \leq 0.05$ was observed in RGR between storage containers, priming treatment and their interaction. However, during early period (30 DAS) to mid growth stages (60 DAS) wetting improved the RGR compared to dry planting. Generally, mean RGR decreased with increase in plant age, but differences were not significant for all storage containers and priming treatments tested. Leaf area partitioning coefficient (LAPC) also decreased with plant age. Significant differences in LAPC between storage containers and priming treatment (between wet and dry planting) were observed during the late stages of growth (90 to 120 DAS). Likewise, shoot partitioning coefficient (SPC), decreased with plant age and significant differences between storage containers and between wetting and dry planting were observed during the growth period 90 to 120 DAS. Seed stored in jute bag had its highest SPC at early to mid growth stages (30 - 60DAS) while seed from deep freezer and basket had its highest SPC at mid to late growth stage (60-90 DAS).

Table 12. Effect of container and priming treatment on relative growth rates and partitioning coefficients at different growth periods

Growth period	30 to 60 DAS				60 to 90 DAS				90 to 120 DAS				
Treatments	RGR	LAPC	SPC	LPC	RGR	LAPC	SPC	LPC	RGR	LAPC	SPC	LPC	HI
Storage													
Freezer	0.06	164.8	0.94	0.60	0.04	133.6	0.97	0.55	0.01	-2.8	0.39	-0.64	0.20
Basket	0.04	108.8	0.96	0.62	0.03	146.7	0.98	0.58	0.02	44.3	0.49	0.38	0.26
Jute bag	0.07	152.7	0.97	0.66	0.03	106.8	0.74	0.52	0.01	2.9	0.43	0.02	0.29
Priming													
Wetting	0.07	108.9	0.94	0.62	0.03	154.6	0.98	0.55	0.01	-34.83	0.22	-0.76	0.28
Dry planting	0.05	143.2	0.98	0.58	0.03	90.0	0.75	0.52	0.01	48.3	0.63	0.33	0.22

DAS = Days after sowing

RGR = Relative growth rate ($g.g^{-1}$/day), LAPC = leaf area partitioning coefficient (cm^2/g), SPC = shoot partition coefficient, LPC = leaf partition coefficient and HI = harvest index

4.1.2.5 Yield variables

Analysis of variance for pods per plant, pod dry matter, shelling percentage seed yield and harvest index is summarized on Table 13. No significant difference between dry planting and wetting prior to sowing was observed in harvest index, shelling percentage and seed yield.

Table 13. Summary of analysis of variance for yield variables

Source	df	No.Pods/plant	PDM	Shelling%	yield (g/plant)	HI
Replication	3					
Storage (m)	2	*	*	ns	ns	ns
Error (a)	6					
Priming (s)	1	ns	ns	ns	ns	ns
Storage X Priming	2	*	*	*	*	ns
Error (b)	9					
Total	23					
CV %		18.96	30.00	6.65	32.94	27

Note: * = significant ($P \leq 0.05$), df = Degree of freedom, CV = coefficient of variation, PDM = Pod dry weight, m = main plot treatment and s = sub plot treatment.

Storage containers and priming treatment interactions had significant influence on yield and yield variables. Significant difference ($P \leq 0.05$) was observed in number of pods per plant and seed yield per plant. Seed stored in jute bag and dry planted had an average of 25 pods per plant and seed yield of 8.9g/plant while seed stored in deep freezer and dry planted performed poorly with 12 pods /plant and seed yield of 2.8g/plant. Significant difference ($P \leq 0.05$) was observed in shelling percentage. Effects of storage containers and priming treatment interactions are summarized on Table 14.

Table 14. Effects of storage containers and priming treatment on yield variables.

Treatment	Yield variables			
	Pods/plant	PDM/Plant	Shelling %	Yield (g/plant)
Deep freezer and wetting	22	10.4	73.5	7.6
Deep freezer and dry planted	12	4.3	64.7	2.8
Basket and wetting	14	7.4	73.0	5.7
Basket and dry planting	18	5.5	75.5	4.1
Jute bag and wetting	21	9.6	66.7	6.3
Jute bag and dry planting	25	12.6	71.0	8.9
Mean	18	8.3	70.7	5.9
CV (%)	17.1	13.0	23.5	19.7
LSD (5%)	5.49	4.18	7.52	3.11

4.1.2.6 Correlations.

Correlation analysis between field germination percentage, seed yield per plant and yield components at 120 DAS (Table 15) indicates that there was a weak positive non significant correlation between field germination percentage with most of the yield components tested. However there were positive significant correlations between final seed yield with root dry matter ($r= 0.66^{**}$), pod yield ($r= 0.88^{**}$) and stem dry matter ($r= 0.56^{**}$). Harvest index and shelling percentage had negative and non-significant correlations with most of the variable recorded during vegetative growth (viz. number of leaves, leaf area, leaf dry matter, plant height and stem dry matter). However, positive significant correlation ($r = 0.41^{*}$) was observed between germination percentage and root dry weight at 120DAS.

Table 15. Correlation matrix between germination percentage, yield components and seed yield.

Character	12	11	10	9	8	7	6	5	4	3	2	1
1	0.04 ^{ns}	0.10 ^{ns}	0.09 ^{ns}	0.09 ^{ns}	0.07 ^{ns}	0.41*	0.11 ^{ns}	-0.11 ^{ns}	0.32 ^{ns}	0.32 ^{ns}	-0.20 ^{ns}	
2	-0.05 ^{ns}	-0.27 ^{ns}	0.39*	0.40*	0.29 ^{ns}	0.38**	0.63**	0.47*	0.62**	0.62**		
3	-0.03 ^{ns}	-0.10 ^{ns}	0.52	0.50 ^{ns}	0.54**	0.65**	0.70**	0.35 ^{ns}	1**			
4	-0.10 ^{ns}	-0.30 ^{ns}	0.52 ^{ns}	0.57**	0.54**	0.65**	0.73**	0.35 ^{ns}				
5	-0.02 ^{ns}	-0.26 ^{ns}	0.19 ^{ns}	0.21 ^{ns}	0.16 ^{ns}	0.16 ^{ns}	0.36 ^{ns}					
6	-0.01 ^{ns}	-0.21 ^{ns}	0.56**	0.63**	0.57**	0.66**						
7	0.25 ^{ns}	-0.04 ^{ns}	0.66**	0.72**	0.67**							
8	0.54**	0.17 ^{ns}	0.86**	0.88**								
9	0.10 ^{ns}	0.54**	0.86**									
10	0.61**	0.22 ^{ns}										
11	0.42*											
12												

** = Very significant ($P \leq 0.01$), * = significant ($P \leq 0.05$) and ns = not significant

Key:

1= Germination%, **2**= Number of leaves per plant, **3**= Leaf area (cm^2/plant), **4**= Leaf dry matter (g/plant), **5**=Plant height (cm), **6**=Stem dry matter (g/plant), **7**=Root dry matter (g/plant), **8**=Number of pods per plant, **9**=Pod yield (g/plant), **10** Seed yield (g/plant), **11** =Shelling percentage, and **12** = Harvest index

4.2 Influence of Seed Priming

4.2.1 Effect of priming on germination and plant growth

Results from priming experiment (field experiment 2) indicated that soaking seed for 24 hours significantly ($P \leq 0.05$) improved seed germination rate and days to first flower compared to control (Table 16). However, all treatments attained 50% flowering at 43 DAS.

Table 16. Effect of seed priming on germination and flowering

Treatment	Days to first germination	Days to 50% germination	Days to first flower
Soaking	6	7	36[b]
Wetting	9	10	38
Dry planting (Control)	9	10	39
Mean	8	9	37
CV %	11.8	5.2	3.0
LSD (5%)	1.6	0.8	2

Number of nodule per plant, stem dry matter per plant, root dry matter per plant and total dry matter per plant at 30 DAS were significantly affected by priming treatment ($P \leq 0.05$) in which soaking for 24 hours improved all the variables compared to control (Table 17). No significant differences at $P \leq 0.05$ were observed in number of leaves, leaf dry matter and plant height.

Table 17. Effect of seed priming on growth variables at 30 DAS.

	Growth variables			
Treatment	RDM (g/plant)	SDM (g/plant)	TDM (g/plant)	Nodules
Soaking	0.12	0.7	2.1	16
Wetting	0.07	0.5	1.7	9
Dry (control)	0.07	0.5	1.3	7
Mean	0.09	0.6	1.8	11
LSD (5%)	0.01	0.18	0.47	3

RDM= Root dry matter, SDM = Stem dry matter and TDM = total dry matter.

At 60 DAS plants from soaked seed had mean of 3.3 pods per plant, wetting had 2 pods per plant and dry planted produced only single pod per plant. These differences were significant ($P \leq 0.05$). However, no significant differences in mean pod weight per plant were observed. No significance differences ($P \leq 0.05$) were observed in all growth variable measured at 60, 90 and 120 DAS, however, soaking seed for 24 hours improved most of growth variables compared to control. Trends of dry matter partitioning to various plant parts over time are indicated in Figure 5.

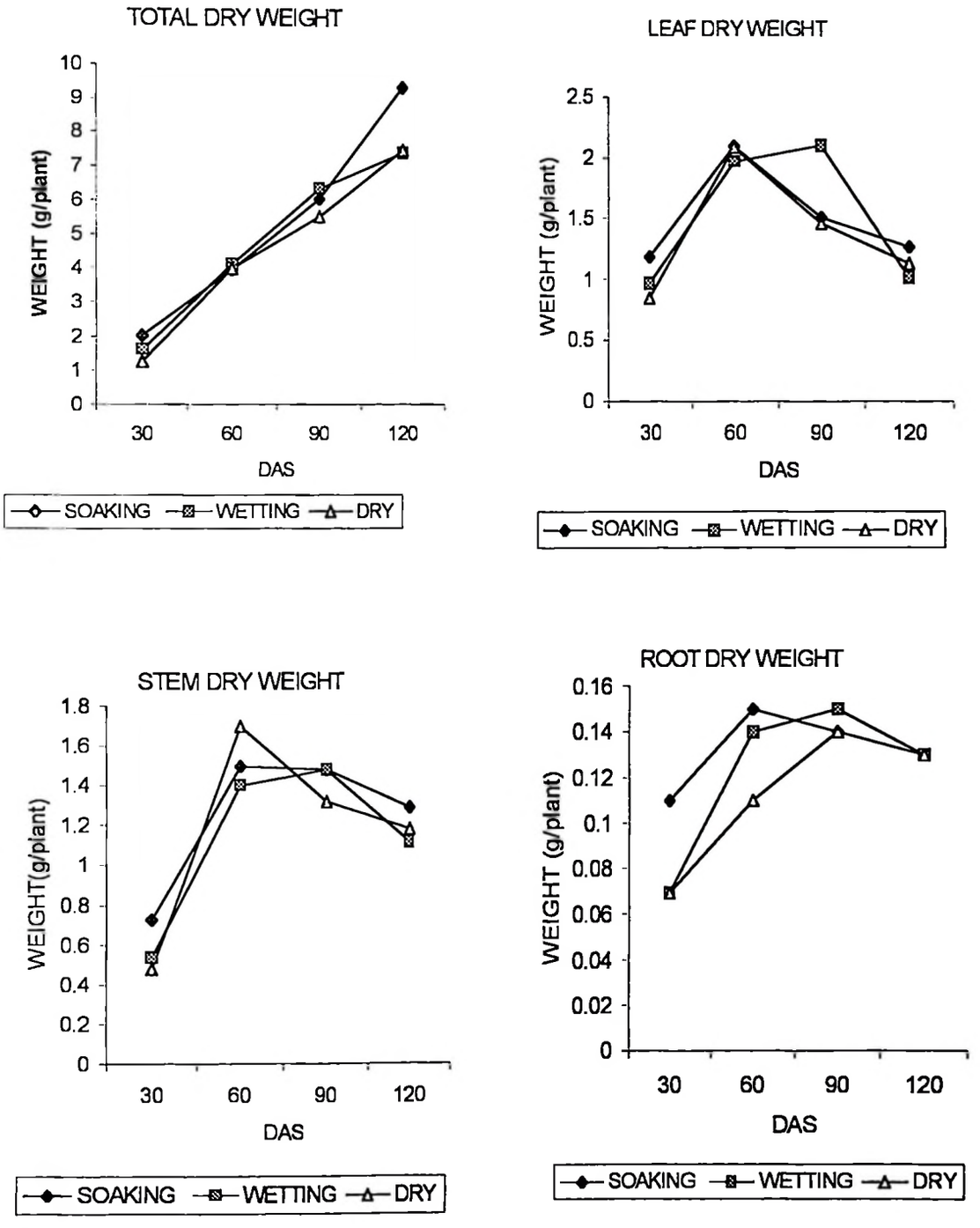

Figure 5. Effect seed priming on dry matter partitioning of bammbara groundnut

4.2.2 Relative growth rate and partitioning fractions

In all sampling periods, no significant differences at $P \leq 0.05$ in mean relative growth rate between treatment means were observed (Table 18)

Table 18. Effect of seed priming on relative growth rate (gg^{-1}/day)

	Growth period (DAS)		
Treatment	30-60	60-90	90-120
Soaking	0.04	0.02	0.02
Wetting	0.03	0.02	0.01
Dry (control)	0.03	0.02	0.01
Mean	0.03	0.02	0.01
CV %	11.18	14.10	15.50
LSD (5%)	0.027	0.001	0.96

At 30 DAS, significant differences at $P \leq 0.05$ were observed in fraction of total dry weight allocated to above ground parts. Soaked seeds allocated 0.94 while soaked seeds and dry planted seeds allocated 0.95. There were no significant differences in fraction of dry matter allocated to above ground parts at 60, 90 and 120 DAS (Table19).

Table 19. Fraction of dry matter allocated to above ground parts

	Days after sowing			
Treatment	30	60	90	120
Soaking	0.94	0.96	0.98	0.98
Wetting	0.95	0.97	0.98	0.98
Dry (Control)	0.95[a]	0.97	0.98	0.98
Mean	0.947	0.96	0.973	0.98
CV %	15.25	18.10	15.18	10.15
LSD (5%)	0.001	0.012	0.002	0.004

Similarly, there were no significant differences ($p \leq 0.05$) between treatment means in specific leaf area (SLA) (Table 20).

Table 20. Effects of seed priming on specific leaf area (cm^2/g)

	Days after sowing			
Treatment	30	60	90	120
Soaking	232.1	177.2	175.8	163.2
Wetting	212.5	177.4	176.8	163.0
Dry (control)	218	177.3	177.0	162.8
Mean	220.6	177.3	176.2	163.0
CV (%)	12.39	11.34	9.52	19.25
LSD (5%)	46.96	0.34	1.58	1.80

At 60 DAS, fraction of above ground dry weight that was allocated to pod showed significant differences (Table 21)

Table 21. Fraction of above ground dry weight partitioned to pod

	Days after sowing		
Treatment	60	90	120
Soaking	0.05	0.50	0.5
Wetting	0.04	0.43	0.5
Dry (control)	0.03	0.42	0.5
Mean	0.038	0.43	0.5
CV (%)	14.30	21.16	20.25
LSD (5%)	0.002	0.16	0.16

4.2.3 Final yield

Differences in seed yield per plant (g) between treatments, 100 seed weight (g), harvest index (HI) and shelling percentage were not significant at $P \leq 0.05$. However soaking seed for 24hours improved most of yield variables compared to control (Table 22).

Table 22. Effect of priming on yield.

	Yield variables			
Treatment	Seed yield (g/plant)	Shelling %	HI	100 seed weight (g)
Soaking	5.4	76	0.5	34.1
Wetting	4.1	76	0.5	33.9
Dry (control)	4.1	75	0.5	33.7
Mean	4.55	75.6	0.5	33.9
CV (%)	10.15	13.48	8.65	25.05
LSD (5%)	1.71	1.32	0.02	4.25

4.2.4 Correlation between seed yields and yield variables.

Correlation analysis between seed yield variables at 120 DAS (Table 23) indicated that characters strongly correlated with seed yield per plant were pod yield per plant ($r = 0.99^{**}$), 100 seed weight ($r = 0.68^{*}$) stem dry matter per plant ($r = 0.48^{ns}$) and number of pod per plant ($r = 0.40^{ns}$). However, all characters tested were positively correlated to seed yield per plant.

Table 23. Correlation matrix between seed yield, yield variables and growth parameters at 120 DAS

Character	1	2	3	4	5	6	7	8	9	10	11	12
12	0.02^{ns}	-0.40^{ns}	0.02^{ns}	0.01^{ns}	-0.24^{ns}	0.56*	0.10^{ns}	0.03^{ns}	0.36^{ns}	0.32^{ns}	0.33^{ns}	
11	0.01^{ns}	-0.03^{ns}	-0.02^{ns}	-0.59^{ns}	0.32^{ns}	0.03^{ns}	-0.06^{ns}	0.61^{ns}	0.76**	0.90**		
10	0.01^{ns}	-0.02^{ns}	-0.01^{ns}	-0.59^{ns}	-0.20^{ns}	0.03^{ns}	-0.02^{ns}	0.60^{ns}	0.76**			
9	0.19^{ns}	0.02^{ns}	0.16^{ns}	-0.22^{ns}	-0.37^{ns}	0.25^{ns}	0.20^{ns}	0.37^{ns}				
8	0.48^{ns}	0.63*	0.45^{ns}	-0.12^{ns}	-0.06^{ns}	-0.19^{ns}	-0.41^{ns}					
7	0.18^{ns}	0.18^{ns}	0.19^{ns}	0.32^{ns}	-0.25^{ns}	0.41^{ns}						
6	0.40^{ns}	-0.40^{ns}	0.43^{ns}	0.44^{ns}	0.10^{ns}							
5	0.08^{ns}	0.40^{ns}	0.08^{ns}	-0.01^{ns}								
4	0.67**	0.30^{ns}	0.66**									
3	0.99**	0.65*										
2	0.68*											
1												

** = Highly significance ($P \leq 0.01$), * = Significance ($P \leq 0.05$) and ns = Not significance ($P > 0.05$)

Key

1 = seed yield (g/plant), 2 = 100 seed weight (g), 3 = pod yield (g/plant), 4 = harvest index, 5 = shelling percentage, 6 = number of pods per plant, 7 = root dry weight (g/plant), 8 = stem dry weight (g/plant), 9 = plant height (cm), 10 = leaf dry weight (g/plant), 11 = leaf area (cm^2/plant) and 12 = number of leaves per plant.

CHAPTER FIVE

5.0 DISCUSSION

Results from this study as indicated in Tables 2, 3, 4 and 5 have shown that generally, as the seed aged there was a decline in germination percentage. The pattern of storage including shell status, storage period and physical structure of the storage facility exerted a significant influence on bambara groundnut seed quality maintenance. From these results it was evident that bambara groundnut seed deteriorated during storage and quality after storage was variable for the different storage containers. These results are similar to that of Nautiyal *et al.* (1990) in India who reported a more serious loss of viability in groundnut produced in post rain season for which more than 50% viability was lost within 4 -5 months of storage.

5.1 Relationship of Quality Indices to Germination

Correlation analysis had indicated that the proportion of carbohydrate in seed food reserve and 100 seed weight were positively correlated with germinabilty of the seed while seed moisture content after storage was negatively correlated to germination percentage. The amount of food reserve in seed plays a role in maintaining viability of seed during storage. There is possibility of involvement of accumulation of toxic metabolites in deterioration process as speculated by Coolbear (1994). Therefore, a good storage method is supposed to suppress metabolic processes but not to the levels of injuring the embryo, since biochemical processes in seed can be part of the natural seed aging process. The differences in the proportion of food reserves at the end of

storage period between various storage methods tested is evidence that metabolic activities were taking place at different rates among the different storage containers during the storage period. Carbohydrate and fat reserves content correlated positively with germination percentage. The results indicated that any storage methods that resulted in a decrease in the proportion of carbohydrates were associated with decrease in germination percentages. The results highlighted here are in agreement with those reported by Locher and Buchel (1998), who found that under simulated tropical storage conditions for soybean seeds, sugar hydrolysis corresponded to diminishing storability and germination capacity.

The water relations of the seed are of primary importance in maintaining viability during storage. The effects of seed moisture content (mc) on food reserve have been reported by Locher and Buchel, (1998) that in soybean seed under accelerated tropical storage conditions (37^0C and 82% RH), increase in seed moisture content induced complete degradation of soluble oligosaccharides. The negative correlation between seed mc and carbohydrate ($r = -0.93^{**}$) and negative correlation between mc with germination percentage ($r = -0.47$), in this study are indications that rise in seed mc during storage had detrimental effects on germination. Abdullah *et al.* (1992) have also reported a similar result in long bean (*Vigna sesquipedalis* L. Fruhw).

Results from this study show that 100 seed weight (seed size) had positive and significant correlation to germination percentage ($r = 0.89^{**}$). Other workers have

similarly reported evidence of positive correlation between seed size and seedling vigor in different plant species. For example, Ram *et al.* (1989) found a positive correlation between 100 seed weight and final field stand in chickpea (*Cicer arietinum* L.) while Sangakkara *et al.* (1985) reported similar results in herbage grasses.

5.2 Influence of Seed Shells and Seed Storage Container on Germination.

Although storage containers played a significant role in seed deterioration, several kinds of environmental stress like mineral deficiency, drought and temperature extremes during seed development and prior to physiological maturity may have impaired the seed longevity. Thus the very low germination percentages in this experiment can be attributed to environmental factors acted on seed before harvest through the parent plant rather than storage containers per se since even seed stored in deep freezer lost more than 50% of its germination capacity.

From earlier work by Sreeramulu (1983a), it was reported that bambara groundnut seed deteriorated during storage. Among storage containers tested in this research work deep freezer was the best in maintaining seed viability (19.9%) compared to the rest storage facilities. However unshelled seed stored under deep freezing conditions (-12 ^{0}C) had significantly low germination parentage (12.6%) compared to unshelled seed stored in basket which was hung over the fireplace (21.9%). With exception of deep freezer, shelled seed did not store well in all other containers tested. With exception of basket hung over the fire place, shelled seed stored in all other remaining

containers imbibed moisture and had an increase in its mc at the end of storage period ranging from 9.4 to 15.9% (Table 4). In addition, the increase in moisture content was slightly associated with the decrease in germination ability ($r = -0.47^{ns}$). Hence the relationship between the type of storage container and reduction in germinability could be considered as a direct result of the changes in seed moisture content of the seed stored in different containers. The plastic bags (commonly used for grocery shopping) were very effective in preventing moisture absorption from the surroundings during storage (with seed mc of 9.12% at the end of storage period compared to 9.4% initial moisture content). However, the low germination percentage of seed stored in the plastic bags is an indication that seed storage container may be good in maintaining ample seed mc during storage. But if storage container fails in preventing other deteriorative events such as excessive respiratory activities and pest damage from taking place, the quality of seeds can be adversely affected. This is indicated in this study by the seed stored in the plastic bags were heavily attacked by bruchid beetles (*Callosobruchus maculatus* and *C.subinnotatuts*). Therefore, despite of the low cost of plastic bags such facility is not appropriate for bambara groundnut seed storage.

For the plastic bucket treatment, there were no moisture exchanges with surrounding atmosphere. However, the final seed mc was higher (13.02%) compared to initial seed moisture content (9.40%). It is assumed that there may have been changes inside the bucket, which could be ascribed to respiration of the seed and associated microorganisms resulting in the rise in internal relative humidity. Consequently, the

seed may have re-absorbed the moisture from the raised humidity (surroundings). Such a situation can be limitation against storing bambara groundnut seed at village level.

Jute bag and plastic bags (polypropylene material) are very commonly used in storing grain in Tanzania. However, among the two, jute bag significantly reduced deteriorative process (Table 2 and Appendix 5). The difference in seed quality after storing in either jute or plastic bag was also reflected in germination (see Table 6).

Among the six storage containers tested, basket hung over the fireplace was the best in maintaining seed viability. Unshelled seed stored relatively better in woven basket as it had germination of 21.94%, which was significantly higher compared to all unshelled seed storage method. This good germinability can be attributed to the heat generated over the fireplace, which reduced seed moisture content. There was no bruchid damage on shelled seeds stored in basket and hanged over the fireplace probably because due to the action of the smoke from the fire in preventing insect pest attack (Forrest *et al.*, 1975; Louwaars *et al.*, 1998). However, only 10.3% of the shelled seeds germinated compared to 21.9% of unshelled seeds. This poor performance of shelled seeds may have been caused by the drying injury of the seed. Seyedin and Burris (1982) reported a similar result in other crops were high temperature drying caused injury that varied from impairment of cell membranes to reduced vigor and complete loss of viability. In this regard seed shells can be regarded as buffer that protected unshelled seed from the effects of strong heat from the fire.

5.3 Influence of Seed Storage Containers on Crop Growth and Final Yield.

Although germination of seed stored in deep freezer, jute bag and in basket hanged over the fireplace differed significantly from the standard germination test, such differences were not reflected under field conditions (Table 9). Seed from all three storage containers took 15 days to germinate with very low germination percentage of 10 % while in laboratory germination test was 19.97%. Poor performance under field conditions can be attributed to the loss of vigor, which was associated with loss of seed viability during storage. These results suggest that laboratory standard germination test alone cannot unequivocally predict the absolute values of field emergence since the field conditions are sometime not optimal for seed germination (Rivas *et al.*, 1984).

It is well known that the number and size of nodules provide morphological evidence for biological N_2 fixation. Rennie and Kemp (1984) emphasized that early nodule development is important in providing N for vigorous vegetative growth and seed yield in field beans. In this study, few and mostly small nodules were observed in early growth stages (30 DAS). This kind of poor nodulation can be attributed to the change of seed quality during storage. Similar results have been reported in soybeans (Smith and Ellis, 1980). Rhizobial infection depends among other factors on root hair availability and total root mass (Rodriguez and McDonald, 1989). Therefore it is assumed that seedling produced from low quality seed may not provided sufficient sites for rhizobial invasion, thus decreasing the infection rate and subsequent nodule

formation. However, the poor nodulation in this research could be due to ineffective native rhizobium species in the soil in an experimental site.

Seed yield potential of the crop is considered a function of rate of biomass accumulation, duration of growth and intensity of partitioning to reproductive sites (Sexton *et al.*, 1994). From the results, seed deterioration during storage resulted in differences in mean relative growth rate (RGR) during the early to mid growth period (30 to 60 DAS), even though these differences did not persist at later stages (Table 12). This implies that initial low rate of growth in bambara groundnut was compensated at later stages of development. Further there were no differences in container influence on dry matter partitioning coefficients to various plant parts during early vegetative growth. The differences observed during late growth stages (90 - 120 DAS) could be due to the differences in partitioning of dry matter to pods and leaf senescence (European Union, EU, 1998). The decline of LAPC with plant age is an indication that at later growth period crop allocated less carbohydrates (dry weight) for production of new leaves, however, leaf senesces which usually begun at 100 DAS may have contribute to the decline of LAPC. Such observations have also reported by other workers such as European Union, EU, (1998). Negative value of LAPC and LPC suggests that at this stage most of the leaves had senesced. Nevertheless, the superiority of large seeds compared to small seeds (in terms of 100 seed weight), which was more pronounced in germination test, was not reflected in single plant grain yields. Seed stored in jute and dry planted gave significantly higher grain yield than

seed stored in deep freezer and woven basket although they were of large size (in terms of 100 seed weight). This suggests that in aged bambara groundnut seed, seed vigor (as indicated by germination percentage) did not necessarily translate to plant vigor. Hence germination could be high but subsequent seedling growth may not be optimal.

Correlation analysis indicated that harvest index and shelling percentage which are among the determinants of intensity of partitioning to reproductive sites had negative correlation with most of the vegetative traits (viz. plant height, number of leaves/plant, leaf area/plant and leaf dry matter/plant). Further, at maturity (120 DAS) the characters most strongly correlated with seed yield per plant were root dry matter ($r = 0.66^{**}$), number of pods per plant ($r = 0.86^{**}$) and pod weight ($r = 0.86^{**}$). According to Goli *et al.*, (1997) the positive correlation between seed yield per plant with number of leaves ($r = 0.39^{*}$), and stem dry matter ($r = 0.56^{**}$) at physiological maturity is an indication that the plant matured late. The shelling percentages obtained in this study are within the range of 70-77 as reported by Linnemann (1992) and Mkandawire (1996), however, the harvest indices reported in this study (i.e. 0.20, 0.26 and 0.29for seed stored in deep freezer, basket and jute bag respectively) are relatively lower compared to that of 0.32 reported by Mkandawire (1996).

5.4 Effects of Seed Priming

Seed priming has been shown to enhance the germination response of a large number of plant species including bambara groundnut (Heydecker and Coolbear, 1977; Masawe *et al;* 1997; Harris, 2000). The germination enhancement by priming may reflect metabolic repair processes (Bray *et al*; 1984), a build up of germination metabolites (Coolbear *et al*; 1980) and /or osmotic adjustment during imbibitions (Bradford, 1986). The first experiment of this study demonstrated detrimental effects of long priming duration on field germination of bambara groundnut. Despite positive responses to seed priming in laboratory test, field germination of bambara groundnut seeds in storage experiment were not improved by 24 hours of soaking in water. Rivas *et al.* (1984) similarly reported in Tabasco pepper (*Capscum frutescens* L.) that emergence of Tabasco pepper in the field was not improved by priming.

The detrimental effects of 24 hours of soaking in germination of bambara groundnut after storage could be ascribed to leakage of metabolites necessary for germination process. In other studies, Sreeramulu (1983b) reported rapid leakage of metabolites by non-viable seeds after 16- 20 hours of imbibitions and the loss of germinability of bambara groundnut seeds was positively correlated with the extent of leakage. However, this can only be assumed, as the primary mechanism of soaking injury in seeds has not yet been clarified (Small *et al.*, 1991).

Seed germinates when it absorbs sufficient moisture from the surrounding soil. Due to its hard seed coat, once it is sown, bambara groundnut seed will spend a great deal of time just absorbing water from the soil to attain required moisture level in the cotyledons. Results of the priming experiment indicated that if this time is minimized by pre sowing soaking of seed, seed germination and seedling emergence could be significantly hastened.

Generally, seed priming improves seedling establishment crop productivity (Kumarao, 2001). In this work, accelerated rate of field emergence of soaked seeds compared to control can be interpreted as a consequence of pre-germination and activation of enzymic processes in seed during hydration (soaking). These results concur with observation of Hofmann *et al.* (1992) in oat (*Avena sativa* L) and laboratory results of Masawe *et al.* (1999) who reported that soaking bambara groundnut Dodoma red landrace for 24 hours accelerated germination and early seedling growth.

From these results, the effects of seed pre sowing soaking in bambara groundnut is limited to germination and early vegetative growth (up to 30 DAS) as the initially faster growth rate of pre soaked seeds approached that of control after 30 DAS and there were no treatment effect on yield. It seems that yield depends much on number of pods, pod weight and 100 seed weight that were strongly correlated to seed yield rather than initial RGR per se.

The results suggest that prior to pod initiation assimilates available for above ground growth are partitioned to the leaves and stems but the fraction starts to decline after pod initiation. At pod initiation the fraction of above ground dry weight partitioned to pod increased with plant age to about 0.5 in all treatments. This is in agreement with earlier findings reported by European Union (1998) that assimilates are preferentially partitioned to the pods, rather than leaves. However, the value 0.5 is relatively higher compared to 0.4 reported by European Union (1998). Reduction in leaf and stem dry weights during the seed filling period can be attributed to the relocation of dry matter to developing seeds (Stephenson and Wilson, 1977) and leaf senescence after 100 DAS (EU, 1998). The specific leaf area in this study which initial was $0.023 m^2 g^{-1}$, declined with plant age. European Union (1998) reported similar results.

Number of leaves and total leaf area per plant are very important in pathways of physiological processes of synthesis of photo assimilates to the production of pods and seeds (Westermann and Crothers, 1977). In this experiment, the strength of the relationship between seed yield with leaf number and leaf area at 120 DAS appeared to be very weak as not to cause variation in yield, which is an indication that the plants matured early. Numbers of pods per plant, number of seeds per pod and seed weight are the primary components of seed yield in legumes (Graf and Rowland, 1987). Thus, failure of priming treatment to cause significant variation in these components can be regarded as the causes for non significant differences in grain yield per plant. However, Harris (2000) reported from preliminary research in Zimbabwe, that soaking

bambara groundnut seed for 8 hours caused the crop to emerge faster, have a vigorous growth, mature earlier and gave higher yield. Therefore, pre sowing seed wetting and 24 hours soaking of bambara groundnut seed in the priming experiment of this work can be regarded as not optimal treatments for significant yield increases.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMENDATIONS

Results obtained in this study have demonstrated a quantifiable relationship between germination rate, seed viability and seed deterioration during storage. From results and discussion it is concluded that;

(i) Shelling of bambara groundnut seeds had detrimental effects on viability during storage. For all types of storage containers, seed stored in unshelled conditions maintained high food (CHO) reserves than shelled seed and hence recorded higher germination percentage.

(ii) Physical structure of storage containers exerted a significant influence on seed quality during storage. Among the storage containers tested, seed stored in the basket, jute bag and deep freezer had more food reserves and hence recorded higher germination percentages. Correlation tests between changes in seed constituents with germinabilty indicated that the changes that occur during storage are associated with seed deterioration.

(iii) Soaking bambara groundnut seeds for 24 hours significantly accelerated germination and early seedling growth of freshly harvested bambara groundnut seed, however, the two pre-sowing hydration treatments tested did not improve field germination of aged bambara groundnut seeds.

From these results, the following recommendations are made;

(i) Bambara groundnut seeds should stored unshelled

(ii) Jute bags and/or basket hanged over the fireplace should be used to store bambara groundnut seeds at farm level, and where possible deep freezing storage facility should be encouraged.

(iii) Pre-sowing soaking for 24 hours should be encouraged in order to enhance germination and early crop vigor.

However, it was difficult to determine the cause of rapid loss of viability in this study, therefore, further research to determine the influence of pod drying methods on storability and viability of bambara groundnut is proposed.

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8.0 APPENDICES

Appendix 1. Summary of weather data during experiment period (2001-02)

Month	Rainfall (mm)	Radiation MJm^{-2}	Mean maximum temperature (^0C)		Sun shine (hours)	Mean RH %
December 2001	72.3	NA	33.2	(22.6)	NA	66
January 2002	35.6	NA	32.0	(22.6)	NA	71
February 2002	116.5	4.15	30.9	(22.0)	7.3	71
March 2002	187.4	19.85	30.5	(21.8)	6.8	80
April 2002	264.6	14.7	28.5	(21.1)	4.3	86
May 2002	24.6	17.4	29.1	(21.5)	7.3	79
June 2002	1.1	NA	29.6	(22.0)	NA	80

NA = Data not available

Values in parentheses represent mean minimum temperatures.

Appendix 2. physical and Chemical characteristics of soil in experimental sites

Characteristics	Site one (Horticulture unit	Site two (Crop museum)
pH (1:2.5) H_2O	6.75	5.3
% Organic carbon	0.8	1.9
% Total nitrogen	0.14	0.14
CEC ($cmolkg^{-1}$)	15.3	
K ($cmolkg^{-1}$)	6.8	1.65
P (ppm)	1.5	4.91
% Sand	38.0	32.0
% Silt	13.7	17.2
% Clay	48.3	50.8
Textural class	Clay	Clay loam

Appendix 3. Leaf area calculation by leaf weight method

Using a sub sample of 4 plants (taken out of the sample of 16 plants for each treatment).

1. Record the leaf dry weight of the sub sample.
2. Calculate specific leaf area (SLA) by dividing the leaf area (LA) with the leaf dry weight (LDW)
3. Determine leaf dry weight of the remaining sample from the dry matter (DM) taken during sequential growth analysis.
4. Calculate leaf area of the remaining sample by multiplying the LDW of remaining sample by the specific leaf area.

$$LA_{\text{remaining sample}} = LDW \times SLA$$

Add leaf area of the remaining sample to leaf area of sub-sample to obtain total leaf area (TLA) of the sample

$$TLA = LA_{\text{sub-sample}} + LA_{\text{remaining sample}}$$

Source: Rhoads and Blooduorr (1964)

Appendix 4. Seed composition after 6 months storage

Treatment	Moisture content%	100 Seed weight (gm)	CHO content (%)	Protein content (%)	Fats content (%)
Shelled	10.93[a]	43.07[b]	54.73[b]	17.766[a]	4.83[a]
	(9.4)	(55.7)	(57.2)	(22.9)	(7.1)
Unshelled	6.67[b]	49.07[a]	60.57[a]	17.14[a]	5.10[a]

Note: Means in the same column followed by the same letter do not differ significantly ($P \leq 0.05$) according to t test.

CHO = carbohydrate

Figure in parentheses indicates seed composition before storage for both shelled and unshelled seeds.

Appendix 5. Seed composition after 6 months of storage using different storage containers.

Storage facility	MC%	100 Seed weight (gm)	CHO content%	Protein content%	Fat content
Earth port	10.29^{b}	44.67^{cd}	54.24^{f}	16.51^{e}	6.05^{a}
Plastic bucket	13.02^{a}	44.00^{cd}	54.23^{f}	17.14^{d}	4.28^{f}
Polyethylene sacks	9.12^{c}	42.63^{d}	55.87^{d}	19.82^{a}	4.06^{g}
Jute bag	7.28^{c}	46.13^{bc}	60.69^{b}	16.53^{e}	4.31^{e}
Basket	4.09^{f}	46.92^{b}	64.76^{a}	17.17^{d}	4.80^{d}
Polypropylene bag	7.75^{d}	45.45^{bc}	57.96^{c}	17.41^{c}	5.54^{c}
Deep freezer (control)	10.10^{b}	52.72^{a}	55.82^{e}	17.59^{b}	5.73^{b}
Mean	8.88	46.07	57.65	17.45	4.96
S.e ±	0.143	0.65	0.01	0.04	0.01
CV (%)	3.25	2.83	0.02	0.53	0.01

Note: Means followed by the same letter(s) in the same column are not significant at $P \leq 0.05$

CHO = carbohydrate, MC = moisture content.

Appendix 6. Analysis of variance table for germination percentage under field conditions.

Source of variation	df	Sum of square	Mean square	F value	Probability
Replication	3	99.00	33.000	1.0693	0.4297
Storage (M)	2	255.056	127.528	4.1323	0.0744
Error	6	185.167	30.861		
Priming (S)	2	718.389	359.194	22.2849	0.000
MS	4	27.444	6.861	0.4276	
Error	18	288.833	16.046		
Total	35	1573.889			

CV 40.28%

M = Main plot treatment

S = Sub plot treatment