This is to certify that the

dissertation entitled

THE INHERITANCE OF COOKING TIME AND CHEMICAL AND AGRONOMIC TRAITS IN SEEDS OF DRY BEAN (Phaseolus vulgaris L.)

presented by

Frank Martin Edward Elia

has been accepted towards fulfillment
of the requirements for

Ph. D. degree in Crop and Soil Sciences

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Date Sept. 6, 1996

THE INHERITANCE OF COOKING TIME AND CHEMICAL AND AGRONOMIC TRAITS IN SEEDS OF DRY BEAN *(Phaseolus vulgaris L.)*

By

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Crop and Soil Sciences

1996

ABSTRACT

THE INHERITANCE OF COOKING TIME AND CHEMICAL AND AGRONOMIC TRAITS IN SEEDS OF DRY BEAN *(Phaseolus vulgaris L.)*

By

Frank M. E. Elia

Cooking time of dry bean *(Phaseolus vulgaris L.)* influences consumer utilization of the crop in countries where beans are a staple in the diet and are cooked in open kettles and stoves. Since dry beans usually require a long cooking time to render the grains palatable, their cooking time determines household fuel needs.

This study was undertaken to determine the inheritance and gain from selection for cooking time, water absorption, protein and tannin content, seed weight and maturity in dry bean. Fifteen genotypes from the Andean gene pool and one form the Middle American were mated in a North Carolina design II scheme. The thirty two progenies from the mating and sixteen parents were grown during the 1993 short rain and the 1994 long rain growing seasons at the Crop Museum and the Morning Site farms in Morogoro, Tanzania. Estimation of genetic variances, heritabilities, and response to selection for the traits were made. Total genetic variance was partitioned into additive and non additive components. Highly significant

differences were observed among progeny for the traits studied. Genetic variance was mostly additive. The single seed descent procedure or pedigree breeding method may be an effective breeding strategy for improving the traits because of their high heritability. Although recurrent selection for general combining ability (RSGCA) or reciprocal recurrent selection (RRS) would lead to the accumulation of favorable alleles with additive effects in the population, the efficiency of recurrent selection would be low because of the slow response to selection.

Beans representing the parents of the Design II mating scheme were stored for nine months to determine the effect of storage on the expression of cooking time, water absorption, and seed germination. Seed germination is important to farmers because they store remnant seed from one year's crop to plant the subsequent year. Results showed significant genotypic differences among the entries.

The influence of accelerated aging on cooking time indicated that genotypes that maintained a short cooking time, and high water absorption and seed germination after extended storage could be identified rapidly for use as parents for population improvement.

Note: This dissertation is presented as a series of two papers written in the style and format required by *Crop Science* and, the *Journal of the American Society for Horticultural Science.*

This work is dedicated to

my father, the late

Martin Edward Elia.

ACKNOWLEDGMENTS

I wish to express my deepest gratitude to Dr. George L. Hosfield, my major professor, for his constant guidance, encouragement and valuable suggestions during the course of my work at Michigan State University. He has been a source of happiness and unlimited stimuli to my professional growth in my field through the many academic challenges he posed to me. His sense of humor and leadership qualities enabled me to tap his rich knowledge ranging from the basics in leadership to the specifics in academics.

Special thanks go to members of my guidance committee Dr. James D. Kelly, Dr. Eunice F. Foster, and Dr. Mark A. Uebersax, Dr. Kelly has been helpful in suggestions and references relating to plant breeding. Dr. Foster has selflessly shared her experience in the MSTAT-C program that I used in the last two chapters. Dr. Uebersax provided useful suggestions and literature that were incorporated in the last two chapters of my dissertation. I would also like to thank Dr. Gill, for his assistance on some statistical work in the last two chapters. Dr. Harvey Dicks, a visiting professor from

South Africa will always be remembered for the invaluable help he provided me at the time I needed it most for the statistical analyses and estimation of components of variance using the GENSTAT software program.

Gratitude is expressed to the African American Institute (AFGRAD III scholarship), and the Bean Cowpea CRSP for their financial support during my studies at MSU. Special thanks are extended to the Rockefeller Foundation that provided financial support which enabled me to conduct the research in Tanzania.

I would also like to thank the University of Dar Es Salaam for granting me a study leave and retaining my teaching position there, while I pursued my Ph.D. studies at MSU. Gratitude is expressed to Sokoine University of Agriculture for providing me with research facilities that enabled the successful completion of this work. I am also grateful to MSU for believing in me and my research, and awarding me the prestigious Thoman fellowship to help me contribute to the alleviation of hunger and poverty in my country and other developing countries.

Sincere thanks go to Crop and Soil Science graduate students with whom I had the privilege to work together and their constant assistance. Thanks also go to Ollie Trotter who spent long hours assisting in data entry and Karen Sussman and Mark Bitman for the final touches in my thesis. Appreciation

and gratitude is expressed to all my friends who selflessly provided love, moral support, and encouragement throughout my study. Special thanks go to Dr. Brian Brunner, Dr. Imru Assefa, and Dr. Elias Sabry, Sandra Jones, and Zipporah Agheneza.

Lastly but not least, I would like to thank and salute my mother Wintyapa, my wife Grace and my children Alfred, Emmanuel and Ester for their unwavering support, tolerance and encouragement during all my student years at Michigan State University.

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GENERAL INTRODUCTION

The availability and affordability of foods which supply an adequate amount of proteins and calories to human diets are of major concern in developing countries. In many poorer countries of Asia, Africa and Latin America, rice (*Oryza sativa* L.), maize (*Zea mays* L.), millet (*Pennisetum americana*), sorghum (*Sorghum bicolor*), banana (*Musa sapientum*), cassava (*Manihot esculenta* Crantz), yams (*Dioscorea cayanensis*), sweet potatoes (*Ipomea batatas* L. Lamb) and cultivated potatoes (*Solanum tuberosum*) are dietary staples. Although these crops are low in protein, they can supply most of the daily energy requirements to consumers. Since meat is scarce and expensive in developing countries, consumers rely heavily on foods from plant sources for their source of protein, vitamins and minerals.

Dry bean *(Phaseolus vulgaris L.)* is an important source of dietary protein and calories for persons in Africa and Latin America. Although beans are low in sulfur amino acids, the bean protein deficiencies are supplemented by cereal protein which is rich in cysteine and methionine. Bean-cereal mixtures offer a balanced protein and energy

diet for consumers. Although beans constitute a large part of the daily diet of consumers in Africa and Latin America, the crop is under utilized as a staple food. One of the factors that detract from the utilization of beans by consumers in developing countries is the long time required to cook them to a desired softness for eating. In developing countries in Africa and Latin America, firewood is the primary source of fuel for cooking beans. The prolonged cooking time required for beans often causes an excessive use of fuel wood which exacerbates deforestation in the countries. In Eastern Africa, fuel wood requirements are largely determined by how often beans are cooked and the cooking time required to render them to desired softness for eating (Shellie-Dessert and Hosfield, 1990). Hence, some consumers due to frequent use of different genotypes are able to distinguish slow cooking genotypes from fast cooking ones in the market by such characteristics as seed size, color and seed coat appearance. They usually purchase beans known to cook more rapidly.

In the Eastern and Central Africa countries of Rwanda, Tanzania, Malawi, Kenya and Uganda, the demand for fuel wood far exceeds the supply (Howe and Gulick, 1980; Bart, 1981); consequently, deforestation far surpasses any

afforestation programs (Sirven, 1981). The scarcity of firewood in bean consuming countries of Africa has made the reduction in resources to prepare beans for eating an important economic consideration (Shellie-Dessert and Hosfield, 1991). Consumers in rural areas in these countries avail themselves of a wide selection of unimproved local bean land races to meet their preferences. Although beans are available there is a cost in terms of human and physical resources because of the relatively long cooking time required by beans compared to most other plant foods. The development of bean cultivars with cooking times shorter than the cultivars currently grown for consumption could be a means to conserve fire wood within the region.

In many studies involving cooking of dry beans the seeds are often soaked in water to improve the hydration characteristics of the seeds for uniform cooking. The amount of water absorbed by beans during soaking prior to cooking may be indicative of the amount of time required to render them to the desired softness for eating (Nordstrom and Sistrunk, 1977, Jackson and Varriano-Marston, 1981, Castellanos *et al.*, 1995).

The storage of dry bean under tropical conditions of high temperature (>30^{0}C) and humidity (>75% RH) typically results in deterioration of seed quality. Moreover, beans

become "hard-to-cook" and require a prolonged cooking time to render the seeds palatable, inactivate heat labile antinutrients, and permit the digestion of starch and protein (Jaffe, 1973). In addition to the increased energy required during preparation, hard-to-cook beans have an inferior nutritional quality and poor acceptance by consumers (Stanley and Aguilera, 1985).

The most important nutritional factor in beans is protein. However, tannin can effectively cross-link with protein and other macromolecules through phenolic hydroxyl and other reactive groups characteristic of tannin molecules (Kakade, 1974). The cross-linking of tannin with protein has been shown to adversely affect the nutrition in animal studies (Kakade and Evans, 1966; Lindgren, 1975; Rannenkamp, 1977).

Traits such as seed weight and maturity are of importance to consumers in East Africa. Seed weight is a central yield component. Adams and Reicosky (1975) reported regression coefficient ($b = 0.99$) between seed weight and yield in dry bean but not for maturity. Moreover, maintaining a preferred seed weight is an important consumer preference characteristic.

The presence of genetic variability is a major prerequisite for making genetic improvement for

quantitative traits in a crop. Genetic variability arises from differences in the assemblage of genes among parents. Selection in segregating generations following hybridization is the current practice to make genetic advance for metric traits in dry bean. However, the basis for selection of a trait depends on a knowledge of its inheritance and heritability.

Bean cultivars that take a short time to cook to a palatable texture have been reported (CIAT, 1986; Shellie, 1986). The utilization of dry bean can be increased in diets of the consuming population in Eastern and Central Africa through plant breeding. Breeding strategies should be directed to the development of cultivars with a relatively fast cooking time, a rapid water uptake during soaking, a low tannin content, protein content between 23-26%, 90 days maturity, and with seed weights between 30-40 g/100 seed.

The objectives of this research were to: (1) determine the inheritance of bean cooking time, water absorption, protein and tannin content, 100-seed weight and maturity, (2) identify traits that are useful for indirect selection of cooking time, (3) measure the effect of accelerated aging on dry bean cooking time and (4) evaluate cooking time, and germination percentage of bean

seed stored for nine months.

GENERAL LITERATURE REVIEW

The dry seeds of common bean *(Phaseolus vulgaris L.)* are an important staple for people in Central and South America and East and Central Africa where animal protein is too costly for the average income consumer. On a dry weight basis, beans have a high protein content, twice that of cereals (Goodhart and Shils, 1980). Beans are also relatively rich in lysine and threonine (Patel *et al.*, 1980; Sahasrabudhe *et al.*, 1981) although deficient in the sulfur amino acids. Beans proteins complement cereals which are deficient in lysine. Beans are an important source of minerals such as iron and calcium, and the water soluble vitamins, like niacin, thiamine, folic acid and riboflavin (Tobin and Carpenter, 1978; Fordham *et al.*, 1975).

Despite the fact that Latin America is the leading producer of dry beans (30%) of the world's 14 million tons/year (van Schoonhoven and Voysest, 1993), the mean per capita consumption is 13.3 kg/capita. Africa produces 10% of the world production but has a mean per capita consumption of 31.4 kg with some countries like Rwanda consuming up to 50.6 kg. Beans in East Africa provide up to 60% of the dietary protein requirements to consuming populations.

Cookability of bean. The definition of 'Cookability' encompasses characteristics that have strong organoleptic appeal to consumers. The time it takes to cook individual grains to a palatable texture, cooked bean wholeness, color, splitting and clumping of beans, and the color and consistency of the broth comprise Cookability. Among the constraints limiting dry bean utilization is the prolonged cooking time of beans and the confounding effect of hardening in storage. Prolonged cooking increases firewood usage and places an economic burden on consumers in areas where firewood is scarce. The aspects of Cookability, the amount of softening and rate of softening are related to cellular breakdown in cell walls and the solubilization of starch. According to Silva *et al.*, (1981), bean softening does not follow first order kinetic reactions. Sefa-Dedah and Stanley (1979) found that water absorption influenced cookability and that it was determined by seed coat texture. Castellanos *et al.*, 1995 reported a negative relationship between cooking time and water absorption and that 'hard shell' rather than the 'hard-to-cook' phenomenon is the main factor delaying the cooking time of newly harvested beans. However, Burr *et al.*, (1968) and Molina *et al.*, (1975) found no correlation between water uptake and Cookability.

Various methods have been employed for the assessment of cooking time. Some investigators (Mwandemele *et al.*, 1984) counted the number of split seed coats after a specified cooking time of two hours. Edmister, (1990) applied pressure to cooked beans placed between the index finger and the thumb and related the force required to rupture the seed to a tactile evaluation. A cut-off point of 450 mean gram force based on a 100 bean sample was determined by squeezing individual cooked beans between the thumb and the index finger and comparing tactile sensations with the grams force registered by those beans using the puncture test cell. A number of instruments have been used to evaluate dry bean cooking time. The Instron (compression type), maturometer (penetration type) and the Mattson pin drop cooker have been used. The pin drop cooker method for assessing cooking time is the most preferred because it is economical, reliable and can evaluate 100 seeds simultaneously.

Dry bean Cookability has been found to be related to seed storage. Observations (Burr *et al.*, 1968) showed that some bean cultivars, when stored for periods of six months or longer become hard-to-cook. A number of factors have been suggested for the hardening phenomenon including lignification of the middle lamella, seed coat thickness

and the level of phytase, calcium and magnesium in the seed. Cooked bean softening is associated with the breakdown of the middle lamella in the seed which allows separation of the cells. Jones and Boulter (1983 a,b) observed that the hard-to-cook phenomenon might be due to reduced water imbibition and reduced pectin solubility resulting in a reduced cell separation rate during cooking. These researchers suggested that the reduced water imbibition and reduced pectin solubility act synergistically. Accompanying symptoms include solute leakage during soaking due to membrane breakdown, phytin catabolism and pectin demethylation. Moscoso *et al.*, (1984) investigating the hard-to-cook phenomenon in red kidney beans, found that the apparent rate constants of cooking decreased with increasing time storage. Jimenez *et al.*, (1989) also observed that when some bean cultivars were stored under high temperature and high relative humidity for nine months, the time required for cooking increased three-fold.

It has been observed that the cooking time of beans is under genetic and environmental control that often interact unpredictably (Shellie and Hosfield, 1991). Hard seed coats especially noticeable in small seeded cultivars (100 seed weights of 18-25 grams), prevent seeds from imbibing water

during soaking leading to prolonged cooking times. It is interesting that the development of a hard seed coat in a cultivar determines the length of time it will take to cook the bean. The hard shell development has been shown to be under genetic and environmental control (Rolston , 1978; Stanley and Aguilera, 1985). Copeland, (1976) established that the heritability of the hard seed coat was high. However, this trait was controlled by a single recessive allele (Kyle and Randall, 1963). Hard seed coat also develops under adverse storage conditions of temperature and relative humidity. Unlike beans with hard seed coats, hard-to-cook seeds imbibe water, but fail to soften adequately during cooking (Burr *et al.*, 1968). Among the early information on the hardness of beans was that of Gloyer, (1928) in which he divided it into hard shell (the condition in which the seed fails to imbibe water) and the hard-to-cook condition (hardness produced by enzymatic action in which the cotyledons darken and harden and become hard to cook. Castellanos *et al.*, 1995 reported that the hard shell plays a major role in extending the cooking time of beans with an initial moisture content of 90 g Kg^{-1} or less.

Protein Content. The protein quality of a food is based on the relative contents of the amino acids. The limited

utilization of a protein is thus based on the essential amino acids present in the lowest amount. Bean proteins though rich in lysine, are limited in the sulfur containing amino acids, cysteine and methionine (Patel *et al.*, 1980; Sahasrabudhe *et al.*, 1981; Sathe *et al.*, 1981). Bean proteins have low digestibility, between 48%-62% compared to meat proteins which range between 82%-86%. The presence of antinutritional factors, lectins (Jaffe, 1973; Barampama and Simard, 1994;) contribute to decreased protein utilization.

Percent protein has been found to be negatively related to seed yield (Leleji *et al.*, 1972; Kelly and Bliss, 1975;) thereby making it difficult to improve protein content through breeding and selection (Bliss and Brown, 1983). Sullivan and Bliss, 1983 reported that it is possible to have an effective breeding strategy either through simultaneous selection for both protein and seed yield employing some form of a selection index, or using tandem selection first for high yield and then for high seed protein. Heritability estimates for seed protein percentage range from 0.10 to 0.85, (Porter, 1972; Leleji *et al.*, 1972; Kelly and Bliss, 1975; Evans and Gridley, 1979;). In a study conducted by Emebiri, 1991, the inheritance of protein content in bean has been shown to be

both controlled by both additive and non additive gene effects. The broad sense heritability of protein content ranged from 0.7 and 0.8 .

Tannin Content. It has been shown that condensed polyphenols in the seed coat (tannins) decrease protein digestibility (Elias *et al.,* 1979) and that high tannin reduces nutritional value by binding with protein (Haslam, 1979; Griffiths and Moseley, 1980). Tannins in beans are located mainly in the testa of colored seeds (Ma and Bliss, 1978a ; Bressani and Elias, 1980; Deshpande *et al.*,1982). Tannins are polyphenolic compounds (high molecular weight 500 to 3,000) that are capable of precipitating proteins to form complexes that are not easy to digest (Griffiths and Moseley, 1980; Aw and Swanson, 1985; Elias *et al.,* 1979; Reddy *et al.,* 1985; Jansman, 1995). Apart from inactivating digestive enzymes and increasing protein insolubility, tannins also lower feed efficiency which lead to a growth depression in experimental animals (Lindgren, 1975; Rannenkamp, 1977; Reddy *et al.,* 1985). Tannins are categorized into hydrolyzable tannins which can be degraded to sugars and phenolic carboxylic acids when treated by acids or alkali, and condensed tannins which are not readily degraded by simple chemical treatments (Haslam, 1979). The tannin content of dry bean seed ranges from 0.0

to 2.0% (Sgarbieri and Garruti, 1986; Reddy *et al.*, 1985). Quantitative variability for tannin has been reported (Ma and Bliss, 1978 a; Lyimo *et al.*, 1992). These researchers showed that beans with colored seeds contained more tannin while tannins were not detected in beans with white seed coats. There was no strong relationship between tannin content of colored seeded genotypes and seed coat color. From a survey of literature on tannin (Allard, 1953; Picard, 1976; Rannenkamp, 1977; Ma, 1978 a,b; Marquardt *et al.*, 1978; Crofts *et al.*, 1980; Dalrymple *et al.*, 1984;), it appears that seed coat color and tannin content are independently inherited. High broad sense heritabilities of 0.80 to 0.97 were obtained by Ma, 1978a when he analyzed populations resulting from crosses between parents that differed in seed coat color and tannin content. He also observed that low tannin was dominant to high tannin. However in a diallel analysis, Wassimi et al., found high tannin dominant to low tannin. Moreover significant estimates of general and specific combining ability were associated for tannin expression.

Maturity and Seed weight. In East and central Africa bean growing regions where short and long rain growing seasons occur, bean growers prefer growing early maturing, large seeded cultivars. The short rains occur between the end of

September to December while the long rains start in March and end in June. The short rain growing season exerts pressure on the farmers who have to plant and harvest within the period. Breeding for large seeded and early maturing genotypes is therefore important in the East and central Africa bean growing region to enable farmers to maximize the short rains.

In their experiment to determine heritability and correlation of biomass, growth rate, harvest index and phenology to the yield in common bean, Scully *et al.* , (1991) reported broad sense heritabilities of 0.96 and 0.87 for days to maturity and seed weight respectively. Cerna and Beaver, (1990) studying inheritance of early maturity in indeterminate dry beans reported a narrow sense heritability of 0.31 to 0.63. Singh *et al.* ,(1990) reported the estimated gain in selection values of < 3% and > 15% for maturity and seed weight respectively.

Beans Stored under adverse conditions: It is recognized that storage environments can profoundly affect bean cookability. Prolonged storage of beans often leads to a long cooking time while freshly harvest beans require shorter cooking time. Jackson and Varriano-Marston, 1981 found that freshly harvested beans cooked for 31 min while beans stored for one year took 45 min to cook. A loss of

nutritive value occurred with prolonged storage of beans (Nordstrom and Sistrunk, 1979).

Beans stored at high relative humidity become increasingly darker and exhibit firmer textures. Beans stored at a relative humidity of 10% or lower maintained their cookability for up to two years (77°F) while those stored at relative humidities above 13% showed a significant deterioration in flavor and textural characteristics within the six months (Morris and Wood, 1956). Storage at high moisture for four months resulted in longer cooking (Morris, 1963). Snyder, (1936) showed that in addition to storage time, temperature and humidity are factors that also lead to the development of hardness in beans.

Bedford (1972) reported that beans stored at low moisture of 8-9%, and temperature of between 68-81°F for four years maintained their cooking quality while those stored at high moisture 15-18% showed a significant increase in cooking time. Unlike the pectase enzyme mechanism leading to the formation of calcium pectate, the increase in seed toughness and hence the increase in cooking time has been suggested to be due to a non-enzymatic entropy increasing pectic chain entanglement that becomes dominant only at certain critical values of water

activity (Schwimmer, 1980). Significant changes in the bean proteins stored in high moisture levels (11-14%) at 90°F were shown to be associated with the cookability of aged dry beans (Rockland, 1963). Cookability studies with sanilac, pinto and large lima beans stored at 70° and 90°F, 6.5-14.4% moisture, for eleven months showed that those at 90° and high moisture required up to five hours to cook to eating soft after the first nine months of storage (Morris, 1963). When seeds with seed coats and those without seed coats were compared in an experiment, Morris, (1963) concluded that seed coats were responsible for most of the increases in cookability in high moisture seeds due to an in increase in lipid acidity. Burr *et al.*,(1968) like Morris (1963) reported loss of cooking time in beans stored at high relative humidity and temperature in addition to seed darkening. Scanning electron microscopy studies (Rockland *et al.*,1973, Rockland and Jones, 1974 and Sefa-Dedah *et al., 1979)* showed that thermal degradation occurred when freshly harvested seeds were cooked. Observations indicated that thermal degradation of the middle lamella was more difficult in cooked seeds stored at high temperature and moisture than in cooked seeds that were freshly harvested.

A number of studies associated the development of hard

seed coat or the hard to cook phenomena to loss of nutritive value. Sada, (1980) reported that dark red kidneys stored at 30°C and 80% RH for eight months, showed decreased contents of total soluble carbohydrates, stachyose and raffinose, tryptophan, lysine, histidine and methionine. Burr *et al.*, (1968) reported that firmer seeds took longer to cook resulting in increased destruction of thiamine and a decrease in biologically available methionine and cysteine. Antunes and Sgarbieri (1979) reported a decrease in the protein efficiency ratio values and protein digestibility. Molina *et al.*, (1976) showed that short heat treatments(2.5 and 10 min. at 121°C and 10,20 and 30 min under steam (98°C) on black beans prior to storage at 25°C, 70% RH for nine months, reduced the development of seed hardness although germination capacity was affected (Molina *et al.*, 1976). This research showed that lignified protein increased during seed storage. Molina and Bressani (1977) attributed similar increases to the migration of polyphenolic compounds from the seed coat to the cotyledon. A study on the effect of different conditions on the development of seed hardness or the hard-to-cook condition in beans was conducted by Mejia (1979). This researcher used seeds with moisture contents of 9, 13, and 17% that were in equilibrium with relative humidities

of 40, 60, and 80%, and stored for six months at temperatures of 4, 20 and 36°C. Mejia (1979) observed a decreased tannin content with higher storage time and greater bean hardening at higher storage temperatures. Jackson and Varriano-Marston (1981) conducted accelerated storage tests which showed that black beans stored at 41°c and 100% RH for 7 to 14 days required longer times to cook. Decorticated beans of the same lot were observed to cook in about half the time. Bressani and Elias (1980) summarized the factors responsible for the development of hardness in legume seeds as influenced by : i) cell structure changes; ii) condensation of tannins that may pose an obstacle to the free permeability of water; iii) reactions between tannins and proteins resulting from adverse storage of the legume seeds; iv) reactions within the cotyledons of organic compounds and calcium and magnesium; v) reactions the cotyledons that are enzymatic.

Srisuma *et al.*, (1989) reported that storage of seeds at 20°C and 73% RH and 35°C and 80% RH induced changes of phenolic acid and promoted the development of the hard-to-cook beans. This work reported that large increases in the amount of hydroxycinnanic acid and increased hardening. Rozo *et al.*, (1990), Hernandez-Unzon and Ortega-Delgado (1987), Shehata *et al.*, (1984) and Hentges *et al.*, (1991)

conducted storage studies on the hard-to-cook phenomenon in beans. Paredes-Lopez *et al.,* (1989) reported an increase in the compact packaging of cotyledon cells and an increase in water activity but a decrease in water absorption capacity in stored beans. Mafuleka *et al.,*(1991) reported increased hardening and higher pectin methylesterase activity with storage. Uebersax (1972) observed that the influence of increased temperature storage became greater at higher relative humidity.

Accelerated Aging. Accelerated aging as a test for seed quality was first developed by Delouche, (1965a) as a method of predicting the viability and germination of seed lots kept in storage. This test was later found to be important as a vigor test (Herrera, 1969; Haya, *et al.,* 1978; Wilson *et al.,,* 1992;). The storage potential of seed lots could be predicted by Delouche (1965b) based on their germination responses after a short exposure to high temperature and high relative humidity. By using the accelerated aging technique, Delouche(1965b) found a high correlation among seed lots of crimson clover *(Trifolium incarnatum L.)* and tall fescue *(Festuca arundinaceae Schreb)*. Pil (1967) found that accelerated aging was an effective test for evaluating the storability of alfalfa *(Medicago sativa L.)*, corn *(Zea mays L.)*, wheat *(Triticum*

aestivum L.) and cotton *(Gossypium hirsutum L.)*. Mercado (1967) found the method to be effective for determining the rate and progress of deterioration of cotton seed during storage. Mercado, (1967) suggested using accelerated aging to supplement the standard germination test to evaluate field performance potential for seed lots. Unlike other researchers Herrera, (1969) reported the possibility that heritable factors may partially control the vigor and deterioration rate of wheat seed lots. He further pointed out that the accelerated aging technique can be used by plant breeders for the selection of breeding materials in a population improvement program. Other researchers Tippayaruk, (1975) while evaluating the usefulness of accelerated aging technique for selecting cotton seed for improved vigor in a segregating population, concluded that F3 cotton seeds that were accelerated aged, failed to show heritable differences in seed vigor in the F4 generation. Wilson *et al.,* (1992) found a high correlation between accelerated aging test and final stand of shrunken-2 sweet corn seed. A similar response was also observed earlier by Baskin, (1970) in peanuts indicating that germinative responses are highly correlated with plant growth and development. Since storability is one aspect of vigor, accelerated aging tests are very effective in the

evaluation of seed vigor (Islam, 1976).

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CHAPTER ONE

INHERITANCE OF COOKING TIME, WATER ABSORPTION, PROTEIN AND TANNIN CONTENT, MATURITY AND SEED WEIGHT IN DRY BEANS *(Phaseolus vulgaris L.)*; THEIR RESPONSE TO SELECTION AND INTERRELATIONSHIPS.

ABSTRACT

This study was conducted to determine the inheritance of cooking time, water absorption, protein and tannin content, maturity and seed weight in a population of dry bean representing the Andean gene pool. The response to selection for the traits and their interrelationships were also determined. Sixteen parents that differed morphologically, phenologically, and agronomically were crossed in a North Carolina design II mating scheme.

General combining ability for males and females were highly significant for all traits. There was a preponderance of additive genetic variance in the population, influencing trait expression. Because of the small gains expected from selection, the use of recurrent selection to improve the traits may be inefficient. It may be feasible to improve the traits under selection using single-seed descent or the pedigree method. Since beans are released commercially as pure lines, pedigree breeding should be considered for improving the traits under study.

Significant correlations were observed between cooking time and maturity, cooking time and tannin content, and cooking time and water absorption. Data indicate that the amount of water absorbed prior to cooking may be indicative of the amount of time required to render them soft for eating. Water uptake may provide a rapid and indirect method for screening genotypes for cooking time.

INTRODUCTION

Dry bean *(Phaseolus vulgaris L.)* is a major source of calories and protein for consuming populations in many African and Latin American countries. Consumers generally eat beans with cereals in developing countries because the bean protein amino acids complement the cereal protein amino acids in cereal-legume diets (Bressani, 1975). Although beans constitute a large part of the daily diet of low and middle income families in many developing countries, the crop is under-utilized as a staple food. One of the factors leading to the under-utilization of dry bean as a food is the prolonged time required to cook beans to a point where they can be digested and their nutrients assimilated (Deshpande, *et al.*, 1982).

In many developing countries, firewood is the primary source of fuel for cooking beans. However, the long cooking time required for beans compared to other foods has a cost in terms of human and physical resources. The cost is reflected in an excessive use of fuelwood which exacerbates deforestation.

In the Eastern and Central African countries of Rwanda, Tanzania, Malawi, Kenya, and Uganda, the demand for fuelwood far exceeds the supply (Howe and Gullick, 1980;

Bart, 1981). Deforestation due to the excessive cutting of trees for fuel renders afforestation programs ineffective (Sirven, 1981). The scarcity of fuelwood in Eastern Africa has made the reduction in resources to prepare beans for eating an important economic consideration (Shellie-Dessert and Hosfield, 1991). Bean varieties with fast cooking times may be a means to conserve fuelwood.

Beans are generally soaked before cooking. Soaking improves the hydration characteristics of the seed so beans cook uniformly. Well soaked beans may also cook more rapidly than ones containing less water. A number of studies in dry bean have indicated an indirect relationship between cooking time and water absorption (Sefa-Dedah, 1979; Mora, 1982; Moscoso *et al.*, 1984; Hernandez-Unzon and Ortega-Delgado, 1987; Paredes-Lopez *et al.*, 1989;, Edmister *et al.*, 1990, Castellanos *et al.*, 1995). Since the degree of hydration of beans prior to cooking may be indicative of the amount of time required to cook them to a palatable texture, the amount of water absorbed by beans may provide an indirect method for screening genotypes for cooking time. Breeders routinely use indirect selection for improving traits when the procedure is more rapid and cost efficient for selecting a particular trait than the protocol necessary to select the trait *per se*.

In addition to cooking time and water absorption, protein and tannin content, maturity and 100-seed weight, are other traits which influence consumer utilization. Cooking time, water absorption, protein and tannin content do not fall into discrete phenotypic classes but show a continuum through a range of expression from a minimum to maximum for the respective trait. The phenotypic expressions of these traits are most often determined by measurement. The current practice to make genetic advances for traits of a metric nature in dry bean is selection insegregating generations following hybridization. However, the efficiency of selection for trait improvement depends on a knowledge of its genetic control and the degree to which the environment influences trait expression. Shellie-Dessert and Hosfield (1991) reported on genetic variability for cooking time of dry bean but there is limited published information on the inheritance of this trait.

An increase in the utilization of dry bean in the diets of consuming population of Tanzania and other major bean consuming countries of the region might be achieved by reducing the cooking time and tannin content and increasing the protein content through testing and selection. The hypothesis for the study was that cooking time in dry bean was highly heritable, and that significant genetic

variability existed in dry bean to develop acceptable cultivars that cook rapidly and conserve fuelwood. Specific objectives were to: (1) estimate additive and dominance variance for cooking time, water absorption, protein and tannin content, seed weight, and maturity in a genetic population of Andean dry bean, (2) estimate the heritability and degree of dominance for the traits and use this information for developing selection strategy, (3) determine the expected gain from selection for the traits and (4) calculate correlations among the traits and evaluate the feasibility of using other seed characteristics as a rapid and indirect method of screening for cooking time.

MATERIALS AND METHODS

Genetic material.

Sixteen genotypes of dry bean that differed morphologically, phenologically, and agronomically (Table 1) were used as parents in the study. Except for 'Sierra', the parents were representative of the Andean gene pool of *P. vulgaris* and represented preferred grain types in Eastern and Central Africa. 'Sierra' is a high yielding bean and representative of the Middle-American gene pool with the medium pinto market class seed size (Table 1).

The genotypes were all adapted to the bean production areas of East and Central Africa. Seeds of the entries are highly favored by consumers in the region because of their meat-like appearance and thick broth/soup quality after cooking. The appearance of the bean complements a stiffened porridge-like staple food prepared from corn called "ugali" in Kiswahili (East Africa), "nsima" in Malawi, "saza" in Zimbabwe, "bogobe" in Setswana, "papa" in South Africa and "fufu" in Nigeria.

Table 1: Description, and combined data for chemical contents, maturity, seed weight, water absorption, and cooking time of 16 dry bean accessions used as parents in a North Carolina Design II mating scheme and grown at two locations in the Morogoro region, Tanzania in the short rain (1993) and long rain(1994) growing seasons.

Entry	Maturity	Protein content	Tannin content	Seed weight (100)	Water absorption	Cooking time
	-days-	g kg^{-1}	mg $100g^{-1}$	g	g kg^{-1}	min
Var 11	72.0	245.0	334.5	35.7	1061.0	25.5
Nyirakizungu	70.5	249.5	307.5	40.3	941.0	30.5
Lyamungu	82.3	220.0	377.0	34.6	786.0	41.5
Kilyumukwe	75.0	253.0	408.5	47.4	699.0	46.5
Yellow eye	68.6	241.0	353.0	36.9	819.0	36.3
UAC 221	79.8	258.0	499.0	45.3	542.5	52.3
TMO 959	82.0	191.5	387.5	34.7	765.0	43.5
Kalima	72.0	204.5	317.5	45.3	991.5	28.3
CIAT 3005	76.0	209.0	479.0	36.8	588.5	49.8
P.I.605621	80.8	253.5	322.5	47.9	1030.0	28.3
GLPX 1125	73.5	260.0	423.0	44.7	649.0	49.0
Jacob's cattle	70.5	216.0	336.5	46.3	818.0	33.5
Montcalm	70.3	245.5	417.5	44.2	675.0	47.3
NY 99	78.8	232.5	401.5	33.1	732.5	45.5
Sierra	68.5	218.5	352.0	28.6	860.0	35.3
Diacol Calima	72.5	253.0	364.5	45.4	805.0	37.0
Mean	74.6	234.4	380.1	40.5	758.3	39.4
LSD (0.05)	3.5	30.3	23.3	1.3	20.0	2.0
CV(%)	3.9	10.9	5.2	4.1	2.0	4.2

Procedures.

The 16 genotypes were intermated in the greenhouse in the fall of 1991 using a North Carolina Design II (Comstock and Robinson, 1948) mating scheme. Eight genotypes were randomly chosen as male parents and the remaining eight genotypes were used as female parents. The genotypes used as male parents were not crossed to each other nor were the genotypes used as females crossed to each other. In order to reduce the number of matings required, two sets were formed. Within each set, each male parent was crossed to four females resulting in a total of 16 crosses per set.

Seeds of the 16 parents were increased, and the 32 F_1 populations were advanced in January, 1992 in a nursery at the University of Puerto Rico substation near Isabela, Puerto Rico on a San Anton clay loam (fine-loam, mixed iso-hyperthemic cumulic haplustolls). Seed of the 48 entries (parents and F_1 populations) were harvested in March 1992, bulked and returned to East Lansing. The 32 F_2 populations were planted in a nursery at the Saginaw Valley Bean and Sugarbeet Research Farm near Saginaw, Michigan in a Misteguay silty clay [fine, illitic (calcareous),frigid typic Haplaquolls]. One hundred F_2 plants from each cross were randomly selected, threshed, and the seed bulked. Because most of the parents were obtained from breeding

programs in Tanzania and other countries in the region of Eastern and Central Africa, the progeny were generally unadapted in Michigan. Hence, the experiments on which data were taken were grown in 1993 and 1994 in Tanzania at the Crop Museum(530 masl) and Morning Site(~1000 masl) research farms in the Morogoro Region. These farms were maintained by the Agricultural University of Sokoine. The soil characteristics at Morogoro are moderately acidic (Appendix Table 1). Rainfall, temperature and solar radiation are given in Appendix Table 22.

The experiments were planted in a randomized complete block design with three replications at each location during the seasons in which the annual bimodal distribution of rain occurs. Because there was not sufficient hybrid seed to plant the experiment at both locations during the short(SR) and long(LR) rainy season, seed of the F_3 was planted at the Crop Museum and Morning Site on September 30th, 1993 during the period of SR and seed produced from this crop (F_4 generation) was planted at both locations in March 10th, 1994 during the LR period. Hence, generation and season effects were confounded. The confounding was not a consideration in the study because the comparison of generations (i.e, F_3 vs F_4) was not made. Moreover, genetic variances can be estimated on populations with any level of

inbreeding.

The field arrangement was based on the assignment of entries to the sets. Although the male groups were originally assigned to each set at random, the integrity of the entries within each set was maintained at each location in each growing season. The four females and four males used as parents of a particular set were included in the planting arrangement along with the 16 F_3 or F_4 hybrid progeny produced by their intermating.

The 48 entries (parents and progeny) making up the two sets were hand seeded into four row plots. Rows were 4 m long and spaced 0.5 m apart. The within row spacing was 0.1 m giving an approximate plant density of 160 plants per plot. On farm practices for herbicide, pesticide and fertilizer applications were followed. Mature plants were harvested by hand from the middle two rows of individual plots when the pods were dry enough to be threshed.

Trait evaluation. Prior to harvesting the plots, physiological maturity was estimated on each plot as the number of days from planting until about 90% of the pods in a plot changed from green to pale yellow or brown(no additional accumulation of dry matter). The freshly harvested seeds from each plot were sun dried for two

weeks. After drying the seed, they were stored in sealed plastic buckets and held at 20°C and 70% RH until evaluated (Shellie, 1990).

Protein content was determined on 30g of raw beans that were ground to 40 μm particle size with a Udy Cyclone mill(Boulder, Colorado). Protein percentage was estimated on the bean flour by the micro-Kjedahl method (Association of Official Analytical Chemists, 1975). The nitrogen content of each sample was multiplied by 6.25 to calculate percent protein.

The vanillin hydrochloric acid method of Burns (1963) was used to estimate tannin content. Since catechin was used as a standard in the tannin assay procedure, tannin content was estimated as catechin equivalent. However in this report, catechin equivalent will be referred as tannin.

The percent water absorption of the entries was determined on triplicate samples of a known weight of 75 seeds from each plot. The seeds were soaked in distilled water for twelve hours at room temperature. The amount of water absorbed was taken as the difference in weight before and after soaking divided by the dry weight of the 75 seed sample.

Bean cooking time was estimated with a 25 seed pin-drop cooker (Jackson and Varriano-Marston, 1981). Cooking time was calculated as the elapsed time from initiation of cooking until 13 of the 25 pins of the instrument had dropped and penetrated seeds in the cooker. Data were taken on triplicate samples for each plot at each location.

Statistical Analysis. Data were collected on both parents and progeny. Data on the parents were analyzed separately using analyses of variance (ANOVA) appropriate to a randomized complete block design RCBD. The progeny data were subjected to ANOVAS appropriate for a (RCBD) pertaining to the Design II mating scheme. The F-tests were straightforward for all sources of variation except for the male and female components (Table 3). An approximate F-test, (Satterthwaite, 1946) was used to test the male and female effects. Mean squares from the ANOVA were used to estimate components of variance pertinent to a Design II mating scheme repeated over environments (Comstock and Robinson, 1948; Cockerham, 1956; Miller *et al.*, 1959; Cockerham, 1963; Hallauer and Miranda, 1988). Replications, locations and genotypes were considered as random effects in the mathematical model used to estimate variance components (Searle, 1971). The values for the variance

components were calculated using the GENSTAT 5 software program (1989).

In the Design II ANOVA, the variability due to crosses was broken-out as variation due to males within sets, variation due to females within sets, and variation due to male x female interaction within sets (Hallauer and Miranda, 1988). Throughout the text, variation due to males within sets, females within sets, and males x females within sets will be referred to interchangeably as GCA males, GCA females, and SCA variation, respectively (Pixley and Frey, 1991). The components of genetic variance were estimated as: $\sigma^2_m = \sigma^2_f = \text{cov HS} = (1/4)\sigma^2_A$, and

$$\sigma^2_{mf} = \text{Cov FS} - \text{Cov HS}_m - \text{Cov Hs}_f = (1/4)\sigma^2_D.$$

Where: σ^2_A ; σ^2_D ; σ^2_m ; σ^2_f ; and σ^2_{mf} are the additive and dominance variance and the variance due to GCA males, GCA females, and SCA, respectively. The Cov HS and Cov FS are the covariances of half sib and full sib progeny.

The degree of dominance governing the genes for trait expression was calculated by the formula: $d = (2\sigma^2_{mf} / \sigma^2_m)^{1/2}$ where d = the degree of dominance.

Heritability in the narrow sense was estimated from the components of variance values pertaining to a Design II mating scheme as: $h^2_N = \sigma^2_A / \sigma^2_{ph}$.

Where σ^2_A is the additive genetic variance and σ^2_{ph} is the phenotypic variance.

The approximate standard error of the narrow sense heritability estimate was calculated as:

$$SE(h^2) = \frac{SE(S^2_f)}{S^2_{pfm}}.$$

Where S^2_f is the variance components due to progeny and S^2_{pfm} is the total phenotypic variance of progeny estimated from variance components (Dickerson, 1969).

Response to selection was estimated from the formula:

$$\Delta G_c = h^2_N D.$$

Where ΔG_c is the genetic gain per cycle, and D is the selection differential. The selection differential is the difference between the mean of the genotypes selected from a population and the overall mean of the population from which they were selected. Since the selection differential can also be expressed as $D = k\ \sigma_{ph}$ where k is the selection differential expressed in standard units and σ_{ph} is the square root of the phenotypic variance (Fehr, 1987), the equation for genetic gain per cycle can be modified by substituting for H^2 and D as follows:

$$\Delta G_c = H^2 D = \frac{\sigma^2_A}{\sigma^2_{ph}}\ k\ \sigma^2_{ph} = \frac{k\ \sigma^2_A}{\sigma^2_{ph}}$$

Phenotypic correlations between the cooking time and maturity, protein, tannin, seed weight and water absorption were calculated using the GENSTAT 5 program.

RESULTS AND DISCUSSION

The means across locations and growing seasons for maturity, protein and tannin content, 100-seed weight, water absorption and cooking time index, of the 16 parents are given in Table 1. Seed weights of the parents ranged from 28.6 to 47.4 g 100^{-1} seeds. There was a range of 26.8 min in the cooking time between the slowest UAC 221 (52.3 min) and fastest VAR 11 (25.5) cooking parent. The range in water absorption for the 16 parents was 472.5 g kg^{-1} with CIAT-3005 and Var 11 imbibing the least and most water, respectively.

Significant location mean squares were detected for the maturity, protein content and water absorption traits in both the SR and LR growing season and for only tannin content in the SR season. Significant variability between locations led to significant location (L) interactions for some traits. Significant GCA males x L and GCA females x L mean squares were noted for maturity in the SR season; a significant GCA males x L interaction for protein content in the SR season and tannin content in the LR season. The GCA females x L mean square was significant for seed weight in the SR season. Significant variability between sets was observed for water absorption and cooking time in both SR

and LR seasons and maturity and seed weight in the SR season.

Mean squares for GCA males and GCA females were highly significant for the six traits in both growing seasons (Table 2). A design II mating scheme provides two independent estimates of GCA, one due to the female and one due to the male parents (Hallauer and Miranda, 1988). Except for the seed weight trait the mean squares for GCA females were larger than the GCA males. The differences between the two GCA reflected the variability for the trait among the parents *per se* (Pixley and Frey, 1991).

The GCA male and GCA female variance components greatly overshadowed the SCA component indicating that the genetic variance influencing trait expression was preponderantly additive. Although there was a difference in the level of inbreeding of the genotypes grown in the SR and LR seasons, the magnitude of variance components were in good agreement for most traits. The exceptions were the GCA male components for maturity and tannin content and the GCA female components for tannin and cooking time (Table 3). The GCA female variance component for cooking time was about 8 times greater in the LR than the SR season.

When the GCA males and GCA females variance components were combined there was good agreement between the SR and

LR growing seasons for most traits except for cooking time and tannin content. For cooking time the combined GCA males and GCA females component of variance was 3 times greater in the LR growing season than in the SR growing season accounting for 51% (i.e. 15.7 + 35.0) and 17% (i.e. 12.3 + 4.4) of the total variance, respectively (Table 3). The combined variance components of these same sources of variation for tannin content in the SR growing season was twice that in the LR season accounting for 76 and 38% of the total variance, respectively.

Table 2. Mean squares from the combined analyses of variance for maturity, protein, tannin, seed weight, water absorption and cooking time index for dry bean progeny grown at two locations in the Morogoro region, Tanzania during the SR (1993) and LR (1994) growing season.

Source of variation	df	Growing season	Maturity	Protein content	Tannin content	Seed weight (100)	Water absorption	Cooking time
Location, L	1	SR †	1801*	339*	324*	98	383*	278
		LR †	1271*	273*	511	203	275*	213
Sets, S	1	SR	768*	6	94	131*	2663*	1117*
		LR	713	9	77	140	2770*	1281*
S(L)	1	SR	0	1	0	0	1	0
		LR	8	0	1	12	1	5
Reps(S)(L)	8	SR	2	0	1	0	1	3
		LR	7	1	3	1	2	1
Males(S), M	6	SR	75**	16**	31**	211**	315**	62**
		LR	53**	21**	15**	245**	322**	114**
Females(S),F	6	SR	132**	40**	326**	203**	1358**	21**
		LR	144**	50**	164**	233**	1310**	248**
M x F(S)	18	SR	2*	1**	3**	1**	23**	1
		LR	12	1	8**	2**	24**	5**
M(S) x L	6	SR	11**	4**	1	1	0	4
		LR	22	1	17**	4	1	1

Table 2: (con't.)

F(S) x L	6	SR	12**	0	0	2*	1	0
		LR	16	1	7	2	0	2
L(S)(M)(F)	18	SR	3*	0.3*	1	1	1	1
		LR	6	1	9	2	1	1
Error	120	SR	1	0	1	0	1	2
		LR	6	1	2	1	1	2
Total 191								

*, ** Significant at the 5% and 1% probability levels, respectively.
† SR and LR = short and long rain growing seasons.

Table 3. Estimates of variance components scaled to sum to 100 for maturity, protein, tannin, seed weight, water absorption and cooking time for dry bean progeny grown at two locations in the Morogoro region, Tanzania during the SR (1993) and LR (1994) growing seasons.

Source of variation	df †	Growing season	Maturity	Protein content	Tannin content	Seed weight (100)	Water absorption	Cooking time
L	1	SR‡	52.7	56.5	17.5	5.4	4.6	15.0
		LR‡	38.2	42.7	31.2	8.4	3.3	7.5
S	1	SR	17.1	56.5	0.0	0.0	12.1	56.0
		LR	16.6	42.7	0.0	0.0	14.1	33.3
S(L)	1	SR	0.0	0.0	0.0	0.0	0.0	0.0
		LR	0.0	0.0	0.0	0.8	0.0	0.3
R(S)(L)	8	SR	0.2	0.0	0.1	0.0	0.0	0.5
		LR	0.1	0.3	0.2	0.0	0.1	0.0
M(S)	6	SR	7.6	8.5	6.2	46.4	14.0	12.3
		LR	3.1	12.1	0.0	42.5	14.4	15.7
F(S)	6	SR	14.1	26.4	69.9	44.3	63.7	4.4
		LR	14.8	30.4	38.4	40.5	62.6	35.0
M x F(S)	18	SR	0.0	0.7	1.9	0.6	4.1	0.4
		LR	2.7	1.3	0.0	0.5	4.4	2.3

Table 3. (con't.)

M(S) x L	6	SR	1.9	4.3	0.0	0.0	0.0	1.5
		LR	3.7	0.0	3.9	0.7	0.04	0.1
F(S) x L	6	SR	2.1	0.0	0.0	0.6	0.01	0.0
		LR	2.3	0.6	0.0	0.1	0.0	0.3
L(S)(M)(F)	18	SR	1.9	0.9	0.0	0.4	0.0	0.0
		LR	0.0	0.0	13.5	0.3	0.0	0.0
Error	120	SR	2.4	2.7	4.5	2.3	1.6	10.0
		LR	18.4	12.5	12.9	6.2	0.1	5.5

*, ** Significant at the 5% and 1% probability levels, respectively.
† L = locations, S = sets, R = replications, M = males, F = females.
‡SR and LR = short and long rain growing seasons.

The narrow sense heritability (H^2_N) for protein and tannin content and water absorption in the SR growing season were 0.71 and 0.77, respectively (Table 4), indicating that the environment had little influence on the expression of the traits. The H^2_N for maturity, 100-seed weight and cooking time in the SR growing season were also high (0.80, 0.98 and 0.97 respectively). In the LR growing season H^2_N for maturity was a low 0.56 while water absorption maintained the same H^2_N of 0.77 as in SR growing season. Protein and tannin content had H^2_N of 0.88 and 0.91 respectively in the LR growing season. Only 100-seed weight and cooking time traits maintained H^2_N >0.90 in both SR and LR growing seasons indicating that they were the most highly heritable in both seasons (Table 4).

The results obtained in this study for H^2_N estimate for days to maturity (0.56) during LR compare favorably with those reported by Singh, (1990) (0.47) and Cerna and Beaver, (1990) who reported H^2_N of 0.31 - 0.63 for the trait. The results reported by Scully, *et al.*,(1991) who obtained a H^2_N estimate of 0.96 for days to maturity were much higher than those reported in this study.

Table 4. Estimates of additive and dominance variance, broad and narrow sense heritability and the degree of dominance for protein, tannin, maturity and seed weight for dry bean progeny grown at two locations in the Morogoro region, Tanzania during the SR(1993) and LR(1994) growing seasons.

Character	Growing season	σ^2_A†	σ^2_D‡	$H^2_{(N)}$§	Degree of dominance
Maturity	SR	10.9	2.7	0.80 ±0.15	0.7
	LR	4.3	3.8	0.56 ±0.03	1.3
Protein	SR	2.1	0.2	0.71 ±0.22	0.4
	LR	3.2	0.4	0.88 ±0.04	0.2
Tannin	SR	4.8	1.4	0.77 ±0.03	0.8
	LR	26.2	2.7	0.91 ±0.11	0.0
100-Seed weight	SR	35.0	0.4	0.98 ±0.04	0.2
	LR	40.2	0.4	0.98 ±0.02	0.2
Water absorption	SR	48.9	14.5	0.77 ±0.05	0.8
	LR	49.6	15.2	0.77 ±0.04	0.8
Cooking time	SR	9.5	0.3	0.97 ±0.10	0.2
	LR	18.1	2.7	0.90 ±0.02	0.5

σ^2_A† additive genetic variance
σ^2_D‡ dominance genetic variance
$H^2_{(N)}$§ narrow sense heritability with the standard errors
SR and LR = short and long rain growing seasons.

The H^2_N estimate obtained in this study for seed weight was high (0.98) in both growing seasons, but Singh *et al.*, (1989) reported a lower value of 0.61 for the trait.

Except for maturity and tannin content in the LR growing season, all the six traits showed a degree of dominance lower than 1.0 but greater than zero (Table 4). The magnitude of degree of dominance indicated that the traits were governed by genes with partial dominance. For a given trait, no progeny had a higher or lower mean than the highest and the lowest parent. The degree of dominance for tannin content in the LR growing season was zero indicating no dominance. For maturity in the LR growing season the degree of dominance was 1.3 indicating overdominance. The magnitude of the degree of dominance in conjunction with gene frequency influences the magnitude of the genetic components of variance in a population (Falconer, 1981). Although knowledge of the degree of dominance is important to the breeder, the dominance relationship of genes probably is of limited usefulness in *P. vulgaris,* a self-pollinated crop. Bean cultivars are released to commerce as pure lines. In a self-pollinated crop, the frequency of heterozygous individuals declines progressively with each generation of inbreeding, and the frequency of homozygous individuals increase, regardless of

the effectiveness of selection (Fehr, 1987). Dominance has its greatest utility in crops released as hybrid varieties.

The means of the progeny grown in the SR and LR were essentially equivalent for cooking time(Table 5). However the range in cooking time was greater in the LR (16 min) versus the SR (10 min). The ranges in cooking time in the SR and LR while not of a great magnitude should be sufficient enough to reduce cooking time through selection and, thus lead to a conservation of fuel wood in improved lines.

Shellie-Dessert and Hosfield (1991) showed an average fuel wood savings of 1.3 kg per cooking session associated with a 15-min reduction in bean cooking time. Consumers of beans in most bean producing East African countries build 14 fires per week for cooking and reheating the food (Shellie-Dessert and Hosfield, 1991). Given a 1.3 kg fuel wood savings per cooking session for beans, the annual fuel savings would be 1050 kg. Since the cooking time of dry beans is of critical importance to energy use and the overall preservation of fuel wood in Tanzania and other bean growing regions of Eastern Africa, even a small reduction of bean cooking time should lead to a greater bean consumption and thus improve the nutritional well-being of consumers.

Table 5. Means and ranges for maturity, protein, tannin, seed weight, and water absorption as related to cookability for dry bean progeny grown at two locations in the Morogoro region, Tanzania in the SR (1993) and LR(1994) growing season.

	Growing season †	Maturity	Protein content	Tannin content	Seed weight (100)	Water absorption	Cooking time
		days	g kg^{-1}	mg 100^{-1} g	g	g kg^{-1}	min
Mean(x̄)							
	SR	76	234	399	42	818	38
	LR	75	245	373	44	823	38
Range							
	SR	66-83	197-340	338-471	34-50	648.3-955.0	33-43
	LR	67-80	205-265	336-438	33-46	676.7-975.0	31-47
LSD(0.05)							
	SR	4	46	11	1	28	2
	LR	3	14	36	1	11	2
CV(%)	SR	5	17	2	5	3	5
	LR	3	5	8	3	1	3

† SR and LR = short and long rain growing seasons.

Phenotypic correlation coefficients between the cooking time and water absorption (based on 192 observations) were high and negative, (-0.87* and -0.78*, in the SR and LR growing seasons, respectively) and consistent across growing seasons. The results obtained in this study were in agreement with those reported by Castellanos *et al.*, 1995 who obtained correlations on non scarified seed of between -0.69** and -0.81** for cooking time and water absorption after an 18 hour soak. However the magnitude of the correlations between cooking time and water absorption obtained in this study and that by Castellanos *et al.*, 1995 differs from the -0.37* value reported by Shellie and Hosfield (1991) for the same traits, based on data from 270 observations.

The negative correlations found in the present study indicated that slow cooking beans imbibed less water than fast cooking beans. The magnitude of the correlations between cooking time and water absorption in this study and that of Castellanos *et al.*, 1995 suggested that the percent water absorption trait should be useful to predict or estimate cooking time in beans. Selection based on the water absorption of a breeding line as an indirect estimation of its cooking time as opposed to measuring the cooking time *per se* can save valuable resources.

The phenotypic correlations between protein and tannin content and cooking time and protein content were negative and non-significant (Table 6) but the correlation between cooking time and tannin was significant and positive indicating that beans with low tannin content cook faster than beans with high tannin content.

Table 7 shows the selection differential and the estimated gain in selection for days to maturity, protein, tannin, and for seed weight. The data indicate that several cycles of selection are needed in order to change protein content, seed weight and water absorption in beans and lower tannin content, days to maturity and cooking time to a point where practical benefits might be noticeable. The estimated gain in selection for cooking time at 25% selection pressure was about two minutes per cycle while that for the water absorption trait was 60 g kg^{-1} per cycle on a weight basis (Table 7). In order to reduce cooking time in beans to a point where a practical benefit might accrue (e.g. 10 minutes in this study), several cycles of selection may be needed. If selection for reduced cooking time is based on the water absorption trait, five years of selection would increase water absorption by 301 g kg^{-1} (about 300 mg per seed). A 300 mg increase in seed weight due to water absorption is almost double the seed weight of

the dry seed. Physical considerations of size impose an upper limit to the amount of water it can absorb and thus, the weight it can attain.

Table 6. Phenotypic correlation coefficients between protein, tannin, maturity, seed weight and cooking time for dry bean progeny grown at two locations in the Morogoro region, Tanzania during the short rains and long rains growing season.

Trait	Protein content	Tannin content	Maturity	Seed weight	Cooking time	Water absorption
Protein content	-	**-0.06**	**-0.36***	**0.45***	**-0.12**	**0.04**
Tannin content	-0.08	-	**0.42***	**0.06**	**0.77***	**0.87***
Maturity	-0.34*	0.42*	-	**-0.14***	**0.37***	**-0.31***
Seed weight	0.45*	0.06	0.16*	-	**-0.04**	**-0.07**
Cooking time	-0.03	0.68*	0.24*	0.05	-	**-0.78***
Water absorption	-0.05	-0.81*	-0.30*	-0.16*	-0.87*	-

* Correlations significant at ($p < 0.05$)
Bold = (SR) short rain growing seasons data.
non-bold = (LR) long rain growing seasons data.

Table 7: Estimation of response to selection for maturity, protein, tannin, and seed weight, water absorption and cooking time for dry bean progeny grown at two locations in the Morogoro region, Tanzania during the short rains (1993) and long rains (1994) growing season.

Trait	$\bar{x}_p$ †	$\bar{x}_s$ ‡	H^2_w §	SD	¶ΔG#
Maturity (days)	74	75	0.56	0.3	0.2
Protein ($g\ kg^{-1}$)	232	244	0.88	12.2	11.4
Tannin ($mg\ 100^{-1}g$)	395	373	0.91	21.9	-19.9
100-Seed weight(g)	42	44	0.98	1.7	1.7
Water absorption ($g\ kg^{-1}$)	744	823	0.77	78.7	60.1
Cooking time (min)	41	39	0.90	2.0	-1.8

$\bar{x}_p$ † mean of the population
$\bar{x}_s$ ‡ mean of the individuals for selection at 25% selection
H^2_w § narrow sense heritability combined over seasons
SD ¶ selection differential
ΔG # genetic gain in selection

Breeding implications.

The significant genetic variation for the six traits found in this study indicated that they can be changed by selection. Recurrent selection (RS), single-seed descent (SSD) or pedigree methods may be used in population improvement. However, RS takes two years per cycle; hence it would take about ten years to make any noticeable progress for most of the traits.

Progress in population improvement depends on the level of heritability of the trait which is used to estimate genetic gain in selection. The amount of genetic gain realized for all traits except maturity were of sufficient magnitude for population improvement. Generally when narrow sense heritability is low for a trait(s) RS is an efficient method to improve a population since this procedure results in an increase in gene frequencies of the desirable trait(s). Since the traits evaluated in this study all had high narrow sense heritabilities, RS as a breeding strategy to improve them would probably be inefficient and used as a last resort although in some cases this procedure may provide the only means for making genetic progress for some of the traits. When the traits in this study are considered, the breeder may wish to practice one cycle of recurrent selection to increase gene frequency

of favorable alleles. Recurrent selection was successful in changing the growth habit and improving seed characteristic and several food quality traits in small-red market class dry bean germplasm (Hosfield *et al.*, 1995).

The SSD procedure is another selection strategy that has been successful in improving quantitative traits in self-pollinating crops (Brim, 1966). The major future of SSD is to make an initial selection (single seed) from each plant in the F_2. No selection is practiced until the F_5 or F_6 when a reasonably high degree of homozygosity is assured. The F_2 derived F_5 or F_6 lines are then subjected to intense evaluation. Although SSD saves labor and time it might not be the most suitable selection procedure to improve food quality traits in bean exhibiting high heritabilities. The SSD method is most efficient for low heritability traits such as yield.

In identifying the most suitable breeding approach one needs to consider efficiency. Since most of the traits in this study had a moderate to high heritability, pedigree or a combination thereof with SSD would be the most efficient breeding strategy because it allows the breeder to practice selection in early generations without increasing the length of time for cultivar development (Fehr, 1987). The fact that the pedigree method is labor intensive and

requires considerable record keeping is no longer a tenable argument against the procedure since high speed electronic computers are used for keeping record.

The breeder might select the best F_2 derived F_3 or F_2 derived F_4 lines and evaluate them in replicated trials. For highly heritable quality traits like water absorption and cooking time the pedigree procedure beginning with $F_{2:3}$ or $F_{2:4}$ lines might prove useful. If the breeder chooses a combination of inbreeding methods to improve the traits evaluated in this study, single seed selection should be started in the F_2 for traits such as maturity, seed size. Further selection among the lines for traits such as yield could be done at the F6, when the individual lines are more homozygous. The selected F6 lines would be replicated, and grown in different locations and seasons or years. The advantage of using a combination pedigree-SSD or pedigree-SSD-RS breeding strategy to improve the traits evaluated in this study, is that when selection is effective inferior bean genotypes may be discarded long before homozygous lines are evaluated in costly replicated tests.

Use of correlated responses in breeding strategy.

The significant and positive correlation (0.77*) obtained in this study between tannin content and cooking time indicated that beans low in tannin cooked faster than

beans with high tannin. The selection and intermating of fast cooking genotypes during the inbreeding process of cultivar development would lead to cultivars with reduced tannin content. The intermating of fast cooking genotypes with high protein content would be useful to improve the three traits simultaneously and lead to the development of cultivars with low tannin, and high protein content that are fast cooking. Although this strategy would lead to improved nutritional quality by increasing protein and decreasing tannin the breeder should proceed with caution and be cognizant of seed coat color. Consumers have preference for particular colors of bean seed coats and seed coats with darker colors such as purple and deep red hues generally are high in tannin (Ma and Bliss, 1978a and 1978b). Whether or not the association between some seed coat colors and tannin is due to pleiotropy or tight linkage is unknown and requires study.

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CHAPTER TWO

THE EFFECT OF STORAGE TIME, TEMPERATURE AND RELATIVE HUMIDITY ON THE COOKABILITY AND SEED QUALITY TRAITS IN DRY BEAN *(Phaseolus vulgaris L.)*.

ABSTRACT

This study was conducted to ascertain the effect of prolonged storage, high temperature and high humidity on seed germination and cooking time of dry beans representative of the Andean gene pool of *P. vulgaris*. The study also investigated the effects that accelerated aging had on beans compared to those stored under unfavorable environmental conditions. Genotypic differences were observed for seed germination, water absorption, and cooking time. Highly significant differences were noted for temperature, humidity, and storage time effects. A genotype x storage interaction was noted for each of the three traits. A significant interaction was detected between temperature and relative humidity for cooking time indicating that cooking time for the eight genotypes was not linear across storage temperatures and humidities. A second order interaction of genotype x storage x temperature for water absorption trait was also detected. Seeds stored in high temperatures took longer to cook. When seeds were stored under the high humidity, they

absorbed less water and had a lower seed germination but took longer to cook than seeds stored at 40% RH.

The mean germination percentage decreased significantly with increasing storage time. The results of this study indicate that seed water absorption is inversely related to the cooking time in dry beans.

Experiment II dealt with the investigation of accelerated aging as a technique to mimic conditions associated with unfavorable storage of sixteen genotypes of dry bean.

Significant differences existed for cooking time index in the entries grown in the SR and LR growing seasons. Significant differences were observed between the two seed conditions (non accelerated aged seed and accelerated aged seed for progeny grown during the SR and LR growing seasons. Differences between the soak and non-soak method for the cooking method of the progeny grown during the SR and LR growing seasons were also significant. Several interactions were detected.

The accelerated aging genotypes took longer to cook than those that stored under adverse conditions for three months. The mean performance of seed stored for six months was 54.8 while that for accelerated aging was 53.7. In this study accelerated aging mimicked the 6 month storage.

INTRODUCTION

Dry bean (*Phaseolus vulgaris L.)* Is the leading food legume with an annual world production of 14 million metric tons. This production accounts for more than 30% of the total world food legume production of 49 million metric tons. Dry bean production accounts for more than 30% of the total world grain legume production of 49 million metric tons. Latin American countries produce 4.0 million metric tons (van Schoonhoven and Voysest, 1993), while Africa produces 1.4 million metric tons.

Throughout Latin America and Africa, beans are a staple in the diets of consumers. Dry bean is a good source of calories and protein in human diets. Bean protein complements cereal protein in diets where little meat protein is available (Antunes and Sgarbieri, 1979). The mean dry bean consumption in the Central and Eastern Africa region is 31 kg. This consumption is about double that of the 13 kg/capita in Latin America (van Schoonhoven and Voysest, 1993). In Africa, the largest per capita bean consumption is in Rwanda (> 50 kg per year) which is four times that in Tanzania.

In developing countries beans are often inadequately stored; hence, considerable storage loss occurs. It has

long been known that dry beans differ in ability to imbibe water. Gloyer, (1921) described two conditions in which beans failed to hydrate after soaking. One kind of failure of water imbibition called "hardshell" was due to the impermeability of the seed coat to water. The second condition affecting water uptake in bean seeds referred to as sclerema (Gloyer, 1921) was due to the inability of the cotyledon to take up water and expand. Snyder (1936) confirmed the condition of sclerema and observed that bean cotyledons would not hydrate even though the seed coat was scarified or removed. The loss of cookability of beans stored under tropical environments in developing countries may be due to one or both phenomena.

Castellanos *et al.,* (1995) reported that "hard shell" is a major contributor to the long cooking time of beans grown in semiarid areas of Mexico. Considerable economic loss occurs in Latin America due to bean hardening during prolonged storage (Mejia, 1979). On the other hand, Jones and Boutler (1983); Burr et al., 1981; observed sclerema which rendered beans hard-to-cook and indigestible. Both "hard shell" and sclerema negatively affect bean seed quality.

Seed quality of a food legume is viewed from two perspectives: 1) characteristics associated with seed

germination and seedling vigor and 2) characteristics that have a direct impact on human nutrition and those related to consumer acceptance and preparation for eating. In dry bean, most growers in developing countries save part of the current season's bean crop to plant in the subsequent year. Hence, seed germination is an important economic consideration. The ability of beans to imbibe water and cook in a reasonable length of time is important to the nutrition well-being of consuming populations in regions where beans are a staple food.

Since seed quality in dry bean is often dependent on its post harvest history, an additional objective was to determine the effect of storage conditions on seed quality. Specific objectives were: 1) show that prolonged storage and high temperature, and high humidity alter seed germination and cookability of the grains and 2) ascertain whether accelerated aging of seeds can be used as a technique to mimic conditions associated with unfavorable storage conditions.

Table 1: Production and consumption figures for major bean growing regions.

Region and country	Percent of total production	Mean per capita consumption (kg/year)
Latin America		
Brazil	55	20.1
Mexico	23	12.6
Argentina	5	2.9
Paraguay	2	24.3
Nicaragua	1	23.8
Total production for (Latin America)	4×10^6 tones	13.3
Africa		
East and Central	59	
Uganda	13	29.3
Kenya	9	21.0
Rwanda	13	50.6
Tanzania	11	12.0
Burundi	12	44.3
Total (East and Central Africa)	0.8×10^6 tones	31.4

From: Shellie-Dessert and Bliss, 1993.

MATERIALS AND METHODS

Genetic material.

This study was conducted in two experiments: Experiment I. It dealt with the investigation of the effect of prolonged storage, high temperature and high humidity on seed germination and cookability of eight genotypes of dry bean. The eight genotypes were representative of the Andean germplasm pool of *P. vulgaris* and named: Var 11, Nyirakizungu, Lyamungu, Kilyumukwe, Yellow eye, UAC 221, TMO 959, and Kalima.

Experiment II. It investigated accelerated aging as a technique to mimic conditions associated with unfavorable storage. In this experiment sixteen genotypes of dry bean, were used. These included the same eight genotypes used in experiment I and CIAT 3005, P.I. 605621, GLPX 1125, Jacob's cattle, Montcalm, NY 99, Sierra, and Diacol Calima. Except for 'sierra', the additional eight genotypes were of Andean germplasm pool origin. 'Sierra' was a representative of the middle-American gene pool with the medium pinto market class seed size.

The dry bean genotypes used as entries in the two experiments differed morphologically, phenologically, and agronomically. The entries were all adapted to the bean production areas of East and Central Africa. Seeds of each

entry are highly favored by consumers in the region because of their meat-like appearance and thick broth/soup quality after cooking.

Procedures.

The experiments on which data were taken were grown in 1993 and 1994 in Tanzania at the Crop Museum(530 masl) and Morning Site (~1000 masl) research farms in the Morogoro Region. Data for aging experiments were combined over locations and growing seasons. These farms used in this study were maintained by the Agricultural University of Sokoine. The soil characteristics at Morogoro are moderately acidic (Appendix Table 1). Rainfall, temperature and solar radiation are given in Appendix Table 22.

The experiments were planted in a randomized complete block design with three replications at each location during the seasons in which the annual bimodal distribution of rain occurs. The short (SR) rain growing season occurs between the end of September and the middle of December. This growing season is suitable for fast maturing bean genotypes as well as other quick maturing crops. Yield of slow maturing bean genotypes generally give low yield during this growing season. The long (LR) rain growing season is characterized by more rainfall, and for longer time (early March to June. Both early and late maturing

bean genotypes are grown during this season. The yield is much higher than in SR growing season. However farmers have to be cautious about the planting date since early planting of beans may result in poor yield due to excessive rainfall.

The entries were hand seeded into four row plots. Rows were 4 m long and spaced 0.5 m apart. The within row spacing was 0.1 m giving an approximate plant density of 160 plants per plot. On farm practices for herbicide, pesticide and fertilizer applications were followed. Mature plants were harvested by hand from the middle two rows of individual plots when the pods were dry enough to be threshed.

Experiment I.

Freshly harvested seed of each entry were cleaned and size-graded. Beans selected for use in the study passed through a 0.675 cm. sieve but were retained on a 0.476 cm. sieve (Bourne, 1967). To prevent mold growth (Sefa-Dedah, 1979) the beans were treated with sorbic acid in absolute methanol (1:8 w/v) per each kg of beans (Monsanto, 1992). The methanol was applied with a thin layer chromatography sprayer using air as the propellant. Seeds were mixed adequately during the treatment. The treatment of beans with sorbic acid was repeated after four and a half months.

Beans were stored in desiccators at 43% and 73% relative humidity (RH). The 43% RH was maintained by using saturated solution of potassium bicarbonate while that of 73% RH was maintained by using a saturated solution of sodium chloride. As an additional safeguard to prevent mold growth, copper sulfate was added to water at a concentration of 1 gram per liter (American Society for Testing Materials, 1951).

The design of the experiment was a split plot in which the cultivar was the whole plot. Storage time, temperature, and relative humidity were sub-plots. The seed lots were stored at two levels of relative humidity for nine months in desiccators, in Gallenkamp temperature-controlled incubators set at three temperature levels i.e. at 20 ^{0}C ± 2 ^{0}C; 25 ^{0}C ± 2 ^{0}C and 30 ^{0}C ± 2 ^{0}C under the following sets of conditions with three replicates per treatment: I) 20 ^{0}C, 40% RH; ii) 20 ^{0}C, 73% RH; iii)25 ^{0}C, 40% RH; iv) 25 ^{0}C, 73% RH; v) 30 ^{0}C, 40% RH; and vi) 30 ^{0}C, 73% RH.

Experiment II.

Accelerated aging is a technique which involves aging seeds for short periods of time, for example between 3 to 14 days depending on the crop species(Delouche and Baskins, 1973). The seeds are aged at high temperatures between 41^{0}C to 45^{0}C and a relative humidity of 100%. During accelerated

aging, seeds deteriorate comparable to that of seed lots stored for several months under adverse conditions.

The seed for accelerated aging study were obtained and pretreated for mold control. The seed lots for the experiment were divided into a control sample (freshly harvested but not aged) and seed sample that underwent accelerated aging. The control and aged seed were cooked with and without soaking. For the soaked sample, beans were soaked for 12 hours. The treatment comparisons of control vs aged seed was as follows:

1) fresh harvested seed, non-soak;

2) fresh harvested seed, soak;

3) accelerated aged, non-soak;

4) accelerated aged, soak

The aging of seed was carried out at 42°C, at 100 RH for 72 hours. A single sample accelerated aging chamber was used to age the seed lots in this experiment. The outer chamber had an immersion type of heating element (Stultz Scientific Equipment Co, Springfield, IL) that was immersed approximately 5 cm deep in the water reservoir.

Temperature was controlled by a Thermistemp temperature control unit. The model used (model 71-A) was capable of controlling temperature to 0.1°C. On the side wall of the chamber, a mercury thermometer was inserted to

about 12.7 cm. into the outer chamber. The inner chambers or seed containers were constructed of plastic. The brass or bronze wire mesh baskets that were used to contain the seeds were locally made to sizes that could accommodate 210 seeds of each of the entries that were used in the experiment for each treatment.

Character evaluation. The actual moisture contents of the seed samples was not determined, but prior to analyses of water absorption and cookability the treatment samples were equilibrated for moisture to each other. This was accomplished by storing the samples for two weeks in sealed plastic buckets held at 20°C and 73% RH. Seeds were kept as per field replications. Seed were analyzed at three, six and nine months. After each storage period (i.e. 3, 6, and 9 months) germination of each genotype of each treatment was determined on a 400 seed sample that was allowed to germinate on moist filter paper.

The amount of water absorbed by each genotype was calculated on triplicate samples of a known weight of 75 seeds from each plot. The seeds were soaked in distilled water for twelve hours. The amount of water absorbed was taken as the difference in weight before and after soaking divided by the difference in the weight of unsoaked seeds and expressed as a percent.

Cookability was determined as the time it took 25-seed sample of beans to cook to a point of softening where a weighted plunger would penetrate the seeds (Jackson and Varriano-Marston, 1981). Beans were considered cooked, when 13 of the 25 pins of the apparatus had penetrated through the seeds. Data were taken on triplicate samples for each plot.

Statistical Analysis. All data were subjected to analyses of variance (ANOVA) appropriate to a split plot design. The cultivar was the whole plot and the storage time, temperature and humidity were sub plots in the splits. Data analyses were conducted using the MSTATC[R] statistical software program (experimental model number 34).

RESULTS AND DISCUSSION

Experiment I.

Genotypic (G) differences among the eight entries were observed for seed germination, water absorption, and cooking time (Table 2 and Appendix Table 17, 18, 19). There were highly significant differences noted for effects of temperature (T), humidity (H), and storage time (S). The significant storage time effects led to several significant interactions. Significant interactions between genotypes and storage effects (G x S) indicated a lack of uniformity in strain response over storage times. There was a genotype x storage interaction noted for each of the three traits. A significant temperature x humidity interaction was detected for cooking time indicating that cooking time responses of the eight genotypes was not linear across storage temperatures and humidities. A second order interaction of G x S x T for water absorption trait was detected. The significant second order interaction for water absorption demonstrated that water absorption for the eight genotypes was not linear across storage times and temperatures (Table 2).

Table 2: Mean squares from analyses of variance for 8 dry bean entries for seed germination, water absorption and cooking time traits over nine months storage at varying temperature and relative humidity.

Source	df	% Seed germination	Water absorption	Cooking time
Replication	2	5	21	6
Genotype(G)	7	449**	12568**	6078**
Error(a)	14	7	27	9
Storage time(S)	2	11159**	9421**	14028**
G x S	14	63**	270**	76**
Error(b)	32	9	13	5
Temperature(T)	2	1376**	1017**	1099**
G x T	14	7	23	6
S x T	4	15	1	6
G x S x T	28	9	24*	5
R.Humidity(H)	1	665*	736**	705**
G x H	7	5	10	4
S x H	2	9	16	10
G x S x H	14	6	16	2
T x H	2	1	0†	14*
G x T x H	14	5	18	1
S x T x H	4	1	10	2
G x S x T x H	28	4	14	2
Error	240	7	15	4
Total	431			

*, ** Significant at the 5% and 1% probability levels, respectively.
† Small positive value < 0.5.

An examination of the Table of means (Table 3)indicated that dry bean seed stored at the 20°C temperature had a significantly higher percent germination, and absorbed more water than seed stored at the 30°C temperature. Seeds stored in high temperatures also took longer to cook. The results of this study agree with previous work (Jackson and Varriano-Marston, 1981; Castellanos *et al.*, 1995) that seed water absorption is inversely related to the cooking time in dry beans. A range of 38 min in cooking time was observed among the genotypes across the different temperatures used to store the seed. The range in percent water absorption and seed germination across storage temperatures were 51% and 14% respectively (Table 3). When seeds were stored under the 73% RH regime, they absorbed less water, had a lower seed germination, and took longer to cook than seeds stored at 40% RH (Table 4). Table 5 shows the effect storage times averaged over temperature and humidities while Table 6 shows the mean performance for the three traits of the eight genotypes stored for nine months under varying temperature and relative humidity conditions.

Table 3: Mean germination percent, water absorption, and cooking time of seed stored at 20°C, 25°C and 30°C and averaged over eight genotypes, two relative humidities and three storage times.

Storage temperature	Seed † germination (%)	Water † absorption (%)	Cooking time (min)
30°C	81a	62a	57c
25°C	84b	65b	54b
20°C	87c	68c	51a
Mean	84	65	54
Range	(77-90)	(33-85)	(33-71)
LSD (0.05)	0.8	1.1	0.6
C.V.(%)	3.2	6.0	3.8

† Means with the same letter in a column are not significantly different at the 0.05 probability level.

Table 4: Mean germination percent, water absorption, and cooking time of seed stored at two relative humidities and averaged over eight genotypes, three temperatures and three storage times.

Relative humidity (%)	Seed † germination (%)	Water † absorption (%)	Cooking † time (min)
73	83a	64a	55b
40	86b	66b	53a
Mean	84	65	54
Range	(78-89)	(34-83)	(34-68)
LSD (0.05)	0.5	0.8	0.4
C.V.(%)	3.2	6.0	3.8

† Means with the same letter in the column are not significantly different at the 0.05 probability level.

Table 5: Mean germination percent, water absorption, and cooking time of seed stored at three storage times and averaged over eight genotypes, two relative humidities and three temperatures.

Storage time (months)	Seed † germination (%)	Water † absorption (%)	Cooking † time (min)
3	93a	73a	44c
6	85b	65b	55b
9	75c	57c	64a
Mean	84	65	54
Range	(69-96)	(32-97)	(28-79)
LSD (0.05)	0.8	0.6	1.1
C.V.(%)	3.2	6.0	3.8

† Means with the same letter are not significantly different at the 0.05 probability level.

Table 6: Mean germination percent, water absorption, and cooking time of eight genotypes averaged over three storage times two relative humidities and three temperatures.

Entry	Seed germination (%)	Water absorption (g)	Cooking time (min)
Kalima	87	81	44
Nyirakizungu	87	76	50
Var 11	86	82	35
Kilyumukwe	86	56	62
TMO 959	84	60	60
UAC 221	82	36	67
Lyamungu 85	82	61	56
Yellow eye	79	68	56
Mean	84	65	54
LSD(0.05)	1	2	1
CV (%)	3	3	6

Experiment II.

From the analyses of variance, significant differences existed for the cooking time of the 16 genotypes (Table 7). The analysis of variance further indicated that the location effects significantly influenced the cooking time in the SR and LR growing seasons. Significant differences were observed between the two seed conditions (control vs accelerated aged seed for progeny grown during the SR and LR growing seasons. The fact that the genotypes differed in their response to accelerated aging implied the existence of significant genetic variability. The differences noted between soaking and non-soaking beans prior to cooking them indicated that soaking of the seeds improved their hydration characteristics. While Burr *et al.*,(1968) and Molina *et al.*, (1975) found no correlation between water uptake and cooking time, Sefa-Dedah *et al.*, (1979) found that water absorption influenced cooking time and that it was determined by seed coat texture. The results obtained in this study for soak and non-soak cooking method agree with those of Sefa-Dedah *et al.*, (1979) and Castellanos, *et al.*, 1995.

Several interactions were detected. In the SR growing season all treatment interactions were significant. Table 8 indicates the mean performance of the bean genotypes that

underwent accelerated aging for 72 hours and control treatment that were stored at 20, 25, and 30 ^{0}C and 40 and 70% RH for 3, 6, and 9 months. Accelerated aging increased the cooking time of each genotype compared to the three months storage period. On the other hand, aging had less of an effect on cooking time except for (UAC 221) than the 9 months storage effects. The average cooking time increase of the eight genotype stored for 9 months compared to the aging treatment was 12 minutes. There were mixed results for the beans stored for 6 months when compared with the aging treatment (Table 8). Table 9 shows that the effect of aging in the bean seed was greater in the SR growing season than in the LR growing season. This suggests that short rains have an adverse effect on the storage of the seed. This is important to the the consumers and farmers, as it concerns the shelf-life of the bean seed during storage.

Table 7: Mean square analyses for cookability of sixteen entries of dry bean seed that were accelerated aged from two locations grown during the SR (1993) and LR(1994) growing seasons at the Morogoro region, Tanzania.

Source	df	Mean squares	
		Short rain	Long rain
Reps (R)	2	1	6
Location (L)	1	598*	858*
Genotype (G)	15	2901*	2400*
G x L	15	5	3
Seed cond.	1	20460*	13184*
L x C	1	32*	44*
G x C	15	74*	62*
L x G x C	15	3	2
Soaking(S)	1	2954*	4874*
L x S	1	71*	81*
G x S	15	97*	59*
L x G x S	15	4*	3
C x M	1	3744*	330*
L x C x S	1	33*	126*
G x C x S	15	55*	16*
L x G x C x S	15	3	2
Error	254	2	2
Total	383		

* significant at the 0.05 probability level

Table 8: Table of means for the cooking time trait of dry bean genotypes under three storage times under normal conditions and under accelerated aging conditions.

Entry	Cooking time (minutes)				
	Storage time in months †				Accelerated aging
	0‡	3	6	9	
Var 11	25	28	33	43	39
Kalima	28	36	46	51	43
Nyirakizungu	30	40	51	58	47
yellow eye	35	44	57	65	50
Lyamungo 85	42	50	61	70	53
TMO 959	42	47	60	72	55
Kilyumukwe	44	52	62	71	57
UAC 221	46	55	67	79	80
Mean	37	37	55	66	54
LSD(0.5)	1	1	1	1	1
C.V.(%)	3.2	3.2	3.2	3.2	2.9

† non aged seed
‡ control storage time

Table 9. Table of means for cooking time of sixteen entries that were accelerated aged during the SR (1993)and LR (1994) growing seasons from two locations at the Morogoro region,Tanzania.

Entry	Cooking time (minutes)		
		Accelerated aged	
	Freshly harvested	SR †	LR ‡
Var 11	25	36	33
605621	27	39	36
Kalima	28	41	37
Nyirakizungu	30	44	39
Jacob's cattle	32	46	40
Sierra	32	48	42
Yellow eye	34	50	44
Diacol calima	30	53	45
Lyamungu 85	43	55	49
TMO 959	42	57	52
NY99	44	60	54
Kilyumukwe	44	62	56
Montcalm	45	64	58
GLPX 1125	45	67	60
CIAT-3005	45	68	62
UAC 221	46	71	65
Mean	37	54	48
LSD (0.05)	1	1	1
CV (%)	3.2	2.4	3.0

† SR = short rain growing season
‡ LR = long rain growing season

Implications. Results of this study indicated that sufficient variability existed in the germplasm for germination and cookability (water absorption and cooking time) of seeds. Rapid water absorption is a favored food quality trait because genotypes that imbibed the most water during soaking i.e. cooked the fastest. This finding held true regardless of the storage conditions imposed.

There was deterioration in the quality of dry beans stored at high temperature and high relative humidity. Measurements of cooking time indicated that beans were increasingly difficult to cook when stored at the higher temperature and relative humidity.

The results from this study indicated that accelerated aging lengthened the cooking time in dry bean and even for beans stored for 3 months (Table 8). Accelerated aging should be useful to predict how well a bean will store under unfavorable environments. However, accelerated aging lost its utility for beans stored more than 6 months. The mean cooking time of the eight entries under accelerated aging was 54 minutes compared to 55 minutes and 66 minutes for beans stored six and nine months respectively. Kalima, Var 11, 605621 and Nyirakizungu out-performed others in maintaining higher percent germination, higher water absorption and fast cooking. The possession of these

attributes make them superior genotypes for population improvement of shortening cooking time, increasing water absorption and seed germination.

Var 11 consistently cooked fast across both SR and LR seasons (Table 9). The same was true for 605621, Kalima and Nyirakizungu although the magnitude between SR and LR was greater in the later genotypes. There was a more pronounced negative effect on cooking time when seed grown in the short rains was subjected to accelerated aging than for seed grown during the long rains season.

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APPENDIX

Table 1: General soil characteristics at Morogoro at 0-12 cm. During the 1993-94 growing seasons.

Characteristic	Determination method	Value
Soil pH:H2O	pH meter	4.60
Soil texture		
% Clay(loam)	Hydrometer	20
% Silt		20
% Sand		60
Textural class: sandy loam		
% Total Nitrogen	Kjedahl (semi micro)	0.10
% Organic carbon		0.83
Phosphorus (ppm)		0.04
% Soil moisture	Volumetric method	13.09
Bulk density (cm3/cm3)	Density method	1.18
Exchangeable cations		
Ca (me/100g^{-1} soil)	Saturation` method	2.20
K " "		1.33
Mg " "		1.31
Al " "		1.03
ECEC " "		5.87
% Al " "	Saturation method	17.54
Drainage class		moderate
Permeability		moderate
Color		reddish brown

Table 2: Form of Analysis of Variance and Mean Square Expectation for Data of a Design II Model II Experiment Repeated over Locations at Morogoro Region, Tanzania in the 1993-94 Growing Season.

Source†	df	Mean square	Expected mean squares ‡
Location, L	l-1		
Sets, S	s-1		
S x L	(l-1)(s-1)		
Reps(S)(L)	ls(r-1)		
M(S),M	s(m-1)	M7	$\sigma^2 + r\sigma^2_{fml} + rf\sigma^2_{ml} + re\sigma^2_{mf} + ref\sigma^2_{m}$
F(S),F	s(f-1)	M6	$\sigma^2 + r\sigma^2_{fml} + rm\sigma^2_{fl} + re\sigma^2_{mf} + rem\sigma^2_{f}$
M x F(S)	s(m-1)(f-1)	M5	$\sigma^2 + r\sigma^2_{fml} + re\sigma^2_{mf}$
M(S) x L	s(m-1)(l-1)	M4	$\sigma^2 + r\sigma^2_{fml} + rf\sigma^2_{ml}$
F(S) x L	s(f-1)(l-1)	M3	$\sigma^2 + r\sigma^2_{fml} + rm\sigma^2_{fl}$
L(S)(M)(F)	s(m-1)(f-1)(l-1)	M2	$\sigma^2 + r\sigma^2_{fml}$
Pooled error	ls(r-1)(mf-1)	M1	σ^2
Total .	lsrmf-1		

† L, S, R, M, and F refer to the number of locations, sets within locations, replications, males, and females, respectively.
‡ σ^2 = pooled error variance; σ^2_{fml} = variance due to the interaction of males and females and locations.

Table 3: Protein Determination.

Prior to estimating protein content the raw dry bean seed were processed to bean flour. The seed were ground to 40 μ with a Udy-Cyclone mill. The flour was then analyzed by the micro- Kjedahl method of nitrogen determination. The nitrogen content of each sample was multiplied by 6.25 to obtain the total protein. Thus % protein = % nitrogen x 6.25.

Table 4: Tannin Content Determination.

Tannin content was determined according to vanillin hydrochloric method of Burns (1971) as modified by Telek (1983). These stages are:

I. Preparation and Extraction of the Samples of the Dry Bean.

1- A 0.2g sample of ground bean seed flour (40μ) was weighed in a 100 ml sample bottle.

2- An acidic methanol solution was then made (V/V/V) by mixing absolute methanol (80 ml): distilled water (19.5 ml): concentrated hydrochloric acid (0.5 ml).

3- 35 ml of the acidic 80% methanol solution were added to the ground bean flour sample.

4- The treated samples were then put in a shaker bath maintained at 70^0C for 30 minutes.

5- The extract was the decanted over a porcelain crucible lined with glass microfiber filter (GF/D whatman, 2.5cm) into a 100 ml volumetric flask.

6- The residual from the filter was decanted two additional times. All the extracts were combined together and volume made up with 80% acidic methanol solution.

7- 5 ml of the extract were pipetted into a 25 ml volumetric flask and brought to volume with a 30% sulfuric acid solution.

8- From this 25 ml volume, 3 ml sample were pipetted into each of three 10 ml volumetric flasks.

9- 3 ml of a 0.5% vanillin solution were added to two of the 10 ml sulfuric acid solution flasks.

10- To the third 10 ml flask only sulfuric acid was added.

11- The 3 flasks were allowed to stand for 20 minutes and then absorbance of each flask read at 500 nm.

12- Two vannilin blanks were then prepared by pipetting 3 ml of 0.5% solution into a 10 ml volumetric flask and bringing up to volume with a 30% sulfuric acid solution.

II. Preparation of the Catechin Standard.

1- A 0.05 g sample of catechin was weighed and dissolved in 2 ml absolute methanol in a 50 ml volumetric flask and brought up to volume with distilled water.

2- A 5 ml sample of this catechin solution was pipetted into 200 ml volumetric flask and brought to volume with a 30% sulfuric acid solution.

3- From this 200 ml solution 3 ml sample were pipetted into 10 ml volumetric flasks in duplicate. To each of the flasks 3 ml of a 0.5% vanillin solution were added. The 0.5% vanillin and catechin solutions were prepared fresh each day prior to pipetting the ground bean seed flour.

III. Reading the Absorbance

1- The spectrometer was set to zero with a vannilin blank by putting the blank in both sample and reference cuvette.

2- The catechin standard is read at 500 nm against a vannilin blank which is left in the reference cell.

3- The Sample blank is placed in both the reference

and sample cell and read. The sample cuvette is rinsed and the actual sample poured into the cuvette and read against the sample blank.

IV. Determining the Catechin Equivalent

A. Day Factor

Day Factor = (wt. Of catechin/ O.D of catechin) x (dilution factor of sample /dilution factor of catechin) x 100

B. % catechin equivalent = (O.D. of sample/ wt. of sample) x Day Factor.

Table 5a: Analysis of variance for protein SR season

Source of variation	d.f.	s.s.	m.s.	v.r.
site	1	339.5	339.5	2036.0
sets	1	5.8	5.8	34.6
site.sets	1	1.1	1.1	6.6
site.sets.reps	8	1.2	0.1	0.9
sets.males	6	98.8	16.5	98.8
sets.females	6	240.4	40.1	240.3
sets.males.females	18	11.0	0.6	3.7
site.sets.males	6	21.2	3.5	21.2
site.sets.females	6	1.3	0.2	1.3
site.sets.males.females	18	6.0	0.3	2.0
Residual	120	20.0	0.2	
Total	191	746.2		

Table 5b: Estimated Components of Variance and standard errors (s.e.)for protein SR season

		s.e.
site	3.525	5.037
sets	0.0000167	0.8679
site.sets	0.0000167	0.1127
site.sets.reps	0.0000167	0.005389
sets.males	0.5275	0.4055
sets.females	1.649	0.9667
sets.males.females	0.04657	0.03860
site.sets.males	0.2672	0.1705
site.sets.females	0.0000167	0.01841
site.sets.males.females	0.05486	0.03750
units	0.1667	0.02152

Table 6a: Analysis of variance for tannin content SR season

Source of variation	d.f.	s.s.	m.s.	v.r.
site	1	324.4	324.4	376.8
sets	1	94.1	94.1	109.2
site.sets	1	0.4	0.4	0.4
site.sets.reps	8	9.0	1.1	1.3
sets.males	6	188.0	31.3	36.4
sets.females	6	1957.2	326.2	378.9
sets.males.females	18	54.3	3.0	3.5
site.sets.males	6	3.9	0.6	0.8
site.sets.females	6	2.9	0.5	0.6
site.sets.males.females	18	15.4	0.9	1.0
Residual	120	103.3	0.9	
Total	191	2752.9		

Table 6b: Estimated Components of Variance and standard errors (s.e.) for tannin content SR season

		s.e.
site	3.376	4.791
sets	0.0000861	5.595
site.sets	0.0000861	0.03889
site.sets.reps	0.01672	0.03594
sets.males	1.189	0.7605
sets.females	13.48	7.857
sets.males.females	0.3600	0.1745
site.sets.males	0.0000861	0.04789
site.sets.females	0.0000861	0.04789
site.sets.males.females	0.0000861	0.1026
units	0.8610	0.1112

Table 7a: Analysis of variance for 90% maturity for SR season

Source of variation	d.f.	s.s.	m.s.	v.r.
site	1	1800.8	1800.8	2041.0
sets	1	768.0	768.0	870.5
site.sets	1	0.2	0.2	0.2
site.sets.reps	8	14.1	1.8	2.0
sets.males	6	451.3	75.2	85.3
sets.females	6	790.3	131.7	149.3
sets.males.females	18	37.7	2.1	2.4
site.sets.males	6	65.1	10.8	12.3
site.sets.females	6	71.1	11.8	13.4
site.sets.males.females	18	51.9	2.9	3.3
Residual	120	105.9	0.9	
Total	191	4156.3		

Table 7b: Estimated Components of Variance and standard errors (s.e.) for 90% maturity SR season

		s.e.
site	18.76	26.83
sets	6.071	11.67
site.sets	0.0000882	0.6400
site.sets.reps	0.05521	0.05563
sets.males	2.715	1.848
sets.females	5.028	3.201
sets.males.females	0.0000882	0.2267
site.sets.males	0.6632	0.5278
site.sets.females	0.7465	0.5754
site.sets.males.females	0.6677	0.3228
units	0.8823	0.1139

Table 8a: Analysis of variance for 100-seed weight SR season

Source of variation	d.f.	s.s.	m.s.	v.r.
site	1	98.2	98.2	222.9
sets	1	130.8	130.8	297.1
site.sets	1	0.01	0.01	0.02
site.sets.reps	8	1.4	0.2	0.4
sets.males	6	1267.6	211.3	479.7
sets.females	6	1218.0	203.0	460.9
sets.males.females	18	23.3	1.3	2.9
site.sets.males	6	3.4	0.6	1.3
site.sets.females	6	11.7	2.0	4.4
site.sets.males.females	18	11.6	0.6	1.5
Residual	120	52.9	0.4	
Total	191	2819.1		

Table 8b: Estimated Components of Variance and standard errors (s.e.) for 100-seed weight SR season

		s.e.
site	1.023	1.475
sets	0.0000440	6.335
site.sets	0.0000440	0.06307
site.sets.reps	0.0000440	0.01424
sets.males	8.752	5.084
sets.females	8.350	4.884
sets.males.females	0.1084	0.08048
site.sets.males	0.0000440	0.03591
site.sets.females	0.1089	0.09567
site.sets.males.females	0.06854	0.07425
units	0.4405	0.05686

Table 9a: Analysis of variance for cooking time SR season

Source of variation	d.f.	s.s.	m.s.	v.r.
site	1	277.9	277.9	144.0
sets	1	1116.5	1116.5	578.4
site.sets	1	0.1	0.1	0.1
site.sets.reps	8	27.7	3.5	1.8
sets.males	6	370.4	61.7	32.0
sets.females	6	127.2	21.2	11.0
sets.males.females	18	22.7	1.3	0.7
site.sets.males	6	25.9	4.3	2.2
site.sets.females	6	2.7	0.4	0.2
site.sets.males.females	18	15.2	0.8	0.4
Residual	120	231.6	1.9	
Total	191	2218.0		

Table 9b: Estimated Components of Variance and standard errors (s.e.) for cooking time season

		s.e.
site	2.894	4.196
sets	10.82	16.55
site.sets	0.0001930	0.2195
site.sets.reps	0.09583	0.1094
sets.males	2.375	1.517
sets.females	0.8472	0.5493
sets.males.females	0.06944	0.1689
site.sets.males	0.2894	0.2654
site.sets.females	0.0001930	0.1074
site.sets.males.females	0.0001930	0.2301
units	1.930	0.2492

Table 10a: Analysis of variance for water absorption SR season

Source of variation	d.f.	s.s.	m.s.	v.r.
site	1	382.5	382.5	277.6
sets	1	2662.6	2662.6	1932.1
site.sets	1	0.6	0.6	0.5
site.sets.reps	8	11.3	1.4	1.0
sets.males	6	1892.0	315.3	228.8
sets.females	6	8148.9	1358.2	985.5
sets.males.females	18	405.8	22.5	16.4
site.sets.males	6	1.8	0.3	0.2
site.sets.females	6	5.2	0.8	0.6
site.sets.males.females	18	14.6	0.8	0.6
Residual	120	165.4	1.4	
Total	191	13690.9		

Table 10b: Estimated Components of Variance and standard errors (s.e.) for water absorption SR season

		s.e.
site	3.978	5.651
sets	10.54	40.13
site.sets	0.004851	0.05852
site.sets.reps	0.002083	0.04549
sets.males	12.22	7.619
sets.females	55.65	32.69
sets.males.females	3.622	1.286
site.sets.males	0.0001378	0.07665
site.sets.females	0.005015	0.07911
site.sets.males.females	0.0001378	0.1643
units	1.378	0.1779

Table 11a: Analysis of variance for protein content LR season

Source of variation	d.f.	s.s.	m.s.	v.r.
site	1	273.4	273.4	328.9
sets	1	8.8	8.8	10.6
site.sets	1	0.1	0.1	0.1
site.sets.reps	8	9.1	1.1	1.4
sets.males	6	123.5	20.6	24.8
sets.females	6	302.8	50.5	60.7
sets.males.females	18	23.5	1.3	1.6
site.sets.males	6	3.9	0.7	0.8
site.sets.females	6	7.7	1.3	1.5
site.sets.males.females	18	13.9	0.8	0.9
Residual	120	99.7	0.8	
Total	191	866.3		

Table 11b: Estimated Components of Variance and standard errors (s.e.) for protein content LR season

		s.e.
site	2.847	4.050
sets	0.0000831	1.086
site.sets	0.0000831	0.05362
site.sets.reps	0.01899	0.03609
sets.males	0.8087	0.5006
sets.females	2.027	1.216
sets.males.females	0.08858	0.08869
site.sets.males	0.0000831	0.04623
site.sets.females	0.04182	0.06817
site.sets.males.females	0.0000831	0.09905
units	0.8311	0.1073

Table 12a: Analysis of variance for tannin content LR season

Source of variation	d.f.	s.s.	m.s.	v.r.
site	1	510.9	510.9	231.5
sets	1	76.8	76.8	34.8
site.sets	1	1.1	1.1	0.5
site.sets.reps	8	21.3	2.7	1.2
sets.males	6	89.6	14.9	6.8
sets.females	6	981.3	163.5	74.1
sets.males.females	18	152.1	8.4	3.8
site.sets.males	6	102.2	17.0	7.7
site.sets.females	6	43.2	7.2	3.3
site.sets.males.females	18	163.7	9.1	4.1
Residual	120	264.8	2.2	
Total	191	2406.9		

Table 12b: Estimated Components of Variance and standard errors (s.e.) for tannin content LR-season

		s.e.
site	5.311	7.773
sets	0.0002207	2.775
site.sets	0.0002207	0.5700
site.sets.reps	0.02845	0.08508
sets.males	0.0002207	0.6067
sets.females	6.541	4.005
sets.males.females	0.0002207	0.7146
site.sets.males	0.6621	0.8578
site.sets.females	0.0002207	0.5053
site.sets.males.females	2.296	1.015
units	2.207	0.2849

Table 13a: Analysis of variance for 90% maturity LR season

Source of variation	d.f.	s.s.	m.s.	v.r.
site	1	1271.0	1271.0	200.4
sets	1	713.0	713.0	112.4
site.sets	1	7.5	7.5	1.2
site.sets.reps	8	53.4	6.7	1.1
sets.males	6	319.1	53.2	8.4
sets.females	6	865.9	144.3	22.8
sets.males.females	18	212.6	11.8	1.9
site.sets.males	6	129.6	21.6	3.4
site.sets.females	6	93.9	15.6	2.5
site.sets.males.females	18	110.6	6.1	1.0
Residual	120	761.3	6.3	
Total	191	4538.0		

Table 13b: Estimated Components of Variance and standard errors (s.e.) for 90% maturity LR season

		s.e.
site	13.16	19.09
sets	5.739	10.91
site.sets	0.0006344	0.9913
site.sets.reps	0.02083	0.2148
sets.males	1.080	1.400
sets.females	5.125	3.502
sets.males.females	0.9444	0.7547
site.sets.males	1.288	1.064
site.sets.females	0.7917	0.7825
site.sets.males.females	0.0006344	0.7561
units	6.344	0.8190

Table 14a: Analysis of variance for 100-seed weight LR season

Source of variation	d.f.	s.s.	m.s.	v.r.
site	1	203.0	203.0	138.0
sets	1	139.7	139.7	95.0
site.sets	1	12.0	12.0	8.2
site.sets.reps	8	8.1	1.0	0.7
sets.males	6	1472.6	245.4	166.9
sets.females	6	1395.3	232.5	158.1
sets.males.females	18	42.3	2.3	1.6
site.sets.males	6	22.3	3.7	2.5
site.sets.females	6	11.3	1.9	1.3
site.sets.males.females	18	30.7	1.7	1.2
Residual	120	176.5	1.5	
Total	191	3513.8		

Table 14b: Estimated Components of Variance and standard errors (s.e.) for 100-seed weight LR season

		s.e.
site	1.989	3.002
sets	0.0001471	7.420
site.sets	0.1783	0.3711
site.sets.reps	0.0001471	0.04755
sets.males	10.05	5.905
sets.females	9.584	5.594
sets.males.females	0.1072	0.1613
site.sets.males	0.1675	0.1850
site.sets.females	0.01528	0.1025
site.sets.males.females	0.07843	0.1999
units	1.471	0.1899

Table 15a: Analysis of variance for cooking time LR season

Source of variation	d.f.	s.s.	m.s.	v.r.
site	1	212.5	212.5	133.7
sets	1	1281.0	1281.0	806.1
site.sets	1	5.3	5.3	3.4
site.sets.reps	8	7.9	1.0	0.6
sets.males	6	682.0	113.7	71.5
sets.females	6	1487.0	247.8	155.9
sets.males.females	18	86.2	4.8	3.0
site.sets.males	6	6.1	1.0	0.6
site.sets.females	6	10.1	1.7	1.1
site.sets.males.females	18	13.7	0.8	0.5
Residual	120	190.8	1.6	
Total	191	3983.0		

Table 15b: Estimated Components of Variance and standard errors (s.e.) for cooking time LR season

		s.e.
site	2.158	3.153
sets	9.596	18.97
site.sets	0.08346	0.2038
site.sets.reps	0.0001590	0.05138
sets.males	4.527	2.756
sets.females	10.09	5.983
sets.males.females	0.6713	0.3244
site.sets.males	0.02083	0.09894
site.sets.females	0.07639	0.1284
site.sets.males.females	0.0001590	0.1895
units	1.590	0.2052

Table 16a: Analysis of variance for water absorption LR season

Source of variation	d.f.	s.s.	m.s.	v.r.
site	1	2.755E+02	2.755E+02	327.08
sets	1	2.776E+03	2.776E+03	3294.93
site.sets	1	0.521E+00	0.521E+00	0.62
site.sets.reps	8	1.692E+01	2.115E+00	2.51
sets.males	6	1.931E+03	3.218E+02	382.05
sets.females	6	7.880E+03	1.313E+03	1559.02
sets.males.females	18	4.238E+02	2.354E+01	27.95
site.sets.males	6	6.458E+00	1.076E+00	1.28
site.sets.females	6	1.875E+00	0.313E+00	0.37
site.sets.males.females	18	1.229E+01	0.683E+00	0.81
Residual	120	1.011E+02	0.842E+00	
Total	191	1.342E+04		

Table 16b: Estimated Components of Variance and standard errors (s.e.) for water absorption LR season

		s.e.
site	2.865	4.088
sets	12.13	41.72
site.sets	0.0000842	0.07958
site.sets.reps	0.07951	0.06643
sets.males	12.41	7.753
sets.females	53.75	31.61
sets.males.females	3.810	1.318
site.sets.males	0.03279	0.06391
site.sets.females	0.0000842	0.04685
site.sets.males.females	0.0000842	0.1004
units	0.8424	0.1087

Table 17: Analysis of variance table for percent seed germination of eight cultivars of dry bean seed stored for nine months at varying temperature and relative humidity

Source	df	Sum of squares	Mean square	F value
Replication	2	9.6	4.8	0.7
Genotype (G)	7	3142.1	448.9	67.0
Error	14	93.8	6.7	
Storage time(S)	2	22318.6	11159.3	1212.2
G x S	14	880.4	62.9	6.8
Error	32	294.6	9.2	
Temperature (T)	2	2751.4	1375.7	192.4
G x T	14	94.7	6.83	0.9
S x T	4	61.3	15.3	2.1
G x S x T	28	259.9	9.3	1.3
R. Humidity (H)	1	665.2	665.1	93.0
G x H	7	32.5	4.6	0.6
S x H	2	17.9	9.0	1.3
G x S x H	14	76.5	5.5	0.8
T x H	2	1.6	0.8	0.1
G x T x H	14	73.5	5.3	0.7
S x T x H	4	3.7	0.9	0.1
G x S x T x H	28	123.2	4.4	0.6
Error	240	1716.0	7.2	
Total	431	32616.4		

Coefficient of Variation: 3.18%

Table 18: Analysis of variance table for water absorption of eight cultivars of dry bean seed stored for nine months at varying temperature and relative humidity

Source	df	Sum of squares	Mean square	F value
Replication	2	42.8	21.4	0.8
Genotype (G)	7	87978.7	12568.4	473.6
Error	14	371.5	26.5	
Storage time (S)	2	18842.0	9421.0	740.5
G x S	14	3781.1	270.1	21.2
Error	32	407.1	12.7	
Temperature (T)	2	2034.1	1017.1	66.2
G x T	14	327.2	23.4	1.5
S x T	4	3.2	0.8	0.1
G x S x T	28	658.6	23.5	1.5
R. Humidity (H)	1	736.3	736.3	47.9
G x H	7	72.7	10.4	0.7
S x H	2	32.2	16.1	1.0
G x S x H	14	220.4	15.7	1.0
T x H	2	0.04	0.02	0.001
G x T x H	14	245.2	17.5	1.1
S x T x G	4	39.9	10.0	0.6
G x S x T x H	28	379.5	13.6	0.9
Error	240	3689.2	15.4	
Total	431	119861.9		

Coefficient of Variation: 6.03%

Table 19: Analysis of variance table for cooking time index of eight cultivars of dry bean seed stored for nine months at varying temperature and relative humidity

Source	df	Sum of squares	Mean square	F value
Replication	2	11.6	5.8	0.7
Genotype (G)	7	42544.5	6077.8	712.7
Error	14	119.4	8.5	
Storage time (S)	2	28055.5	4027.8	3028.5
G x S	14	1062.9	75.9	16.4
Error	32	148.2	4.6	
Temperature (T)	2	2198.8	1099.4	259.3
G x T	14	82.6	5.9	1.4
S x T	4	23.7	5.9	1.4
G x S x T	28	125.7	4.5	1.1
R. Humidity (H)	1	705.3	705.3	66.4
G x H	7	26.6	3.8	0.9
S x H	2	19.3	9.6	2.3
G x S x H	14	32.7	2.3	0.6
T x H	2	27.5	13.8	3.2
G x T x H	14	18.6	1.3	0.3
S x T x H	4	8.8	2.2	0.5
G x S x T x H	28	56.2	2.0	0.5
Error	240	1017.4	4.2	
Total	431	76285.5		

Coefficient of Variation: 3.81%

Table 20: Analysis of variance for cooking time of 16 entries of accelerated aged SR dry bean seed grown in SR growing season

Source	df	Sum of squares	Mean square	F value
Replication	2	2.69	1.34	0.80
Location (L)	1	597.50	597.50	355.72
Genotype (G)	15	43522.21	2901.48	1727.37
G x L	15	67.79	4.52	2.69
Seed cond.	1	20460.44	20460.44	12180.95
L x C	1	32.09	32.09	19.10
G x C	15	1114.35	74.29	44.23
L x G x C	15	38.71	2.58	1.54
Soaking(S)	1	2953.71	2953.71	1758.47
L x S	1	70.90	70.90	42.21
G x S	15	1454.41	96.961	57.72
L x G x S	15	67.23	4.48	2.67
C x S	1	3743.75	3743.75	2228.81
L x C x S	1	33.25	33.25	19.81
G x C x S	15	817.87	54.51	32.46
L x G x C x S	15	50.37	3.36	2.00
Error	254	426.65	1.68	
Total	383	75454.00		

Coefficient of Variation: 2.41%

Table 21: Analysis of variance for cooking time of 16 entries of accelerated aged dry bean seed grown in LR growing season

Source	df	Sum of squares	Mean square	F value
Replication	2	12.56	6.28	3.13
Location (L)	1	858.01	858.01	428.36
Genotype (G)	15	35993.66	2399.52	1197.97
G x L	15	38.49	2.57	1.28
Seed cond.	1	13183.59	13183.59	6581.81
L x C	1	44.01	44.01	21.97
G x C	15	928.41	61.89	30.90
L x G x C	15	27.49	1.83	0.91
Soaking(S)	1	4873.50	4873.50	2433.06
L x S	1	80.67	80.67	40.27
G x S	15	885.67	59.04	29.48
L x G x S	15	50.00	3.33	1.66
C x S	1	330.04	330.04	164.77
L x C x S	1	126.04	126.04	62.93
G x C x S	15	242.13	16.14	8.06
L x G x C x S	15	26.63	1.76	0.87
Error	254	508.77	2.00	
Total	383	58210.00		

Coefficient of Variation: 2.94%

Table 22: Daily Weather Data for 1993/94 Growing Season at Sokoine University of Agriculture (SUA).

Day of Year	Month	Day	Temperature °C Maxim.	Minim.	Solar radiation (MJ m^{-2})	Rainfall (mm)
94060	March	01	31.0	22.0	16.6	1.2
94061	March	02	31.5	20.5	18.4	0.0
94062	March	03	30.5	21.5	15.8	1.2
94063	March	04	31.2	19.5	21.8	18.0
94064	March	05	30.5	21.2	17.1	4.2
94065	March	06	29.7	20.8	17.5	6.2
94066	March	07	31.0	22.0	18.5	0.0
94067	March	08	31.7	21.6	20.2	0.0
94068	March	09	31.5	21.3	22.2	0.0
94069	March	10	31.5	19.8	17.3	0.0
94070	March	11	32.0	20.0	22.7	0.0
94071	March	12	32.4	20.9	17.1	0.0
94072	March	13	32.0	20.0	17.6	0.0
94073	March	14	30.7	19.5	13.4	0.0
94574	March	15	31.1	20.6	17.4	7.4
94065	March	16	32.5	20.7	19.2	0.0
94076	March	17	28.5	21.0	7.2	1.5
94077	March	18	30.7	19.5	7.2	7.2
94078	March	19	29.6	20.0	18.1	6.4
94079	March	20	30.0	19.0	15.0	0.0
94080	March	21	30.5	20.5	14.6	0.6
94081	March	22	31.0	19.5	15.3	1.7
94082	March	23	31.6	20.2	14.6	2.2
94083	March	24	30.4	20.0	14.3	0.0
94084	March	25	31.5	19.5	16.3	2.1
94085	March	26	30.3	21.9	14.3	12.0
94086	March	27	30.5	18.4	16.4	6.4
94087	March	28	31.6	20.0	21.3	0.0
94088	March	29	32.0	21.5	20.3	0.0
94089	March	30	31.7	21.5	16.2	0.3
94090	March	31	30.7	22.5	15.6	0.0
Mean	**values**		**29.0**	**21.1**	**17.3**	**2.2**

Table 22 (cont.)

Day of Year	Month	Day	Temperature °C Maxim.	Minim.	Solar radiation (MJ m^{-2})	Rainfall (mm)
94091	April	01	30.5	21.4	19.5	0.0
94092	April	02	32.0	17.1	16.3	0.0
94093	April	03	32.0	18.8	22.0	0.0
94094	April	04	30.0	19.9	12.5	0.0
94095	April	05	28.0	20.5	11.8	1.3
94096	April	06	28.0	20.3	10.6	31.9
94097	April	07	26.4	20.3	9.7	11.1
94098	April	08	29.5	18.1	13.2	6.9
94199	April	09	29.0	20.0	14.7	24.4
94100	April	10	27.5	20.5	10.9	24.2
94101	April	11	27.1	20.0	10.6	4.1
94102	April	12	29.4	19.8	15.0	15.0
94103	April	13	29.8	18.0	17.9	1.0
94104	April	14	30.1	19.2	15.7	0.0
94105	April	15	30.5	20.8	20.6	7.1
94106	April	16	30.5	20.4	12.7	2.6
94107	April	17	30.5	19.5	20.1	0.0
94108	April	18	33.3	20.0	17.8	0.0
94119	April	19	31.5	18.3	17.5	5.9
94110	April	20	28.5	20.2	12.5	7.2
94111	April	21	26.5	20.2	7.8	16.0
94112	April	22	31.0	20.6	19.1	14.0
94113	April	23	30.0	19.4	15.6	1.2
94114	April	24	27.0	20.3	9.0	26.0
94115	April	25	29.0	19.6	18.1	26.5
94116	April	26	29.0	18.8	11.7	35.0
94117	April	27	27.6	19.5	14.4	5.9
94118	April	28	28.4	17.0	14.5	0.0
94119	April	29	26.5	17.5	11.0	13.4
94120	April	30	23.5	19.6	3.7	33.0
Mean values			**29.0**	**19.5**	**14.2**	**10.6**

Table 22 (cont.)

94121	May	01	25.2	19.2	9.2	0.5
94122	May	02	24.5	19.8	6.8	8.0
94123	May	03	27.5	17.5	13.7	17.2
94124	May	04	28.5	19.5	14.4	16.1
94125	May	05	30.3	20.5	13.3	0.6
94126	May	06	30.7	19.8	18.1	0.6
94127	May	07	29.5	19.4	19.7	0.8
94128	May	08	27.0	19.5	10.6	0.0
94129	May	09	26.5	19.6	11.6	0.0
94130	May	10	27.3	18.8	11.1	0.0
94131	May	11	25.5	20.0	7.5	15.0
94132	May	12	25.5	19.0	6.7	8.4
94133	May	13	29.2	19.8	11.8	9.6
94134	May	14	27.5	19.4	11.0	0.0
94135	May	15	28.4	19.8	14.8	18.5
94136	May	16	29.5	19.5	14.1	0.0
94137	May	17	28.9	18.9	14.5	0.8
94138	May	18	29.2	19.0	16.0	1.1
94139	May	19	25.6	18.8	9.8	3.7
94140	May	20	28.6	19.0	16.2	6.9
94141	May	21	29.5	19.1	16.2	1.9
94142	May	22	27.8	18.7	14.3	0.5
94143	May	23	29.5	17.6	19.5	0.0
94144	May	24	28.6	17.1	18.8	0.0
94145	May	25	29.0	17.5	16.5	0.5
94146	May	26	26.0	19.0	11.2	0.0
94147	May	27	26.5	16.0	11.4	0.0
94148	May	28	26.0	15.1	11.1	0.0
94149	May	29	26.0	16.2	11.0	0.0
94150	May	30	25.0	16.0	6.4	1.9
94151	May	31	26.8	17.5	11.1	0.0
Mean values			**27.6**	**18.6**	**12.9**	**5.9**

Table 22 (cont.)

94152	June	01	26.8	13.2	18.6	0.0
94153	June	02	28.0	12.6	17.0	0.0
94154	June	03	28.5	14.5	19.0	0.0
94155	June	04	28.6	13.9	19.4	0.0
94156	June	05	29.0	15.7	17.1	0.0
94157	June	06	29.5	14.8	21.2	0.0
94158	June	07	29.3	14.1	20.7	0.0
94159	June	08	28.5	15.4	15.5	0.0
94460	June	09	27.5	15.0	14.3	0.0
94161	June	10	27.2	14.0	15.9	0.0
94162	June	11	27.5	12.4	19.5	0.0
94163	June	12	26.5	11.2	18.9	1.2
94164	June	13	28.3	11.0	17.1	0.0
94165	June	14	27.7	13.9	16.2	0.0
94166	June	15	26.2	16.3	11.5	0.0
94167	June	16	27.0	14.4	15.2	0.0
94168	June	17	28.5	13.0	18.7	0.0
94169	June	18	27.0	13.2	15.4	0.0
94170	June	19	25.0	13.8	12.6	0.0
94171	June	20	26.6	14.1	15.0	0.0
94172	June	21	25.5	13.7	13.2	0.2
94173	June	22	23.0	16.1	5.8	6.6
94174	June	23	28.5	16.2	17.1	0.0
94175	June	24	28.8	16.3	17.5	0.0
94176	June	25	28.5	14.2	16.9	0.0
94177	June	26	28.6	13.2	14.0	0.2
94178	June	27	29.0	17.0	17.5	0.0
94179	June	28	27.8	17.0	14.3	0.0
94180	June	29	27.0	16.6	12.9	0.0
94181	June	30	28.5	12.5	27.2	0.0
Mean values			**27.6**	**14.3**	**16.4**	**0.3**

Table 22 (cont.)

94182	July	01	29.0	13.9	17.8	4.7
94183	July	02	24.4	18.2	6.1	0.0
94184	July	03	27.4	17.1	12.9	0.2
94185	July	04	27.5	16.1	13.8	13.6
94186	July	05	28.0	18.0	16.5	1.1
94187	July	06	27.0	16.5	12.9	17.2
94188	July	07	25.5	17.4	10.6	0.0
94189	July	08	27.4	14.5	17.1	0.0
94190	July	09	28.0	14.5	17.4	0.0
94191	July	10	29.3	13.5	18.7	0.0
94192	July	11	29.2	15.1	16.9	0.0
94493	July	12	28.1	15.0	19.0	0.0
94194	July	13	25.0	16.1	10.8	0.0
94195	July	14	27.0	11.4	19.1	0.0
94196	July	15	26.5	10.2	19.0	0.0
94197	July	16	28.4	11.1	21.0	0.0
94198	July	17	27.0	10.8	16.2	0.0
94199	July	18	27.5	14.0	16.2	0.0
94200	July	19	27.6	13.7	18.3	0.0
94201	July	20	28.0	16.1	12.7	0.0
94202	July	21	27.0	17.5	15.9	0.0
94203	July	22	27.9	16.0	13.4	0.0
94204	July	23	29.0	17.4	10.3	0.0
94205	July	24	24.9	19.2	16.1	0.0
94206	July	25	28.7	14.8	16.1	0.0
94207	July	26	30.0	15.5	18.6	0.0
94208	July	27	30.0	16.2	19.3	0.0
94209	July	28	27.6	15.0	18.7	0.0
94210	July	29	27.0	16.0	18.2	0.0
94211	July	30	27.7	14.6	16.2	0.0
94212	July	31	26.2	16.8	7.0	0.0
Mean values			**27.5**	**15.2**	**16.6**	**1.2**

Table 22 (cont.)

94213 August 01	27.5	16.0	18.4	0.0
94214 August 02	26.5	15.1	11.0	8.4
94215 August 03	24.6	16.1	8.6	0.5
94216 August 04	25.9	6.6	12.5	0.0
94217 August 05	28.0	13.1	19.3	0.0
94218 August 06	28.0	14.6	22.9	0.0
94219 August 07	27.0	15.4	14.2	0.0
94220 August 08	27.5	15.5	18.1	0.0
94221 August 09	29.0	17.0	17.9	0.0
94222 August 10	30.3	14.1	18.1	0.0
94223 August 11	28.2	5.0	17.7	0.0
94224 August 12	28.5	17.4	14.0	0.0
94225 August 13	30.0	16.0	19.3	0.0
94226 August 14	29.0	14.7	19.9	0.0
94227 August 15	28.3	15.5	13.4	0.0
94228 August 16	28.2	16.8	16.8	0.0
94229 August 17	30.0	16.0	17.1	0.0
94230 August 18	29.0	14.8	17.8	0.0
94231 August 19	30.5	17.6	16.9	0.5
94232 August 20	24.5	17.2	6.3	0.5
94233 August 21	27.5	15.6	16.2	0.0
94234 August 22	28.6	14.1	19.9	0.0
94435 August 23	28.8	15.2	19.5	4.7
94236 August 24	27.5	16.8	15.7	0.0
94237 August 25	28.6	15.1	16.2	2.7
94238 August 26	28.5	17.4	12.4	1.0
94239 August 27	28.2	16.6	16.0	0.4
94240 August 28	28.6	15.8	16.2	0.0
94241 August 29	29.0	16.1	18.1	0.0
94242 August 30	29.5	15.2	18.5	0.0
Mean values	**28.1**	**15.8**	**16.0**	**0.6**