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INHERITANCE OF ANTIOXIDANT ACTIVITY AND ITS ASSOCIATION WITH SEED COAT COLOR IN COWPEA (*Vigna unguiculata* (L.) Walp.)

A Thesis

by

MAGNIFIQUE NDAMBE NZARAMBA

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2004

Major Subject: Horticulture

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Approved as to style and content by:

J. Creighton Miller, Jr. (Chair of Committee)	Jeffrey D. Hart (Member)
Frank M. Hons (Member)	Tim Davis (Head of Department)

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ABSTRACT

Inheritance of Antioxidant Activity and Its Association with Seed Coat Color in Cowpea, (*Vigna unguiculata* (L.) Walp). (May 2004)

Magnifique Ndambe Nzaramba, B.Sc., Makerere University;

Chair of Advisory Committee: Dr. J. Creighton Miller, Jr.

Analysis of antioxidant activity (AOA) of entries in the 2002 Regional Southernpea Cooperative Trial revealed not only significant differences among entries, but that entries with pigmented (black and red) seed coats were clustered among the highest, cream types were the lowest, while pinkeye and blackeye types were intermediate. Red colored peas were higher in antioxidant activity than black types. These findings provided strong evidence that compounds responsible for pigmentation were involved in AOA. The objectives of the present investigation were to investigate the inheritance of AOA in cowpea and further study the relationship between AOA and seed coat color.

Four advanced selections, ARK95-356 (black), ARK98-348 (red), ARK96-918 (cream), and LA92-180 (cream), were crossed in a complete diallel mating design, generating F_1, F_1', F_2, F_2', BC_1, and BC_2 populations. Individual seeds were ground and samples were extracted in methanol and analyzed for AOA using the free radical 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) method.

Combining ability tests using Griffing's Method I Model I indicated presence of highly significant general combining ability (GCA), specific combining ability (SCA),

and reciprocal (REC) and maternal (MAT) effects, with pigmented lines exhibiting positive GCA and MAT, while non-pigmented lines exhibited negative GCA and MAT. AOA in the F_1 was not significantly different from the maternal parent, with seed coat color also resembling the maternal parent. Segregation for seed coat color was observed in the F_2 and F_2'. Additive, dominance, and epistatic effects were significant. The broad sense heritability estimate was 0.87. Minimum number of genes responsible for AOA was estimated at about five. Factors governing high AOA appeared to be the same as those responsible for seed coat color, with apparent pleiotropic effects. In conclusion, breeding for high AOA is possible using highly pigmented parental lines.

ACKNOWLEDGEMENTS

Sincere appreciation is expressed to Dr. J. Creighton Miller, Jr. for the advice, guidance, encouragement and support offered to me during my education. I am grateful to my committee members, Dr. Jeffrey D. Hart and Dr. Frank M. Hons, for their advice and time put into my education. Thanks are extended to Dr. Jeannie Miller for proofreading and valuable revisions made to this thesis.

Special thanks to Douglas C. Scheuring and Dr. Anna L. Hale for their profound technical help, for without it this study could not have been completed. My thanks to the Vegetable Legume Improvement Group, Lavanya, Tyann, Robin and Kristen for their help.

I must thank the PEARL-RWANDA project for funding my studies. And, of course, I am grateful to my parents, Rev. Canon Martin and Espérence Nzaramba, and my brothers and sisters for their prayers and moral support.

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CHAPTER I

INTRODUCTION

"Eating healthy" has been a common slogan among educated consumers. This means eating a balanced diet that provides sufficient proteins, carbohydrates, fats, and vitamins. In recent years, dietary fiber was added to the list. Plant scientists, through breeding, have expended substantial effort in increasing the availability of all these compounds in food crops.

In countries where availability of these nutrients is still an issue due to insufficient crop production and yield, especially developing countries, the meaning of "healthy foods" is still the same. However, in developed nations, the meaning is changing as consumers become more health conscious. With the discovery of health benefits of certain plant compounds that have antioxidant capabilities, the term is changing and now includes such compounds as carotenoids, flavonoids, anthraquinones etc., which are believed to possess tremendous health benefits such as antioxidant activity.

These antioxidant compounds are still referred to as non-nutrients, implying that they are not yet qualified to be in the same category as proteins, carbohydrates, vitamins, and fats. Duyff (2002) referred to these compounds as phytonutrients, meaning plant chemicals. He also categorized them differently from vitamins and minerals.

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In all likelihood they will soon be upgraded to the level of essential nutrients, as more research findings support and /or confirm their ultimate necessity in the livelihood of animals and humans as well as plants that produce them.

Since the early 1980s, research has intensified in investigating the health benefits of phytonutrients (Duyff, 2002), and several research reports have already indicated that the benefit of plant foods is not solely due to the levels of vitamins or other nutritive factors they contribute to the diet, but may actually be due to activity of the non-nutritive factors found in many plants. Many of these plant-secondary components are antioxidants (Riedl et al., 2002), and it is believed that they underlie the correlation between consumption of diets rich in fruits, vegetables and grains and a reduced risk of diseases such as cancer and heart disease (Hertog et al., 1993).

Seeds are the most economically valuable plant part of cowpea, as with other grain legumes. Recent studies have indicated that legume seeds possess compounds with antioxidant activity (Narasinga, 1995; Beninger et al., 1998; Troszyńska et al., 2002).

At Texas A&M University, the Vegetable Legume Improvement Group evaluated a number of entries in the 2001 and 2002 Regional Southernpea Cooperative Trials for antioxidant activity. Preliminary results indicated that cultivars differed in antioxidant activity levels, with colored types showing higher activity than the less colored and cream types (Warrington et al., 2002).

Variability among cultivars under similar conditions is an indication of genetic control, offering an opportunity to breed cowpeas with enhanced antioxidant levels. Yamaguchi et al. (1983) reported that if there is wide variation in the content of any

nutrient in a given population grown under the same conditions, it is possible by breeding and selection to increase the content of that nutrient greater than the mean of the original population.

In order to breed for increased antioxidant activity in cowpea seed, determination of the inheritance of antioxidant activity is necessary. Also, ascertaining the relationship between antioxidant activity and seed coat color would help breeders improve cowpea varieties while keeping in mind color preferences of consumers. Most of the preferred cultivars on the market are blackeye, pinkeye, and cream types that are not highly colored and also have moderate to low antioxidant activity, respectively.

The principal aim of this research was to elucidate the genetics governing antioxidant activity in cowpea seeds, which could therefore be utilized to improve cowpea varieties for this trait. The specific objectives of the study were to a) elucidate the nature of the inheritance of antioxidant activity in cowpea seed by estimating heritability, number of genes involved, their additive and dominance effects, and non-allelic effects and b) further investigate the relationship between antioxidant activity and seed coat color.

CHAPTER II

LITERATURE REVIEW

Background

Cowpea (*Vigna unguiculata* (L) Walp.) is one of the most ancient food sources and has probably been used as a crop plant since Neolithic times (Summerfield et al., 1974). The precise location of the center of origin of cowpea has been difficult to determine. It is a broadly-adapted and highly-variable crop cultivated around the world primarily as a pulse, especially in tropical and subtropical areas, but also as a vegetable legume for both greens and grain and as a cover crop and fodder.

The economic cowpea species (*Vigna unguiculata*) exhibits a number of attributes that make it valuable in many cropping systems. It is grown successfully in extreme environments such as high temperature and low rainfall, and does well on poor soils with few economic inputs. Cowpea is a nutritious source of food, providing proteins, vitamins, minerals and carbohydrates. The dry seed contain about 20.5 - 31.7% protein, 1.14 – 3.03% fat, 1.70 – 4.5% fiber, 56.0 – 65.7% carbohydrate, a moisture content of 6.20 - 8.92% (Onwuliri and Obu, 2002), and 3.6% ash (Smatt, 1976). The protein profile complements roots, tubers or cereals, especially in areas where people cannot afford higher protein foods such as meat.

The need to improve the human diet has motivated researchers to examine the levels of essential and non-essential nutrients and anti-nutritional factors in crop plants. Much emphasis has been placed on evaluating vegetables, fruits and legumes for all

available nutrients, including antioxidant compounds. Many plant foods such as grains, oilseeds and legumes, as well as herbs, spices and tea, contain phenolic compounds with potential antioxidant activity (Shahidi et al., 1992).

There is an increasing demand to utilize antioxidants from direct plant extracts or isolated products of plant origin rather than developing synthetic products. This is due to the current interest in low-density lipoproteins and protection of important cells and organs, as well as food systems, against oxidative damage caused by superoxide, hydroxyl and peroxyl radicals (McClements and Decker, 2000).

The challenge and responsibility of horticulturists is to design approaches for improving the nutritional quality and visual appeal of the food supply in order to provide a sustainable, inexpensive complement to medical and social programs that are striving to prevent human diseases (Simon, 1997).

Importance of cowpea

Like other legumes, cowpea plays an important role in the traditional diets in many regions of the world, especially Asia, Africa and South America, which include most of the world's developing countries. Pulses are the main source of protein in the primarily vegetarian Indian diet (Narasinga, 1995). In Western countries, they tend to play a minor dietary role, despite the fact that they are low in fat and excellent sources of protein, dietary fiber, minerals and a variety of micronutrients.

All parts of cowpea plants are used for food or fodder. Fresh young leaves, immature pods, and peas are used as vegetables, while dry grain is used to prepare main meal dishes and snacks (Quin, 1997). The edible parts are nutritious, providing protein,

vitamins, and minerals, thus making cowpea extremely valuable to many people who cannot afford animal protein (Karakaya et al., 2001).

After harvest of the pods, the haulms are used for fodder. The whole plant is used for hay or grazing where lack of rainfall or irrigation water does not permit the production of fine-stemmed and easily cured fodder such as alfalfa and clover. Sellschop (1962) reported that cowpea hay is as equally digestible as alfalfa, and that cowpea fiber is more digestible than that of alfalfa. Van Wyk (1955) found that cowpea hay is richer in total digestible nutrients than alfalfa. Cowpea is also used for ensiling in mixtures with sorghum and maize in the U.S.A (Bogdan, 1977).

In some countries in western and central Africa, farmers cut and store cowpea fodder for sale at the peak of the dry season, making the residues (haulms) a highly remunerative commodity. It has been reported that these farmers can obtain as much as 25% of their annual income by selling the haulms (Quin, 1997).

Cowpea is used as a cover crop, mainly in cropping systems where intercropping is practiced. An increase of 8.3 % was observed in the weight of single plants of sorghum inter-cropped with cowpea compared with that of sorghum grown alone (Chundawat, 1971). As a plant of diverse habit with spreading indeterminate and semi determinate bushy growth, it provides a good ground cover that protects the soil against erosion and suppresses weed growth. The cowpea crop withstands moderate shading, making it valuable as a cover crop in orchards.

Cowpea has been used as a restorative crop throughout the southern states of the U.S. (Wheeler, 1950). It grows well on soils that do not produce profitable yields of

other legumes and cereals. Wheeler (1950) reported that cowpea improves the physical condition of the soil, making heavy clay soils more open and sandy soils more compact.

Some cowpea varieties have been reported to cause suicidal germination of the seeds of *Striga hermonthica*, which is a notorious weed that infests cereals, often with devastating effects (Quin, 1997).

Cowpea plants are capable of fixing atmospheric nitrogen through symbiosis with bacteria (*Bradyrhizobium spp.*) (Miller et al., 1986; Pemberton et al., 1990). Miller (1989) reported that levels of biological nitrogen fixation in cowpea can be improved with genetic manipulation of the plants. Bogdan (1977) reported that in areas previously cultivated with cowpea, no seed inoculation is necessary. Cowpea fixes a larger fraction of total nitrogen accumulated by the crop than common beans, and its total fixation of nitrogen compares favorably with that of soybean.

Burkitt and Trowell (1975) suggested that the dietary fiber present in legumes plays a role in the etiology of chronic diseases. They reported that dietary fiber may be beneficial in preventing several diseases that are common in western societies.

Several legumes, cowpea included, have been found to contain non-nutrient, bioactive phytochemicals (Anderson et al., 1999; Messina, 1999). Some of the important health promoting, non-nutrient compounds present in pulses and legumes include non-starch polysaccharides, phytosterols, saponins, isoflavone (a class of phytoestrogens), phenolic compounds (Narasinga, 1995), and antioxidants (Warrington et al., 2002; Nzaramba et al., 2003).

Cowpea production

Cowpea is grown under a wide range of climatic conditions, but mostly in the tropics and subtropics. It is tolerant to heat and relatively dry conditions. A study conducted by Bagnall and King (1987) showed that maximum yields are obtained at $27/22^{0}$C (day/night) temperatures.

Cowpea is able to maintain some growth, or at least survive, under dry soil conditions (Bogdan, 1977; Quin, 1997). It is generally considered that dehydration in cowpea is markedly delayed due to morphological or physiological modifications that reduce transpiration or increase adsorption. Under drought conditions, cowpea can maintain high leaf and xylem water potential by complete stomatal closure. Drought resistance in some varieties is explained partially by the deep rooting system of these varieties, and it also accounts for the crop's ability to grow in the semidesert conditions of the African Sahel and northern Brazil (Quin, 1997).

Cowpea is adapted to a wide range of soils, from sands to clays. The primary soil requirements are good drainage and presence of or inoculation with the proper nitrogen-fixing bacteria (Miller, 1989). Fageria (1991) reported that cowpea can thrive on acid soils. Good yields were obtained around pH 6 in an Oxisol of central Brazil. Wheeler (1950) reported that no other legume can be grown so successfully on such a variety of soils under adverse conditions as the cowpea. It grows as well on sandy soils as on heavy clays, and on thin soils and soils poor in lime.

It has been reported that a very rich soil is not necessary for cowpea (Wheeler, 1950). On such soils, the crop exhibits abundant vine growth, while grain yield is low. Poor soils produce little growth of vines, but generally yield a good proportion of seed.

World cowpea production statistics are difficult to obtain, as most countries do not keep separate records due to the many different ways cowpeas are utilized, most of which do not permit estimating yield, e.g., green fodder, hay, grazing by wild game and for ensiling in mixtures with sorghum or maize. It is estimated that cowpea is cultivated on at least 12.5 million hectares, with an annual production of over 3 million tons worldwide (Singh, et al., 1997). FAO unpublished estimates indicate that 3.3 million tons of dry cowpea grain were produced worldwide in 2000. The largest acreage is in Africa, with Nigeria and Niger predominating, but Brazil, West Indies, India, U.S.A., Burma, Sri Lanka and Australia all have significant production. Production figures in the U.S.A. were estimated at about 800,000 hectares/year. Georgia, California and Texas are the leading producers, accounting for about 60% of the total production in the U.S.A. (Fery, 1981).

In the U.S.A, most of the horticultural cowpea cultivars are classified according to seed and seed-eye color and closeness of spacing of seeds in the pod. Fresh market peas are classified as blackeye, crowder or cream types. Each type has a distinctive appearance and flavor and appeals to a unique market (Fery, 1985).

Blackeye peas are the most popular cultivars, serving most of the dry-seed industry. A variant, the pinkeye, is more popular as a processing vegetable crop because it contains less water-soluble pigments in its seed coat and when cooked produces a

lighter-colored liquor. The blackeye/pinkeye types are noted for good yield (Fery, 1985). Among the crowders, there are a number of seed coat colors and patterns, with brown crowders as the most popular. Cooked crowder peas have a strong or astringent flavor, a granular texture, and are dark in color with a dark liquor. They are the least popular. Cream peas have a much milder, less starchy flavor, are succulent and have a better appearance than the blackeye or crowder peas. The creams have long been popular with the American market.

Importance of antioxidants

Antioxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions (Velioglu et al., 1998; Klein and Kurilich, 2000). The Food and Nutrition Board of the National Academy of Science (1998) defined a dietary antioxidant as a substance in foods that significantly decreases the adverse effects of reactive oxygen species, reactive nitrogen species, or both on normal physiological function in humans.

Antioxidants function by scavenging free radicals via donation of an electron or a hydrogen atom, or by deactivation of prooxidant metal ions and singlet oxygen (Shahidi, 2002). Morello et al. (2002) stated that the primary role of antioxidants is to prevent degredation induced by free radical reactions. They noted that antioxidants function by hydrogen abstraction and metal ion assisted electron transfer. The antioxidant donates hydrogen atoms to the free radicals, thus inhibiting the propagation of the autocatalytic chain reaction.

An effective antioxidant is one that has a high scavenging ability for the target radical, and if the antioxidant is oxidized or rearranged, forms a non-radical species (Hertog et al., 1993). Antioxidants exert their effects by different mechanisms and functions. Therefore, essential in evaluating antioxidant capacity, it is essential to clarify which function is being measured by the method employed (Niki and Noguchi, 2000).

There are two basic categories of antioxidants, synthetic and natural. In general, synthetic antioxidants are compounds with phenolic structures of various degrees of alkyl substitution, whereas natural antioxidants can be phenolic compounds (tocopherols, flavonoids, and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids, and amines), or carotenoides, as well as ascorbic acid (Larson, 1988; Hudson, 1990; Hall and Cuppett, 1997). Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been used as antioxidants since the beginning of the twentieth century. Restrictions on the use of these compounds are being imposed, however, because of their carcinogenicity (Branen, 1975; Ito et al., 1983).

Antioxidants are also classified according to their mechanisms of action: free-radical inhibitor (chain breaker), peroxide decomposer, metal ion inactivator, or oxygen scavenger (Yagi, 1970; Dziezak, 1986).

Aerobic organisms are protected by an array of defense systems against oxidative stress, which is detrimental to life. Various antioxidants with versatile functions constitute an elegant, yet complex, defense network to cope with such oxidative stress. Under certain circumstances, however, the natural defenses can be insufficient, and

administration of exogenous antioxidants as food constituents or therapeutic agents may be beneficial. The assessment of in vivo antioxidant status or antioxidant capacities of biological samples and natural and synthetic compounds has been the subject of extensive studies and arguments (Niki and Noguchi, 2000).

Recently, there has been increasing interest in the protective biochemical function of phytochemicals, especially flavonoids and their related compounds, for the prevention of oxidative damage to organisms caused by reactive oxygen species (ROS) and reactive nitrogen species (RNS). These reactive species are associated with lipid peroxidation and DNA damage, and with malignant transformation in vitro (Klein and Kurilich, 2000). There is considerable evidence that imbalance between ROS/RNS and antioxidant defense systems may lead to chemical modification of biologically relevant macromolecules like DNA, proteins, carbohydrates or lipids (Troszyńska et al., 2002).

There are natural antioxidants present in foods as endogenous constituents. These antioxidants belong to the phenolic group of compounds that includes phenolic acids, phenylpropanoids, flavonoids and isoflavones, anthocyanins and anthocyanidins, phytates, sterols and carotenoids. Vitamins C and E and phospholipids can act as antioxidants in foods (Shahidi, 2002). Efforts have been undertaken to isolate, characterize and extract these phenolics and polyphenols from natural plant sources (Morello et al., 2002). Polyphenolic anthocyanins are responsible for the orange, red, blue, violet and purple color of most plant species and their products. Food industries use colorants extracted from the skin of grapes and other plants (Francis, 1993).

Plants vary in composition of phytochemicals with concomitant protective functions. Hence, for maximum health benefits, sufficient amounts of phytochemicals from a variety of sources such as fruits, vegetables and whole grain-based foods are recommended (Kafui and Rui, 2002). About 5,000 of the phytochemicals present in plants have been identified, and a large percentage still remains unknown (Shahidi and Naczk, 1995).

It has been established that phenolic compounds such as flavonoids, anthraquinones, anthocyanidins and xanthones commonly present in the plant family leguminosae, possess remarkable antioxidant activity (Siddhuraju et al., 2002). In live plants, these compounds protect against oxidative stress and attack by herbivores and act as UV filters and healing agents.

The unique attributes of an antioxidant lead to its unique role in the plant that produces it, in the animal that consumes it, or the processed foods that contain it (Reidl et al., 2002). Phenolic compounds are essential for growth and reproduction of plants and also act as antifeedants and antipathogens. Recognition of symbionts may also be related to the presence of polyphenols in plants (Shahidi and Naczk, 1995). Many properties of plant products are associated with the presence and content of their polyphenolic compounds, and they differentiate plants from one another. The astringency of foods (Clifford, 1997) and the beneficial health-related effects of certain phenolics (Huang and Ferraro, 1992) are important to consumers when present in plant foods.

Investigations have suggested that diets rich in polyphenolic compounds are associated with longer life expectancy (Hertog and Hollman, 1996). These compounds have many health-related properties such as anticancer, antiviral, and anti-inflammatory activities, effects on capillary fragility, and ability to inhibit human platelet aggregation (Benaveto-Garcisa et al., 1997).

Several studies have indicated that polyphenolic compounds in higher plants such as flavonols (Salah et al., 1995), flavonoids (McBride, 1996; McBride, 1999; Comis, 2000), zeaxanthin (Stelljes, 2001), anthraquinones (Yen et al., 2000), xanthones and proanthocyanidins (Minami et al., 1994), act as antioxidants or agents of other mechanisms that contribute to their anticarcinogenic or cardioprotective effects. Several studies have shown that increased dietary intake of natural phenolic antioxidants correlates with reduced coronary heart disease (Deshpande et al., 1996; Stampfer et al., 1993).

Presence of phenolics in foods has an important effect on the oxidative stability and microbial safety of products. Many phenolics in foods have important biological activity related to their inhibitory effects on metagenesis and carcinogenesis (Shahidi, 2002).

Many of the natural antioxidants, especially flavonoids, exhibit a wide range of biological effects, including antibacterial, antiviral, anti-inflammatory, antiallergic, antithrombotic and vasodilatory actions (Cook and Samman, 1996). Antioxidant activity is a fundamental property important for life. Many of the biological functions, such as

antimutagenicity, anticarcinogenicity and antiaging, among others, originate from this property (Huang and Ferraro, 1992).

Recent studies have shown that complex mixtures of phytochemicals in foods provide better protective health benefits than single phytochemicals through a combination of additive and/ or synergistic effects (Eberhardt et al., 2000). Previous studies on health benefits of single antioxidants gave inconsistent results in human clinical trials (Rapola et al., 1997), hence supporting the mixture of phytochemicals theory.

An increasing number of epidemiological studies have shown an inverse correlation between the consumption of antioxidants and the incidence of various diseases such as cancer and heart disease (Block et al., 1992; Hertog et al., 1993). With the increasing intake of dietary antioxidants, legume seeds have also been investigated for their antioxidant properties, with most of these works focusing on beans (Troszyńska et al., 2002).

The importance of antioxidant constituents in the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists, food manufacturers and consumers, as the trend of the future is moving toward functional foods with specific health effects (Velioglu et al., 1998; Robards et al., 1999)

Besides the well-known and traditionally used natural antioxidants from teas, wines, fruits, vegetables and spices (Kanner et al., 1994; Madsen and Bertelsen, 1995; Cao et al., 1996; Wang et al, 1996; Velioglu et al., 1998; Fogliano et al., 1999), many

other plant species have been investigated for novel antioxidants (Economou et al., 1991; Kim et al., 1994). Some natural antioxidants (rosemary and sage) are already exploited commercially either as antioxidant additives or as nutritional supplements (Schuler, 1990).

Antioxidants are incorporated in fats and oils or in foods that contain fats and oils to help retard the oxidation of lipids. Yen and Duh (1994) mentioned that oxidative deterioration occurs spontaneously when materials containing lipids or lipid-containing foods are exposed to air. This interferes seriously with the efficiency of processing, as it can result in organoleptic rancidity in the finished products, making them unacceptable to consumers. Oxidation can also cause degrading effects such as vitamin destruction, nutritional losses, and food discoloration (Sherwin, 1978).

There is an outstanding interest in replacing synthetic antioxidants, which are of safety concern, with natural antioxidants (Chang et al., 2002). People and animals have consumed chemicals that occur naturally in plants for years with no concern raised about their usage (Namiki, 1990). Currently used synthetic antioxidants have, however, been suspected to cause or promote negative health effects (Barlow, 1990), hence stronger restrictions have been placed on their application. There is a trend to replace these synthetic antioxidants with naturally occurring antioxidants (Koleva et al., 2002).

Numerous reports have been published aimed at measuring reactivity toward radicals of antioxidant compounds and complex mixtures such as biological fluids and plant extracts (Prior and Cao, 1999). The reactivities can be measured by following the reaction of antioxidants with stable free radicals such as DPPH (Brand-Williams et al.,

1995), galvinoxyl (Shi and Niki. 1998), and 2,6-di-tert-butyl-4-(4-methoxyphenyl) phenoxyl radical (Mukai et al., 1987). The reactivities toward various radicals can also be measured by the inhibition method in which a free radical species is generated and the effect of the antioxidant is measured from the inhibition of the reference reaction. Various indices such as total reactive antioxidant potential (TRAP), total antioxidant activity (TAA), oxygen radical-absorbing capacity (ORAC), and total antioxidant reactivity (TAR) have been proposed and measured (Prior and Cao, 1999; Arnao et al., 1999).

Function and structure of the seedcoat

The seed coat (testa) is the interface between the embryo and the exterior environment. Its function is to promote seed dispersal, survival in adverse environments and protection from pests and pathogens. It gauges the environment for conditions that favor germination, and is also physiologically and metabolically dynamic providing nourishment for the developing embryo (Murray, 1988). Photoassimilate flow from the maternal tissue to the developing embryo is facilitated by testa-associated invertases that convert sucrose to glucose (King et al., 1997).

It is believed that morphogenic signals for embryo development and seed filling include the metabolites, their gradients across maternal to embryo tissue, and any associated differences in osmotic potential (Wobus and Weber, 1999). In such cases, the testa plays a major role in ensuring a proper signal flow, in addition to nutritionally supporting embryo development.

The seed coat has economic implications in several crops. Seed coat color determines the classification of the different cowpea grain types as well as their popularity (Fery, 1985). The seed coat contributes to seed mass of rape seed/canola (*Brassica napus*; *Brassica rapa*) and thus reduces the oil and protein content of the crop. It persists in oil-extracted meal, and its dark color and the causal phenolic substances are considered anti-nutritional in animal feeds (Heneen and Brismar, 2001).

The seed coats, or testa, of the grain legumes are similar in structure. That of mature soybean has been well characterized, and contains an epidermal layer of palisade cells, or macrosclereids, a sub-epidermal layer of hourglass cells, or osteosclereids, a few layers of parenchyma, and an aleurone layer (Williams, 1950; Corner, 1951).

Several reports have indicated that the seed coat of various grains is of maternal origin, but there are still contradictory results as regards the origin of some of the layers of the seed coat, especially the aleurone layer. In developing seeds, which are physically and physiologically connected to the parent, the testa is the maternal conduit to the embryo (King et al., 1997). The embryo and endosperm develop embedded in maternal tissues of sporophytic origin which eventually form the seed coat, but there are interactions between the various seed tissues that are still complex and unresolved (Grossniklaus et al., 2001). Wan et al. (2002) also reported that the testa is a maternal organ derived from the outer and inner integuments of the ovule. The inner integument becomes compressed and impregnated with pigments imparting the characteristic brown color in Brassicaceae seeds.

Many consider the origin of the aleurone layer in soybean to be derived from the endosperm (Winton and Winton, 1932; Williams, 1950; Carlson and Lerstein, 1987; Yaklich et al., 1992), while others maintain that this layer is an endothelium of maternal origin (Thorne, 1981; Baker et al., 1987). Schmidt et al. (1994) found convincing biochemical evidence of the endosperm origin of the aleurone. They demonstrated by analysis of the expression of proline-rich proteins in seed coats and aleurone layers dissected from F2 seeds, that the seed coats had the F2 (maternal) genotype, whereas the aleurone was F3 (zygotic). This work presented further evidence that the aleurone is actually the outermost layer of the endosperm, which persists at maturity. They also found that the aleurone is a single-celled layer that originates from the embryonic generation, as well as in the outer seed coat. Because the layer is one generation removed from the rest of the seed coat, which is maternal in origin, it may have a different genotype.

Inheritance of seed coat color

The genetics of seed coat color in cowpea has been reported, but there are interactions and modifier genes that are not yet understood (Fery, 1980). The first attempt to explain the inheritance of cowpea seed coat color was by Spillman in 1912. He postulated that a general color factor, C, is responsible for color and its absence results in white seeds. The C factor in combination with R, U, Br, Br and N, and N and B conditions red, buff, brown, black, and blue seed coat, respectively. Harland (1919) proposed a model with R as a general color factor conditioning red seed coat. He

suggested that the R factor with B, N, M, and N and M conditions black, buff, maroon, and brown, respectively.

Spillman and Sando (1930) designated the general color factor as R and described N as an anthocyanin pigment factor. They used symbols B, F, P and U for brown, fine and dense speckling, purple, and buff, respectively, and showed how these genes interacted to produce ten different seed coat colors. Saunders (1960) reported that most of the common colors and color patterns of the cowpea seed coat result from interactions between two or more genes. He stated that the gene responsible for black color is dominant to all but the purple seed color. Calub (1968) suggested that black is epistatic to all colors regardless of the presence of other color genes.

Seed coat patterns are inherited independently of seed coat color, but the appearance of any pattern is dependent upon the presence of the general color factor C (Calub, 1968; Fery, 1980). Drabo et al. (1988) noted that incomplete dominance of several seed coat pattern genes makes classification difficult in progeny segregating for the Holstein, Watson, small eye, and hilum ring traits.

Prakken (1970, 1972) working with a similar legume, *Phaseolus vulgaris*, found that eight major loci contributed to color inheritance. The loci were designated P, C, D, J, G, B, V and Rk. He also reported that C, D and J are the color genes, whereas G, B, V and Rk are modifying genes having an intensifying effect or darkening influence upon pale colors formed by color genes. Kooiman (1931) reported that the complex interactions of major loci affect the seedcoat colors of common beans, rather than the

genetic background. The seedcoat colors of *Phaseolus vulgaris* are believed to be qualitatively inherited (Beninger and Hosfield, 1999).

Bassett (1991) stated that a 'ground factor' gene P is needed to avoid white seed coats (with gene p) or gray white (with gene p^{gri}), and also color genes C and J are needed to fully express the color modifying genes G (yellow brown), B (greenish brown), V (violet to black), and Rk (recessive red from red kidney). He reported close linkage between the red color gene and C, and the bracket convention, [C r], is used to indicate this linkage.

Although the genetics of seed coat color in common bean is well established, the nature of the pigments giving rise to color is less well understood. Today researchers accept the fact that the pigments responsible for seed coat color in *Phaseolus vulgaris* are flavonoids. Many of the flavonoid pigments that give rise to seed coat color in bean may also impart positive health benefits as antioxidants (Hertog et al., 1993).

Miller et al. (1999) reported that the tissues of the developing seed coat are logical targets for modification of gene expression in any plant to modify properties of the mature seed. They also indicated that to manipulate pigmentation or change constituents in the seed coat, or in the seed itself, it is important to understand the developmental sequence and try to elucidate the functions of the different tissues.

Antioxidant activity and seed coat color

Many studies have associated phenolic antioxidants with the color pigments of food plants. Comis (2000) stated that people who pay attention to the colors of foods

they cook and serve are enhancing not only visual and gustatory pleasure, but nutritional effect as well.

Tsuda et al. (1993) screened various species of beans (*Phaseolus vulgaris* L.) for antioxidant activity, and several of them were markedly active, particularly the red and black types. The white beans revealed very weak activity. He stated that anthocyanins in bean seed coats were responsible for high activity of the colored types. Another study conducted by Amarowicz et al. (1996) indicated that extracts obtained from five species of legumes with colored seed coat, pea, faba bean, lentil, everlasting pea, and broad bean, are characterized by high antioxidant activity. They also concluded that antioxidant substances in legumes are present mainly in the seed coat.

Accumulation of flavonoid intermediates has been reported in unpigmented seed coats of legumes, barley (*Hordeum vulgare* L.) and *Arabidopsis thaliana* (L) Heynh. Flavones, flavonols and dihydroflavonols were reported to accumulate in the seed coats of white bean (*Phaseolus vulgaris*) and *Vicia faba* L. cv Blandine, whereas only proanthocyanidin was present in the dark-seeded *Vicia faba* cv. Alfred (Bekkara et al., 1998; Beninger and Hosfield, 1998; Beninger et al., 2000). Flavonoids found in an extract from the bean seed coat were strong antioxidants. A genetic link was also found between bean color and flavonoids (Comis, 2000).

Beninger and Hosfield (1999) stated that the normally colorless proanthocyanidin in beans undergoes secondary changes during seed maturation to form insoluble compounds with the cell wall and other phenolics in the seed coat, causing darkening.

This darkening process was also proposed for proanthocyanidins in the brown testa of the caryopses of sorghum (Stafford, 1990).

Seeds are exposed to oxidative damage due to oxygen, UV light, and other environmental factors. The seed coat contains numerous bioactive compounds, including polyphenols, which have antioxidant properties that protect the seeds against oxidative damage (Osawa et al., 1985).

Troszyńska et al. (2002) reported that extract from pea (*Pisum sativum* L.) seed coat exhibited pronounced antioxidant activity. Fractional separation of the extract indicated that it consists of various phenolic antioxidants such as tannins, flavonoids: flavone and flavonol glycosides and some phenolic acids (benzoic and cinnamic acids and cinnamic acid derivatives). They stated that the colored seed of pea is protected against oxidative damage by its seed coat antioxidant constituents. They concluded that phenolic substances occurring in the seed coat of colored pea cannot be neglected as a source of antioxidants.

There are tannins in the seed coats of beans, with negligible amounts in the cotyledons (Deshpande et al., 1982). Bean varieties have different amounts of condensed tannins depending on the color of their seed coats. The white varieties contain lower concentrations of tannins than those with red, black or bronze seed coats (Elias et al., 1979; Bressani and Elias, 1980). Kadam et al. (1982) reported that there are less tannins in mature bean seeds than in younger ones due to the polymerization of polyphenolic compounds to high-molecular weight insoluble polymers.

The diallel analysis

The term "diallel cross" has been attributed to a Danish geneticist, J. Schmidt (Wricke and Weber, 1986), who used it in livestock breeding to designate a cross of two males with two females. The term came into use in plant breeding and genetics during the 1950s (Christie and Shattuck, 1992), with the first written report of a diallel cross applied in plants released by Jinks and Hayman in 1953.

The diallel cross is defined as all possible crosses among a group of parents. A diallel cross with n parents would generate n^2 families (Jinks and Hayman, 1953). This is also called a complete diallel (Griffing, 1956). Later generations i.e. F_2 and BCs, can also be included in a diallel cross (Hayman, 1959). Since the advent of the diallel mating design, it has been widely used in plant breeding research to obtain genetic information. It is used in both self-pollinating and cross-pollinating species, as well as homozygous or inbred parents (Jinks and Hayman, 1953; Griffing 1956b) and non-inbred parents (Gardner and Eberhart, 1966).

Christie and Shattuck (1992) concluded that diallel analysis is a sophisticated form of progeny testing from which information can be obtained that is not available from any other analysis, and can be used by plant breeders as an aid in selection.

According to Hallauer and Miranda (1981), the diallel mating design has been used and abused more extensively than any other mating design. However, they noted that it is very useful if properly analyzed and interpreted. Sokol and Baker (1977) suggested that genetic interpretation of data from diallel experiments is valid only if the following assumptions about the parental material are true: diploid segregation,

homozygous parents, gene frequencies are equal to one-half at all segregating loci, genes are independently distributed between parents, and no non-allelic interaction.

Some of the assumptions regarding diallel analysis are easily accepted while others are more critical. Kempthorne (1956) suggested that no valid information would be derived from genetic analysis of diallel crosses if genes are not independently distributed between parents. According to Hayman and Mather (1955), gene frequencies that are not equal to one-half confound the statistical estimates. Horner et al. (1955) and Gilbert (1958) and Cockerham (1959) agree that the absence of epistasis cannot be assumed when dealing with quantitative traits until experimentally proven otherwise. Sokol and Baker (1977) further asserted that no epistatic assumption is biologically unrealistic. According to Hallauer and Miranda (1981) the assumptions of independent distribution of genes in the parents used and no epistasis are not valid for the small number of parents usually used in diallel crosses. They stated that independent distribution of genes cannot occur unless a minimum of 2^n parents are included in the diallel set of crosses. Gilbert (1955) reported that certain assumptions are not justified in self-pollinating cereals.

There are several methods of diallel analysis and modification but the basic methods have been described by Jinks and Hayman (1953), Griffing (1956b), Gilbert (1958), and Gardner and Eberhart (1966). Each analysis requires certain assumptions that may limit its use or interpretations of its results, therefore, criticism of diallel analysis and perceptions of abuse arise from the interpretations of results (Baker, 1978; Christie and Shattuck, 1992). Nevertheless diallel analyses are of great benefit to

breeders and geneticists. Plant breeders and geneticists have used diallel mating designs extensively to investigate genetic properties of plant cultivars and populations. Hayman's (1954) and Griffing's (1956b) analyses are frequently used together to complement interpretation of data.

Diallel analyses differ in three main ways (Hayman, 1960a) 1) in the material under investigation 2) in the postulated underlying genetic mechanism and 3) in the methods of estimation. For example, some studies are aimed at a particular set of lines while others target populations from which these lines are sampled.

Diallel analysis provides information on average performance of individual lines in crosses known as general combining ability (GCA). It also gives information about the performance of crosses relative to the average performance of parents involved in the cross known as specific combining ability (SCA). Ghosh and Das (2003) explained that a cross between two lines has an expected value, which is the sum of the general combining abilities of its two parental lines. However, some crosses deviate from this expected value to a greater or lesser extent, and this deviation is what is known as the specific combining ability of the two parents in combination.

In statistical terms, general combining abilities are the main effects and specific combining ability is an interaction. Zary (1980) elaborated that the term 'interaction' should not be confused with any form of genetic interaction between postulated genes. The term is used to refer to the departure from additivity represented by main effects. The analysis is similar to that in factorial experiments.

There are no genetic assumptions with Griffing's analysis on combining ability (Wright, 1985), and several scientists believe that this method conveys reliable information on the combining potential of parents (Gill et al., 1977; Nienhuis and Singh, 1986). Griffing (1956b) proposed four methods of diallel analysis:

Method 1: Complete diallel. Includes parents, F_1 and reciprocals (n^2 entries, where n is the number of parents).

Method 2: Half diallel. Parents and F_1's without reciprocals [n(n+1)/2 entries].

Method 3: F_1's and reciprocals used but not parents [n(n-) entries].

Method 4: Only F_1's used, no reciprocals or parents [n(n-1)/2 entries].

Griffing (1956b) also postulated two models for analysis of variance of the diallel design. Model I (fixed effects) is used in assumption that the parents are the population, i.e. parents are a fixed set of lines. Estimates from this model apply only to the genotypes included and cannot be extended to some hypothetical reference population (Hallauer and Miranda, 1981). In this model, estimation of components of variance is not appropriate, but estimation of GCA and SCA effects is valid and informative. Model II (random effects) is used where parents are a sample of randomly chosen lines from a reference population and the estimates are interpreted relative to the reference population. With this model, estimates of variance components are the main interest.

According to Griffing (1956a) and Bhullar et al. (1979) a large GCA:SCA variance ratio suggests importance of additive gene effects, while a low ratio signifies presence of dominant and/or epistatic gene effects. Baker (1978) reported, in reference

to Griffing's models, that for inbred parents the closer the following equations are to unity, the greater the predictability based on general combining ability; model I $2g_i^2/(2g_i^2 + s_{ij}^2)$ and model II $2\delta_g^2/(2\delta_g^2 + \delta_s^2)$, where g_i^2, δ_g^2 represent GCA mean square, and variance, respectively, and s_{ij}^2, δ_s^2 refers to SCA mean square and variance, respectively. Therefore, performance of a single cross progeny can be predicted on the basis of the GCA of the parents if SCA is small relative to the GCA. Cockerham (1963) suggested partitioning of reciprocal effects into maternal and non-maternal effects. This is helpful in determining whether maternal or extranuclear factors are involved in the expression of a trait.

Wassami et al. (1986) noted that the GCA component contains additive effects in addition to additive x additive effects when present. Christie and Shattuck (1992) concluded that it is easy to select the appropriate analysis if the breeder decides on the purpose or level of the analysis desired and reference population before initiating a diallel cross. They also reported that diallels or other complicated designs do not assure success in reaching plant breeding goals, but will increase the chances of success if properly utilized.

Generation mean analysis

The main function of generation mean analysis (GMA) is to obtain information about a particular pair of lines. Means of different generations are used to estimate genetic effects in a cross. Several models of generation mean analysis have been developed (Hayman, 1958, 1960b; Van der Veen, 1959; and Gardner and Eberhart, 1966).

According to Kearsey and Pooni (1996), there are very simple relationships between the expected means of different generations. However, these relationships hold only if the generation means depend solely on additive and dominance effects. In the presence of maternal effects, epistasis, and differential viability the expected relationships do not hold. In the presence of epistasis, the estimates of additive and dominance effects are biased by some of the epistatic effects.

Comparison among generation means provides a test for the presence of epistasis. Mather (1949) derived scaling tests (A, B, and C) to test the adequacy of the additive –dominance model in explaining variation among generation means. Cavalli (1952) introduced a joint scaling test, which includes any combination of families simultaneously following multiple linear regression.

In the case of a single gene, GMA allows computation of the dominance ratio [d]/[a], where [a] is the additive component and [d] is the dominance component. However, with two or more genes, this ratio is referred to as the potence ratio indicating which parent has the most dominant alleles hence the more potent in the cross (Kearsey and Pooni, 1996). The numerator of the ratio could be zero due to ambi-directional dominance while the denominator could be zero as a result of gene dispersion, and hence the ratio can take any value irrespective of the true degree of dominance.

The errors of estimate in generation mean analysis (GMA) are smaller as means (first degree statistics) are used instead of variances (second degree statistics). Also GMA is equally applicable to cross- and self-pollinating species, and requires smaller experiments to obtain a good degree of precision (Hallauer and Miranda, 1981).

Different generations can be included in the analysis (Mather and Jinks, 1977; Kearsey and Pooni, 1996), and also extended to more complex models including epistasis, linkage and trigenic. GMA permits estimation of heterosis and inbreeding depression. Adequate sampling of segregating generations is necessary to produce a representative sample of genotypes and hence better estimates of the generation means.

Generation mean analysis does not permit estimation of heritability and genetic gain which are important in crop improvement. Also it does not reveal opposing effects, i.e. cancellation of positive and negative gene effects.

CHAPTER III

MATERIALS AND METHODS

Plant material

Advanced inbred lines in the 2002 replicated and observational Regional Southernpea Cooperative Trials were evaluated for antioxidant activity (AOA) (Tables 1 and 2). Four lines from the trials were selected as parental lines for this study. The selected lines had extreme levels (2 lowest and 2 highest) of antioxidant activity. Lines ARK96-918 (Fig. 1) and LA92-180 (Fig. 2) selected from the replicated trial (Table 1) were the lowest in AOA, while lines ARK98-348 (Fig. 3) and ARK95-356 (Fig. 4) selected from the observation trial (Table 2), were the highest.

Selected parental lines were planted in the vegetable legume greenhouse in the fall of 2002. Seeds were inoculated with *Rhizobium spp.* and planted in 7.57 liter plastic pots filled with Metro-mix® 366 growing medium. The pots were arranged in a completely randomized block design (Lentner and Bishop, 1993), consisting of 3 blocks with 5 pots per block and four seeds per pot. After germination, seedlings were thinned leaving two plants per pot.

Crosses were made among the parental lines (Table 3) in all possible combinations, including reciprocals, forming a 4-by-4 complete diallel design (Griffing, 1956b). Flowers from which pollen was collected were picked in the morning and crosses were made either in the morning or evening. Flowers pollinated were one-day preanthesis buds. Buds were opened along the keel using forceps and emasculated by

removing the stamens. After pollination, the flowers were closed and secured with scotch tape to prevent contamination and desiccation.

F_1s and reciprocals, together with their parents from each block, were harvested and bulked. Thirty seeds were randomly chosen from F_1 and reciprocal populations and stored for antioxidant analysis. The remaining F_1, reciprocals and parental seeds were planted in the greenhouse during the spring of 2003. Pollen from F_1 flowers was used to make backcrosses to recurrent parents, at the same time letting F_1s self to generate F_2 and F_2' populations.

Table 1. Antioxidant activity of entries in the 2002 replicated Regional Southernpea Cooperative Trial as determined by the DPPH assay[1].

		Fresh Seed	Dry Seed
Entry	Type	µg Trolox equivalents/gfw[2]	µg Trolox equivalents/gdw[3]
LA 92-86	Pinkeye	589.4	479.4
LA 96-21	Pinkeye	537.3	418.1
TX 159 BE^{gc}	Blackeye gc	533.6	304.8
TX 148 PE^{gc}	Pinkeye gc	529.8	362.7
TX 164 PE^{gc}	Pinkeye gc	454	297.2
ARK 96-1022	Pinkeye gc	444.1	335.4
Coronet	Pinkeye	409.1	240.9
TX 128 BE	Blackeye	394.8	267.3
US-1033	Blackeye	389.3	289.8
TX 123 BE	Blackeye	385.4	260.6
ARK BE #1	Blackeye	368	226.4
US-1070	Cream gc	346.8	191.3
Early Acre	Cream	346	212.4
US-1031	Cream	344	259

Table 1 – Continued.

		Fresh Seed	Dry Seed
Entry	Type	µg Trolox equivalents/gfw[2]	µg Trolox equivalents/gdw[3]
US-1069	Cream gc	310.1	201.1
TX 139 CRM	Cream	296.8	200.9
LA 95-62	Cream	258.5	204
ARK 96-918	Cream	255.5	216.4
LA 92-180	Cream	232.9	206.4

[1]The assay used to evaluate antioxidant activity was based on 'a free radical method' by Brand-Williams et al., 1995, Lenensm. Wiss. Technol. 28:25-35.

[2]µg Trolox equivalents/gfw - Absorbance was converted to equivalent activity of trolox per g of fresh weight based on a standard curve using the following equation: $Y=888.12*\Delta A_{515} + 3.488$

[3]µg Trolox equivalents/gfw - Absorbance was converted to equivalent activity of trolox per g of dry weight based on a standard curve using the following equation: $Y= 888.12*\Delta A_{515} + 3.488$

Table 2. Antioxidant activity of entries in the 2002 observational Regional Southernpea Cooperative Trial as determined by the DPPH assay[1].

		Fresh Seed	Dry Seed
Entry	Type	µg Trolox equivalents/gfw[2]	µg Trolox equivalents/gdw[3]
ARK 95-356	Black	833.2	1098.6
ARK 98-348	Red	837.5	1083.7
TX 160 BE^{gcgt}	Blackeye gc/gt	493.0	419.3
LA 94-55	Pinkeye	534.7	357.8
LA 96-18	Pinkeye	523.3	332.8
TX 158 PE^{gc}	Pinkeye gc/gc	358.9	312.6
LA 94-1	Pinkeye	368.0	297.2
Coronet	Pinkeye	409.1	240.9
LA 96-4	Cream	309.4	236.4
ARK BE #1	Blackeye	368.0	226.4
TX 162 PE^{gc}	Pinkeye gc/gc	495.2	218.3
Early Acre	Cream	346.0	212.4
US-1076	Pinkeye	335.9	176.9

[1]The assay used to evaluate antioxidant was based on 'a free radical method activity' by Brand-Williams et al., 1995, Lenensm. Wiss. Technol. 28:25-35.

[2]µg Trolox equivalents/gfw - Absorbance was converted to equivalent activity of trolox per g of fresh weight based on a standard curve using the following equation: $Y= 888.12*\Delta A_{515} + 3.488$

[3]µg Trolox equivalents/gfw - Absorbance was converted to equivalent activity of trolox per g of dry weight based on a standard curve using the following equation: $Y= 888.12*\Delta A_{515} + 3.488$

Sample extraction

Individual seeds from each generation were selected at random (Nzaramba et al., 2003). Thirty seed samples were chosen from parental, F_1 and reciprocals, while 200 seeds were chosen from each of the segregating populations, i.e. BC_1, BC_2, and F_2.

Individual seeds were ground with a Braun KSM2 coffee grinder. The product was transferred to a porcelain mortar and further ground to a fine powder. The powder was weighed and placed in 1.5 ml microcentrifuge tubes. One ml of HPLC grade methanol was added to each sample, homogenized using a 29H4 UL laboratory mixer, and centrifuged for 15 min. at 13,000 rpm using a Biofuge 13 microcentrifuge.

DPPH assay

Total antioxidant activity of the sample extracts was analyzed using DPPH (2, 2-Diphenyl-1-picrylhydrazyl) (Brand-Williams et al., 1995). DPPH is a stable radical with strong oxidizing capacity. DPPH free radicals are used to determine antioxidant activity of plant extracts by measuring radical scavenging capacity or reduction potential of samples. This method is based on the change in absorbance of DPPH radicals in methanol solution as they reduced by antioxidants. The DPPH methanol solution is dark purple in color and when reacted with an antioxidant the color fades, ranging from light purple to light yellow depending on the strength of the antioxidant. The change in color is measured as a reduction in absorbance using a spectrophotometer.

Table 3. Description of cultivars selected for the study.

Parent	Cultivar	Antioxidant activity	Color
P_1	ARK98-348	High	Red
P_2	ARK95-356	High	Black
P_3	ARK96-918	Low	Cream
P_4	LA92-180	Low	Cream

Figure 1. Inbred line ARK96-918 selected from the replicated Regional Southernpea Cooperative Trial.

Figure 2. Inbred line LA92-180 selected from the replicated Regional Southernpea Cooperative Trial.

Figure 3. Inbred line ARK98-348 selected from the observational Regional Southernpea Cooperative Trial.

Figure 4. Inbred line ARK95-356 selected from the observational Regional Southernpea Cooperative Trial.

The DPPH method requires very mild experimental conditions, which is an advantage compared to other commonly used methods that require preliminary sample treatment to accelerate tests by employing high temperatures and or oxygen supply that may risk bringing about undesirable alterations (decomposition, evaporation, polymerization) of the studied antioxidants (Koleva et al., 2002).

The DPPH assay was prepared by dissolving 24 mg of 2, 2-Diphenyl-1-picrylhydrazyl in 100 ml of HPLC grade methanol. The solution was diluted several times with methanol until its absorbance was 0.5 units at 515 nm on a Shimadzu Biospec-1601 spectrophotometer.

Fifteen µl of supernatant were pipetted into a scintillation vial in which 2850 µl of methanolic DPPH solution was added and left to react for 15 min. on a shaker. The mixture was transferred to a plastic UV-cuvette and its absorbance recorded at 515 nm on the spectrophotometer. Absorbance of a blank containing 150 µl of methanol with DPPH was recorded after every 12 samples were analyzed. Change in absorbance of each sample was computed as the difference between the blank and sample readings.

A known antioxidant, trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), was used as a standard to express AOA of seed extracts as trolox equivalents (Brand-Williams et al., 1995; Koleva, 2002). Known concentrations of trolox were used to prepare a standard curve with linear regression (Fig. 5), which was used as a reference for comparing the sample extracts. The regression curve computed was $Y = 892.98X$, where Y was the concentration of trolox in µM, and X the change in absorbance of DPPH due to reduction by trolox.

Antioxidant activity of seed extracts was expressed as trolox equivalents using the following equation; Y= 892.98 x ΔA_{515} x (PM_{Trolox}/1000) x [(M_{seed} + Vol_{MEOH})/ M_{seed}], where Y is the AOA in trolox equivalents [µg Trolox equi/ g of dry seed], ΔA_{515} is the change in absorbance due to antioxidants in the seed extract, PM_{Trolox} is the molecular weight of trolox (250 g/gmol), M_{seed} is the weight of seed samples in g, Vol_{MEOH} is the volume of methanol in ml used for extraction.

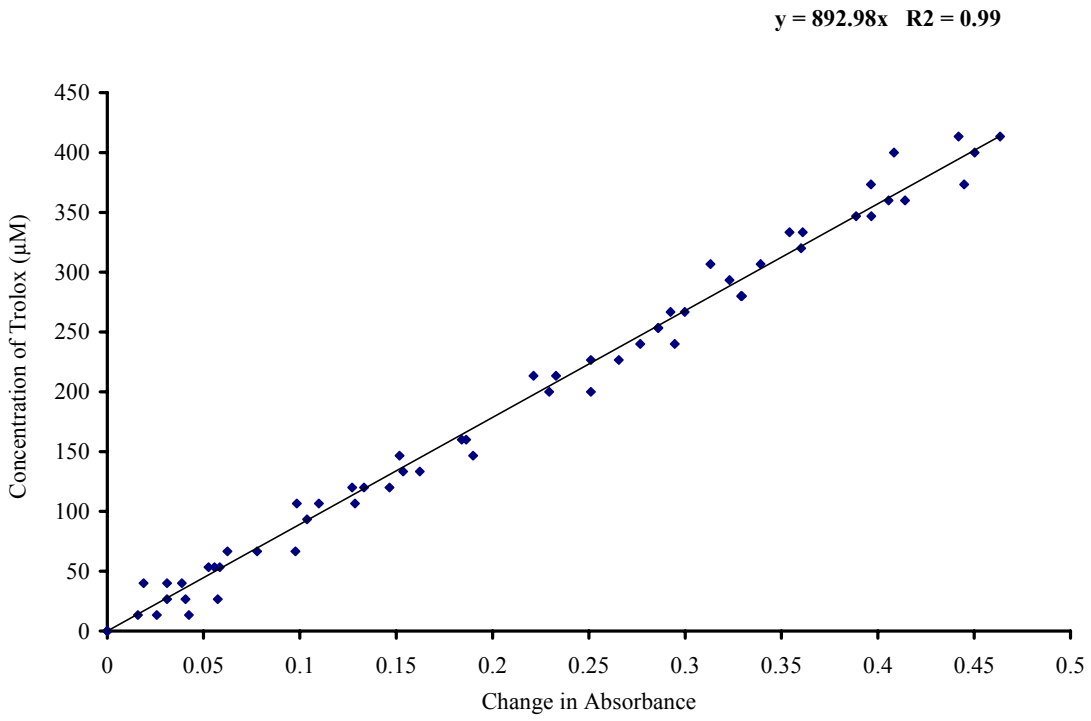

Figure 5. Standard curve used to convert antioxidant activity of seed methanolic extracts to trolox equivalents.

Statistical analysis

Analysis of variance was performed with the PROC GLM procedure from SAS (SAS, 1999). Parents, crosses, and blocks were considered fixed effects, while replications within blocks were considered as random factors. The model used in ANOVA was; $Y_{ijk} = \mu + b_i + r_j + g_k + \varepsilon_{ijk}$, where Y_{ijk} is the observed value in the j^{th} replication of the k^{th} genotype in the i^{th} block, μ is the general mean, b_i is the block effect, r_j is the replication effect, g_k is the effect of the k^{th} genotype and ε_{ijk} is the residual effect.

Comparisons among parents, F_1s and reciprocals were done following Griffing's (1956b) method I model I, where all genotypes including parents were considered fixed. The diallel analysis of variance was done by the DIALLEL-SAS program developed by Zhang and Kang (1997). The sum of squares for the crosses was partitioned into general combining ability (GCA), specific combining ability (SCA) and reciprocal effects (RE). The reciprocal effects were partitioned into maternal (MAT) and non-maternal (NONM) effects following Cockerham's (1963) method using SAS codes adopted from Kang (2003).

Broad sense heritability was computed according to Hallauer and Miranda (1981), $H^2 = 2\hat{\sigma}^2_{GCA} / \left(\hat{\sigma}^2 / r + \hat{\sigma}^2_{SCA} + 2\hat{\sigma}^2_{GCA}\right)$. The GCA:SCA ratios with a theoretical maximum of unity were computed according to Baker (1978) as follows: $GCA : SCA = 2MS_{GCA} / \left(2MS_{GCA} + MS_{SCA}\right)$; where MS_{GCA} is the GCA mean square and MS_{SCA} is the SCA mean square.

Generation means and variances were used to determine gene actions by performing scaling tests (Mather and Jinks, 1971). These tests assume that the genes exhibit simple autosomal inheritance, i.e. there are no sex-linkage or maternal effects in determining the character under study. They also assume an additive-dominance model which stipulates that the genes involved are independent of each other, i.e. total effects of genes affecting the trait is the sum of their individual effects. Three scaling tests were performed, $A = 2\overline{BC_1} - \overline{P_1} - \overline{F_1}$, $B = 2\overline{BC_2} - \overline{P_2} - \overline{F_1}$, and $C = 4\overline{F_2} - 2\overline{F_1} - \overline{P_1} - \overline{P_2}$; where $\overline{P_1}$, $\overline{P_2}$, $\overline{F_1}$, $\overline{F_2}$, $\overline{BC_1}$, and $\overline{BC_2}$ are the means of parents , F_1 crosses, F_2's, and backcross generations, respectively.

A joint scaling test was performed using regression (Cavalli, 1952). This test combines all the scaling tests into one and also can cover any combination of generations, making it more convenient and informative. A three parameter model was used to estimate mid-parent values (m), additive gene effects [a] and dominance deviation [d] among generation means. Non-allelic interactions additive x additive [aa], additive x dominance [ad], and dominance x dominance [dd] epistatic effects were estimated using a six parameter model.

The effective or minimum number of genes (n_E) controlling antioxidant activity in seeds was estimated with the equation, $n_E = \frac{\left(\overline{P_1} - \overline{P_2}\right)^2}{\left[8\left(2\sigma^2_{F_2} - \sigma^2_{BC_1} - \sigma^2_{BC_2}\right)\right]}$ where $\overline{P_1}$ and $\overline{P_2}$ refer to means of the parents, $\sigma^2_{F_2}$ refers to the variance of F_2, $\sigma^2_{BC_1}$ and $\sigma^2_{BC_2}$ are the variances of backcross generations with P1 and P2 as recurrent parents, respectively.

The standard error of estimate for minimum number of genes was computed using the following formula (Lande, 1981),

$$\sqrt{Var(n_E)} \cong \sqrt{n_E^2}\left(\frac{4\left[\sigma_{P_1}^2 / N_{P_1} + \sigma_{P_2}^2 / N_{P_2}\right]}{\left[\overline{P_1} - \overline{P_2}\right]^2} + \frac{\left[8\sigma_{F_2}^4 / N_{F_2} - 2\sigma_{BC_1}^4 / N_{BC_1} - 2\sigma_{BC_2}^4 / N_{BC_2}\right]}{\left(2\sigma_{F_2}^2 - \sigma_{BC_1}^2 - \sigma_{BC_2}^2\right)^4}\right)$$

where, σ^2_{P1}, σ^2_{P2}, σ^2_{F2}, σ^2_{BC1} and σ^2_{BC2} are variances of parents, F_2s and backcrosses. *N* is the number of individuals in the generation corresponding to its subscript.

CHAPTER IV

RESULTS AND DISCUSSION

Diallel analysis

Significant differences among crosses were revealed by the analysis of variance (Table 4). Diallel analysis showed highly significant general combining ability (GCA), specific combining ability (SCA) and reciprocal (REC) mean squares. The significance of both GCA and SCA implies that additive and dominance effects contributed to the genetic control of antioxidant activity in the set of lines used in this study. Results also show that additive effects were more important than dominance effects, since the mean square for GCA was greater than that for SCA. According to Goffman and Becker (2001) the relative amount of GCA variance may have been overestimated since the parents used in this study were not randomly selected, but rather selected for extreme (highest and lowest) values of total antioxidant activity.

Combining abilities reported could be biased by the lack of independent distribution of genes in the parental lines as a result of the small number of lines used in the study (Baker, 1978). Despite the limitations mentioned above, information from this study is helpful in identifying the best sources of antioxidant activity among the lines used. Estimates of general combining abilities were significant for all parents (Table 5). These results revealed that parents behaved genetically as expected, with the high antioxidant activity parents (ARK98-348 and ARK95-356) exhibiting large positive GCA effects, while the low antioxidant activity parents (ARK96-918 and LA92-180)

had negative GCA effects. In addition, pigmented lines exhibited positive GCA while non-pigmented ones exhibited negative GCA effects. Therefore, there seems to be a strong relationship between seed color and antioxidant activity. Both negative and positive GCA effects are of interest since they may permit breeding for higher quantities of antioxidant activity.

Specific combining ability effects were observed only in the cross ARK98-348 x LA92-180 (Table 6). These results indicate that non-additive gene effects were significant in this cross. All crosses showed highly significant REC effects for antioxidant activity, especially crosses between pigmented and non-pigmented parents. The mean square for REC effects was partitioned into MAT and NONM effects (Tables 4, 5 and 6). Maternal effects were highly significant (Tables 4 and 5) while nonmaternal effects not significant (Tables 4 and 6). These results indicate that reciprocal effects were mostly due to maternal effects since the nonmaternal component of the REC effects was not significant.

Table 4. Analysis of variance in a 4-by-4 complete diallel following Griffing' Method I Model I.

Source[¥]	df	Sum of squares	Mean squares	F
Block	2	83459.3	41729.6	0.9
Rep(Block)	42	1295068.3	30835	0.7
Crosses	15	101719829.3	6781322	160.1**
GCA	3	57158374	19052791.3	449.7**
SCA	6	809269	134878.2	3.2**
REC	6	33952320.8	5658720.1	133.6**
MAT	3	33375098.7	11125033	262.6**
NONM	3	186921.4	62307.1	1.5
Error	440	18640863.1	42365.6	

[¥]GCA = general combining ability; SCA= specific combining ability; REC= reciprocal effects; MAT= maternal effects; NONM= nonmaternal effects.

** indicates significance at $P \leq 0.01$.

Table 5. Estimates of parental general combining ability (GCA) and maternal (MAT) effects in a 4-by-4 complete diallel.

Parent	Seed color	GCA	MAT
ARK98-348	Red	353.1** ± 11.1	333.5** ± 13.0
ARK95-356	Black	49.2** ± 11.1	40.4** ± 11.9
ARK96-918	Cream	-177.3** ± 11.1	-214.9** ± 13.0
LA92-180	Cream	-225.0** ± 11.1	-159.0** ± 12.1

** indicates significance at $P \leq 0.01$

The GCA: SCA ratio computed was 0.99, which is near the theoretical maximum of unity. This provided further evidence for presence of additive gene action among the loci that control antioxidant activity (Baker, 1978).

Heritability on individual plant basis (broad sense) was estimated to be 0.87. According to Bernardo (2002), individual plant measurements of quantitative traits are prone to large nongenetic effects making estimates of heritability higher. This estimate of heritability pertains to the conditions of this study and the four lines used since they were not randomly selected. However, this high value suggests that improvement for antioxidant activity in cowpea can be realized through breeding if some of this genetic variation is additive. Some reports have referred to this kind of estimate (ratio of genetic variation to phenotypic variation) as repeatability when nonrandom genotypes are evaluated (Fehr, 1987).

Table 6. Estimates of specific combining ability (SCA), reciprocal (REC), and nonmaternal (NONM) effects of the crosses.

Cross	SCA	REC	NONM
ARK98-348 x ARK95-356	-2.8 ± 20.6	286.8** ± 26.5	-6.4 ± 20
ARK98-348 x ARK96-918	24.7 ± 24.5	590.6** ± 34.3	42.2 ± 22.1
ARK98-348 x LA92-180	-82.5** ± 21.6	456.7** ± 28.7	-35.8 ± 20.5
ARK95-356 x ARK96-918	-37.3 ± 21.4	244.1** ± 28.3	-11.2 ± 20.4
ARK95-356 x LA92-180	-0.7 ± 21.1	204.2** ± 27.4	17.7 ± 35.2
ARK96-918 x LA92-180	38.5 ± 21.1	-25.2 ± 27.6	53.5 ± 37.3

** indicates significance at $P \leq 0.01$

Generation mean analysis

All F_1 hybrids exhibited the same color as their female parent. AOA from crosses ARK98-348 x ARK95-356, ARK98-348 x ARK96-918, ARK95-356 x ARK96-918 and ARK96-918 x LA92-180 was not significantly different from the female parent (Tables 7, 8, 10 and 12). Crosses in which AOA levels in F_1's were significantly different from the higher parent were ARK98 x LA92 and ARK95 x LA92, but AOA levels were closer to the higher parent than the lower parent (Tables 9 and 11). Backcrosses were similar in color to their recurrent parents and also not significantly different in antioxidant activity.

Segregation of seed coat color was observed in the F_2 generation. F_2 and F_2 reciprocal seeds from crosses involving pigmented parents were a slightly different shade from the colored parent (Tables 7, 8, 9, 10 and 11). F_2 seeds from the cross between red (P_1) and black (P_2) lines were black (Table 7), indicating dominance of the gene responsible for black color to that responsible for red seed coat color. Similar results were observed by Saunders (1960) and Calub (1968).

Antioxidant activity of F_2 seeds from the cross ARK98-348 (red) x ARK95-356 (black) was significantly different from that of the red line but not significantly different from the black line. These results showed that factors controlling antioxidant activity in ARK95-356 were dominant to those in ARK98-348, with dominance being negative in relation to ARK98-348 since it exhibited more activity than ARK95-356 (Table 7).

Crosses between pigmented (red or black) and non-pigmented (cream) lines showed dominance of pigmented lines over non-pigmented lines for both seed coat color and antioxidant activity (Tables 8, 9, 10 and 11), indicating an apparent relationship

between seed coat color and antioxidant activity. Pigmented lines seem to possess favorable factors that enhance antioxidant activity.

The inheritance pattern among factors governing antioxidant activity in the lines studied is similar to that of factors responsible for seed coat color, hence suggesting a very strong relationship between these traits.

Table 7. Generation means and their standard errors for antioxidant activity for the cross ARK98-348 x ARK95-356.

Generation	Color	No. of seeds	Mean*	Std. Error
P_1	Red	45	1346.3^{A}	255.2
P_2	Black	45	718.8^{D}	97.3
F_1	Red	30	1272.2^{A}	196.5
F1'	Black	30	698.2^{D}	158
F_2	Black	192	836.6^{C}	160.5
F_2'	Black	201	764.5^{CD}	143.6
BC_1	Red	25	985.3^{B}	499.2
BC_2	Black	149	727.1^{D}	184.7

*Means with same letter superscript are not significantly different by LSD test at 0.05 level.

Table 8. Generation means and their standard errors for antioxidant activity for the cross ARK98-348 x ARK96-918.

Generation	Color	No. of seeds	Mean*	Std. Error
P_1	Red	45	1346.3^{A}	255.2
P_2	Cream	44	202.3^{D}	37
F_1	Red	27	1378.8^{A}	659.9
F_1'	Cream	13	193.9^{D}	35.7
F_2	Grayed red	194	877.5^{C}	248.4
F_2'	Grayed red	200	846.1^{C}	207.5
BC_1	Red	142	990.3^{B}	218.5
BC_2	Cream	165	173.6^{D}	28.7

*Means with same letter superscript are not significantly different by LSD test at 0.05 level.

Table 9. Generation means and their standard errors for antioxidant activity for the cross ARK98-348 x LA92-180.

Generation	Color	No. of seeds	Mean*	Std. Error
P_1	Red	45	1346.3^{A}	255.2
P_2	Cream	45	174.4^{E}	27.4
F_1	Red	23	1089.2^{B}	340.3
F_1'	Cream	30	174.9^{E}	26.4
F_2	Ash gray	209	786.5^{D}	216.2
F_2'	Ash gray	208	920.1^{C}	138.5
BC_1	Red	164	1063.4^{B}	239.4
BC_2	Cream	180	176.3^{E}	26.9

*Means with same letter superscript are not significantly different by LSD test at 0.05 level.

Table 10. Generation means and their standard errors for antioxidant activity for the cross ARK95-356 x ARK96-918.

Generation	Color	No. of seeds	Mean*	Std. Error
P_1	Black	45	718.8^{AB}	97.3
P_2	Cream	44	202.3^{C}	37.0
F_1	Black	24	665.6^{B}	57.9
F_1'	Cream	30	176.6^{C}	50.8
F_2	Black with white spots	201	729.4^{A}	115.0
F_2'	Black with white spots	201	635.4^{B}	91.6
BC_1	Black	173	669.7^{B}	130.9
BC_2	Cream	147	191.3^{C}	76.1

*Means with same letter superscript are not significantly different by LSD test at 0.05 level.

Table 11. Generation means and their standard errors for antioxidant activity for the cross ARK 95-356 x LA92-180.

Generation	Color	No. of seeds	Mean*	Std. Error
P_1	Black	45	718.8^{AB}	97.3
P_2	Cream	45	174.4^{DE}	27.4
F_1	Black	30	613.8^{C}	97.4
F_1'	Cream	27	207.1^{D}	24.2
F_2	Black with white spots	201	697.8^{B}	114.8
F_2'	Black with white spots	175	734.8^{A}	102.3
BC_1	Black	192	700.3^{AB}	119.5
BC_2	Cream	87	147.1^{E}	19.9

*Means with same letter superscript are not significantly different by LSD test at 0.05 level.

Table 12. Generation means and their standard errors for antioxidant activity for the cross ARK 96-918 x LA92-180.

Generation	Color	No. of seeds	Mean*	Std. Error
P_1	Cream	41	202.1^{A}	30.6
P_2	Cream	45	174.4^{BC}	27.4
F_1	Cream	28	200.1^{A}	18.8
F_1'	Cream	12	210.1^{A}	12.5
F_2	Cream	197	168.5^{C}	25.2
F_2'	Cream	198	179.3^{B}	25.6
BC_1	Cream	147	167.3^{C}	20.4
BC_2	Cream	160	166.2^{C}	20.7

*Means with same letter superscript are not significantly different by LSD test at 0.05 level.

Gene effects

All scaling tests (A, B and C) were not significant in the crosses ARK98-348 x ARK95-356 (Table 13) and ARK96-918 x LA92-180 (Table 18), implying that the additive-dominance model was satisfactory in explaining the variation among generations. A three-parameter model (m, [a] and [d]) was fitted for these crosses and all parameters involved were significant (Tables 13 and 18). Greater additive than dominant effects were observed in the cross ARK98-348 x ARK95-356 (Table 13) which involved both pigmented parents.

Some of the scaling tests in crosses ARK98-348 x ARK96-918, ARK98 x LA92-180, ARK95-356 x ARK96-918, and ARK95 x LA92-180 were not significant (Tables 14, 15, 16 and 17). Therefore, a simple additive-dominance model would not be adequate in explaining variation among generations from these crosses (Kearsey and Pooni, 1996).

Table 13. Estimates of mid-parent (m), additive [a] and dominance [d] pooled effects and standard errors of the estimates for the cross ARK98-348 x ARK95-356.

Parameter		Estimate	Standard error
m		959.2**	29.9
[a]		293.1**	19.6
[d]		-100.6**	36.4
Scaling test	A	-645	1035.1
	B	-536.9	429.5
	C	-1263.3	800.8

** indicates significance at $P \leq 0.01$

Table 14. Estimates of mid-parent (m), additive [a], dominance [d], and non-allelic interactions ([aa], [ad], and [dd]), pooled effects and standard errors of the estimates for the cross ARK98-348 x ARK96-918.

Parameter		Estimate	Standard error
m		1755.2**	86.4
[a]		572.4**	26.8
[d]		-3278.6**	221.2
[aa]		-1079.1**	78
[ad]		532.3**	79.4
[dd]		2437**	162.3
Scaling test	A	-744.5	831
	B	-1233.9*	663.4
	C	-796.2	1664.7

* indicates significance at $P \leq 0.05$ ** indicates significance at $P \leq 0.01$

Table 15. Estimates of mid-parent (m), additive [a], dominance [d], and non-allelic interactions ([aa], [ad], and [dd]), pooled effects and standard errors of the estimates for the cross ARK98-348 x LA92-180.

Parameter		Estimate	Standard error
m		1650.4**	70.1
[a]		585.9**	22.6
[d]		-2271.1**	178.9
[aa]		-911.9**	63.3
[ad]		606.9**	64.7
[dd]		1174.1**	128.7
Scaling test	A	-308.7	640.5
	B	-911.1*	428.8
	C	-553.1	1130

* indicates significance at $P \leq 0.05$ ** indicates significance at $P \leq 0.01$

Table 16. Estimates of mid-parent (m), additive [a], dominance [d], and non-allelic interactions ([aa], [ad], and [dd]), pooled effects and standard errors of the estimates for the cross ARK95-356 x ARK96-918.

Parameter		Estimate	Standard error
m		1479.2**	41.3
[a]		258.2**	12.9
[d]		-2081.6**	104.8
[aa]		-1003.5**	37.1
[ad]		436.3**	37.7
[dd]		1011.1*	74.9
Scaling test	A	-45	285.2
	B	-485.3**	189.7
	C	665.2	485.6

* indicates significance at $P \leq 0.05$ ** indicates significance at $P \leq 0.01$

Table 17. Estimates of mid-parent (m), additive [a], dominance [d], and non-allelic interactions ([aa], [ad], and [dd]), pooled effects and standard errors of the estimates for the cross ARK95-356 x LA92-180.

Parameter		Estimate	Standard error
m		1568.5**	42.1
[a]		272.2**	12
[d]		-2411.2**	108.6
[aa]		-1149.3**	38
[ad]		548.8**	38.2
[dd]		1238.9**	76.6
Scaling test	A	68	275.9
	B	-494**	108.7
	C	670.4	508

** indicates significance at $P \leq 0.01$

Table 18. Estimates of mid-parent (m), additive [a] and dominance [d] pooled effects and standard errors of the estimates for the cross ARK96-918 x LA92-180.

Parameter		Estimate	Standard error
m		172.4**	3.5
[a]		7.6**	2.0
[d]		-0.4	4.7
Scaling test	A	-67.7	54.3
	B	-42.2	53.1
	C	-102.7	115.2

** indicates significance at $P \leq 0.01$

Failure of the simple additive-dominance model to fit the data in some crosses (Tables 13 and 18) implies that some of the assumptions on which the model was constructed were not valid (Mather and Jinks, 1982). Scaling tests (A, B and C) assume simple autosomal inheritance of the factors concerned. Therefore, they do not hold if differential viability, maternal effects and non-allelic (epistatic) interaction between genes exist (Kearsey and Pooni, 1996). A six-parameter model, including non-allelic interaction, was fitted using joint scaling test to determine the type and magnitude of gene action involved in the crosses in which the simple additive-dominance model was inadequate (Cavalli, 1955). All the effects including non-allelic interactions (m, [a], [d], [aa], [ad] and [dd]) were significant (Table 14, 15, 16 and 17). This confirmed the presence of non-allelic interactions, thus explaining the failure of the simple additive-dominance model observed in these crosses.

The dominance effects [d] were positive while the dominance x dominance interactions [dd] were negative for cross ARK98-348 x ARK96-918, ARK98-348 x LA92-180, ARK95-356 x ARK96-918, and ARK95-356 x LA92-180 (Tables 14, 15, 16 and 17). This suggests the presence of duplicate gene interactions in these crosses (Mather and Jinks, 1977).

The minimum number of factors or genes controlling antioxidant activity was estimated to be 4.9 ± 0.3 (Table 19). This estimate is in the range proposed by Lande (1981). He reported that the effective or minimum number of freely segregating genetic factors involved in producing a large difference between populations in a quantitative trait is estimated to be about 5 or 10. Harland (1919) and Spillman and Sando (1930)

proposed about six genes (C, B, F, P, R and U) to be responsible for seed coat color in cowpea. Several other studies on the genetics of seed coat color were published, however, many of the genes identified in these studies appear to be redesignations for genes in the Harland (1919) or Spillman and Sando (1930) models (Fery, 1980). Estimates from crosses ARK98-348 x ARK95-356 and ARK96-918 x LA92-80 were less than one because the range of genetic variance in the F_2 generations exceeded the mean difference of the parental generations. Hence, the method used for estimation was of little value in these crosses.

Table 19. Estimates of the minimum number of genes controlling antioxidant activity.

Cross	Number of genes	Standard errors
ARK98-348 x ARK95-356	*	
ARK98-348 x ARK96-916	2.2	0.2
ARK98-348 x LA92-180	4.5	0.3
ARK95-356 x ARK96-916	9.5	0.6
ARK95-356 x LA92-180	3.2	0.2
ARK96-916 x LA92-180	*	

* Estimate of minimum number of genes was less than one.

CHAPTER V

CONCLUSIONS

There is a very strong relationship between antioxidant activity and seed coat color. Pigmented varieties of cowpea possess favorable factors that enhance antioxidant activity. Factors governing high antioxidant activity in cowpea seed appear to be the same factors responsible for seed coat color, with apparent pleiotropic effects. The inheritance pattern of factors governing antioxidant activity is similar to that of factors governing seed coat color.

There are differences in antioxidant activity levels among colored cowpea varieties. Color factors impart different amounts of antioxidant activity; therefore, different levels of activity can be obtained from cowpea since different colors are exhibited by cowpea varieties.

Antioxidant activity is highly heritable in cowpea, as indicated by a large estimate of heritability. Therefore, selection for this trait can be achieved with minimal effort. However, a larger population needs to be studied with several colors involved to determine more precise estimates of heritability and the number of genes governing antioxidant activity.

Breeding for enhanced antioxidant activity in cowpea is possible with pigmented varieties as preferred parental material. However, the available information applies to only a few selected varieties. Therefore, screening of the core cowpea collection (~700

varieties) for antioxidant activity would provide a more reliable estimate of the potential availability of antioxidant activity in cowpea.

LITERATURE CITED

Adom, K. K. and R. H. Lui. 2002. Antioxidant activity of grains. J. Agric. Food Chem. 50:6182-6187.

Amarowicz, R., U. N. Wanasundara, M. Karamac, and F. Shahidi. 1996. Antioxidant activity of ethanolic extract of mustard seed. Nuhrung 40:261-263.

Anderson, J.W., B.M. Smith, and C.S. Washnock. 1999. Cardio-vascular and renal benefits of dry bean and soybean intake. Am. J. Clin. Nutr. 70(suppl.):464S-474S.

Arnao, M.B., A. Cano, and M. Acosta. 1999. Methods to measure the antioxidant activity in plant material. A comparative discussion . Free Radic. Res. 31:89-96.

Bagnall, D.J. and R.W. King. 1987. Temperature and irradiance effects on yield of cowpea (*Vigna unguiculata*). Field Crops Res. 16:217-229.

Baker, D.M., H.C. Minor, and B.G. Cumbie. 1987. Scanning electron microscopy examination of soybean testa development. Can. J. Bot. 65:2420-2424.

Baker, R. J. 1978. Issues in diallel analysis. Crop Sci. 18:533-536.

Barlow, S.M. 1990. Toxicological aspects of antioxidants used as food additives. p.253-307. In: B.J.F. Hudson (ed.). Food antioxidants. Elsevier, London.

Bassett, M. J. 1991. A revised linkage map of common beans. HortScience 27:834-836.

Bekkara, F., M. Jay, M.R. Viricel, and S. Rome. 1998. Distribution of phenolic compounds within seed and seedlings of two *Vicia faba* cvs differing in their seed tannin content , and study of their seed and root phenolic exudations. Plant Soil 203:27-36.

Benavente-Garcisa, O., J. Castillo, F. R. Marin, A. Ortuno and J. A. D. Rio. 1997. Use and properties of citrus flavonoids. J. Agric. Food Chem. 45: 4505-4515.

Beninger, C. W. and G.L. Hosfield. 1999. Flavonol glycosides from Montcalm dark red kidney bean: Implications for the genetics of seed coat color in *Phaseolus vulgaris* L. J. Agric. Food Chem. 47:4079-4082.

Beninger, C. W., G. L. Hosfield, and M. G. Nair. 1998. Flavonol glycosides from a new manteca-type dry bean (*Phaseolus vulgaris* L.). J. Agric. Food Chem. 46:2906-2910.

Beninger, C. W., G. L. Hosfield, and M. J. Bassett. 1999. Flavonoid composition of three genotypes of dry bean (*Phaseolus vulgaris* L.) differing in seed coat color. J. Amer. Soc. Hort. Sci. 124:514-518.

Beninger, C. W., M. J. Bassett, and S. Owens. 2000. Chemical and morphological expression of the B and Asp seedcoat genes in *Phaseolus vulgaris*. J. Amer. Soc. Hort. Sci. 125:52-58.

Bernardo, R. 2002. Breeding for quantitative traits in plants. Stemma Press, Woodbury, MN.

Bhullar, G.S., K.S. Gill, and A.S. Khehra. 1979. Combining ability analysis over F_1-F_2 generations in diallel crosses of bread wheat. Theor. Appl. Genet. 55:77-80.

Block, G., B. Patterson, and A. Subar. 1992. Fruits, vegetables, and cancer prevention: A review of the epidemiological evidence. Nutr. Cancer 18:1-29.

Bogdan, A.V. 1977. Tropical pasture and fodder plants (grasses and legumes). Longman, New York.

Brand-Williams, W., M. E. Cuvelier, and C. Berset. 1995. Use of free radical method to evaluate antioxidant activity. Lebensm. Wiss. Technol. 28: 25-30.

Branen, A. L. 1975. Toxicology and biochemistry of butylated hydroxyl anisole and butylated hydroxyl toluene. J. Am. Oil. Chem. Soc. 52:59-63.

Bressani, R. and L.G. Elias. 1980. The nutritional role of polyphenols in beans. p.6. In: J. H. Hulse. (ed.). Polyphenols in cereal and legumes. The International Development Research Centre, Ottawa, Canada.

Burkitt, D.P. and H. C. Trowell. 1975. Refined carbohydrate foods and disease: Some implications of dietary fiber. Academic Press, London.

Calub, A.G. 1968. Inheritance of seed color and color pattern in *Vigna sinensis* (Linn.). Cowpeas (*Vigna unguiculata* L. Walp): Abstracts of World Literature. Vol. 1: 1950-1973. The International Grain Legume Information Centre, IITA, Ibadan, Nigeria. p.14.

Cao, G., E. Sofic, and R. L. Prior. 1996. Antioxidant capacity of tea and common vegetables. J. Agric. Food Chem. 44:3426-3431.

Carlson, J. B. and N. R. Lerstein. 1987. Reproduction morphology. p. 95-133. In: J. R. Wilcox (ed.). Soybeans: Improvement, production and uses. 2nd ed. Amer. Soc. Agron. and Academic Press, Madison, WI.

Cavalli, L.L. 1952. An analysis of linkage in quantitative inheritance. p.135-144. In: E.C.R. Reeve and C.H. Waddington (eds.). Quantitative inheritance. HMSO, London.

Chang, L. W., W. J. Yen, S. C. Huang, and P.D. Duh. 2002. Antioxidant activity in sesame coat. Food Chem. 78:347-354.

Christie, B. R. and V. I. Shattuck. 1992. The diallel cross: Design, analysis and use for plant breeders. Plant Breed. Rev. 9:9-36.

Chundawat, G. S. 1971. Note on growth of sorghum as affected by methods of sowing, crop mixtures and phosphate levels. Indian J. Agric. Res. 5:212-214.

Clifford, M. N. 1997. Astringency. p. 87-107. In: F.A. Tomas-Barberan and R.J. Robins (eds.). Phytochemistry of fruits and vegetables. Oxford Science Publications, Oxford, UK.

Cockerham, C. C. 1959. Partitions of hereditary variance for various genetic models. Genetics 44:1141-1148.

Cockerham, C. C. 1963. Estimation of genetic variances. p. 53-94. In: W.D. Hanson and H.F. Robinson (eds.). Statistical genetics and plant breeding. National Academy of Science-National Research Council, Washington, D.C.

Comis, D. 2000. Bringing you better beans. Agric. Res. Mag. 48(9): 14-17.

Cook, N. C. and S. Samman. 1996. Flavonoids-chemistry, metabolism, cardio protective effects, and dietary sources. Nutr. Biochem. 7:66-76.

Corner, E. J. H. 1951. The leguminous seed. Phytomorphology 1:117-150.

Deshpande, S. S., U. S. Deshpande and D. K. Salunkhe. 1996. Nutritional and health aspects of food antioxidants. p. 361-469. In: D. L. Madhavi, S. S. Deshpande and D. K. Salunkhe (eds.). Food antioxidants. Marcel Deckker, New York.

Deshpande, S.S., S.K. Shate, D. K. Salunkhe, and D.P. Cornforth. 1982. Effect of dehulling on phytic acid, polyphenols and enzyme inhibitors of dry beans (*Phaseolus vulgaris* L.). J. Food Sci. 47:1846-1849.

Drabo, I., T. A. O. Ladeinde, and R. Redden. 1988. Inheritance of eye pattern and seed coat color in cowpea (*Vigna unguiculata* (L.) Walp). Plant Breed. 100: 119-123.

Duyff, R. L. 2002. American dietetic association complete food and nutrition guide. 2nd ed. Wiley and Sons, New York.

Dziezak, J. D. 1986. Preservatives: Antioxidants. Food Technol. 40:94-102.

Eberhardt, M. V., C.Y. Lee, and R.H. Liu. 2000. Antioxidant activity of fresh apples. Nature 405:903-904.

Economou, K. D., V. Oreopoulou, and C. D. Thomopoulos. 1991. Antioxidant activity of some plant extracts of the family Labiatae. J. Am. Oil Chem. Soc. 68:109-111.

Elias, L. G., D. G. Fernandez, and R. Bressani. 1979. Possible effects of seed coat polyphenolics on the nutritional quality of bean protein. J. Food Sci. 44:524.

Fageria, N.K., V.C. Baligar, and C.A. Jones. 1991. Growth and mineral nutrition of field crops. Marcel Dekker, New York.

Fehr, W. R. 1987. Principles of cultivar development Vol. 1: Theory and technique. Macmillan, New York.

Fery, R. L. 1980. Genetics of *Vigna*. Hort. Rev. 2:311-394.

Fery, R. L. 1981. Cowpea production in the United States. HortScience 16:473-474.

Fery, R. L. 1985. The genetics of cowpea: A review of the world literature. p. 25-62. In: S. R. Singh and K. O. Rachie. (eds.). Cowpea research, production and utilization. John Wiley & Sons, Chichester, UK.

Fogliano, V., V. Verde, G. Randazzo, and A. Ritieni. 1999. Method for measuring antioxidant activity and its application to monitoring the antioxidant capacity of wines. J. Food Chem. 47:1035-1040.

Food and Nutrition Board. 1998. Dietary reference intakes: Proposed definition and plan for review of dietary antioxidants and related compounds. Natl. Acad. Press. http://www4.nas.edu/IOM/IOMHome.nsf/Pages/Ongoing+studies.

Francis, F. J. 1993. Polyphenols as natural food colorants. p.209. In: A. Scalbert (ed.). Polyphenolic phenomena. Institut National de la Recherché Agronomique, Paris.

Gardner, C.O. and S. A. Eberhart. 1966. Analysis and interpretation of the variety cross diallel and related populations. Biometrics 22:439-452.

Ghosh, H. and A. Das. 2003. Optimal diallel cross designs for estimation of heritability. J. Stat. Plann. Infer. 116:185-196.

Gilbert, N.E.G. 1958. Diallel cross in plant breeding. Heredity 12:477-492.

Gill, K.S., G.S. Bhullar, G.S. Mahal and A.S. Khehra. 1977. Combining ability for the sedimentation and pelshenke valve in F_1-F_5 diallel crosses in wheat (*Triticum aestivum* L.). Z. Pflanzenzucht. 78:73-78.

Goffman, F. D. and H. C. Becker. 2001. Diallel analysis for tocopherol contents in seeds of rapeseed. Crop Sci. 41:1072-1079.

Griffing, B. 1956a. A generalized treatment of the use of diallel crosses in quantitative inheritance. Heredity 10:31-50.

Griffing, B. 1956b. Concept of general combining and specific combining ability in relation to diallel crossing systems. Aust. J. Biol. Sci. 9:463-493.

Grossniklaus, U., C. Spillane, D.R. Page, and C. Köhler. 2001. Genomic imprinting and seed development: Endosperm formation with and without sex. Curr. Opin. Plant Biol. 4:21-27.

Hall, C. A. and S. L. Cuppett. 1997. Structure-activities of natural antioxidants. p.2-29. In: O. I. Aruoma, and S. L. Cuppett (eds.). Antioxidant methodology *in vivo* and *in vitro* concepts. AOCS Press, Champaign, IL.

Hallauer, A.R. and J.B. Miranda Fo. 1981. Quantitative genetics in maize breeding. Iowa State Univ. Press, Ames.

Harland, S. C. 1919. Inheritance of certain characters in the cowpea (Vigna sinensis). J. Genet. 8:101-132.

Hayman, B. I. 1954. The analysis of variance of diallel tables. Biometrics 10:235-244.

Hayman, B. I. 1958. The separation of epistasis from additive and dominance variation in generation means. Heredity 12:371-390.

Hayman, B. I. 1960a. The theory and analysis of diallel crosses. III. Genetics 45:155-172.

Hayman, B. I. 1960b. The separation of epistasis from additive and dominance variation in generation means. II. Genetica 31:113-146.

Hayman, B.I. and K. Mather. 1955. The description of genic interactions in continuous variation. Biometrics 11:31-50.

Heneen, W. K. and K. Brismar. 2001. Maternal and embryonal control of seed color by different *Brassica alboglabra* chromosomes. Plant Breed. 120:325-329.

Hertog, M. G. L. and P. C. H. Hollman. 1996. Potential health effects of dietary flavonoid quercetin. European J. Clin. Nutri. 50: 63-64.

Hertog, M. G. L., E. J. M. Feskeens, C. H. Hollman, M. B. Katan and D. Kromhout. 1993. Dietary antioxidants and risk of coronary heart disease: De Zutphen elderly. Lancet 342: 1007-1011.

Horner, T.W., R.E. Comstock and H.F. Robinson. 1955. Non-allelic gene interactions and the interpretation of quantitative genetic data. N.C. Agric. Tech. Bull. 118.

Huang, M. T. and T. Ferraro. 1992 Phenolic compounds in food and cancer prevention. p.318. In: M. T. Huang, C. T. Ho and C. Y. Lee. (eds.). Phenolic compounds in food and their effects on health II. Antioxidants and cancer prevention. ACS Symposium Series 507, Am. Chem. Soc., Washington, DC.

Hudson, B. J. F. 1990. Food antioxidants. Elsevier Applied Science, London.

Ito, N., A. Higawara, M. Shibata, T. Ogiso, and S. Fukushima. 1983. Induction of squamous cell carcinoma in the stomach of F344 rats treated with butylated hydroxyanisole. Gann Manographe Cancer Res. 73:332-334.

Jinks, J.L. and B.I. Hayman. 1953. The analysis of diallel crosses. Maize Genet. Coop. Newsletter 27:48-54.

Kadam, S. S., L. S. Kute, K. M. Lawade, and D. K. Salunkhe. 1982. Changes in chemical composition of winged beans during seed development. J. Food Sci. 47:2051.

Kang, M. S. 2003. Handbook of formulas and software for plant geneticists and breeders. Food Products Press, Binghamton, NY.

Karakaya, S., S. N. El, and A. A. Tas. 2001. Antioxidant activity of some foods containing phenolic compounds. Intern. J. Food Sci. Nutri. 52:501-508.

Kearsey, M.J. and H.S. Pooni. 1996. The genetic analysis of quantitative traits. Chapman & Hall, London.

Kempthorne, O. 1956. Theory of the diallel cross. Genetics 41:451-459.

Kennar, J., E. N. Frankel, R. Granit, J.B. German, and J. E. Kinsella. 1994. Natural antioxidants in grapes and wines. J. Agric. Food Chem. 42:64-69.

Kim, S.Y., S. K. Kim, M. J. Oh, and M. Y. Jung. 1994. Antioxidant activity of selected oriental herb extracts. J. Am. Oil Chem. Soc. 71:633-640.

King, S.P., J.E. Lunn, and R.T. Furbank. 1997. Carbohydrate content and enzyme metabolism in developing canola siliques. Plant Physiol. 114:153-160.

Klein, B. P. and A. C. Kurilich. 2000. Processing effects on dietary antioxidants from plant foods. HortScience 35:580-584.

Koleva, I. I., T. A. van Beek, J. P. H. Linssen, A. de Groot, and L. N. Evstatieva. 2002. Screening of plant extracts for antioxidant activity: A comparative study on three testing methods. Phytochem. Anal. 13:8-17.

Kooiman, H. W. 1931. Monograph on the genetics of *Phaseolus vulgaris* (especially *P. vulgaris* and *P.multiflorus*). Bibliographia Genet. 8:295-413.

Lande, R. 1981. The minimum number of genes contributing to quantitative variation between and within populations. Genetics 99:541-553.

Larson, R. A. 1988. The antioxidants of higher plants. Phytochemistry 27(4):969-978.

Lentner, M., and T. Bishop. (1993). Experimental design and analysis. Valley Book, Blacksburg, VA.

Madisen, H.L. and G. Bertelsen. 1995. Spices as antioxidants. Trends Food Sci. Technol. 6:271-277.

Mather, K. 1949. Biometrical genetics, 1st ed., Methuen, London.

Mather, K. and J.L. Jinks. 1971. Biometrical genetics. Cornell Univ. Press, Ithaca, NY.

Mather, K. and J.L. Jinks. 1977. Introduction to biometrical genetics. Cornell Univ. Press, Ithaca, NY.

McBride, J. 1996. Plant pigments: Paint a rainbow of antioxidants. Agric. Res. Mag. 44(11): 4-8.

McBride, J. 1999. Can foods forestall aging. Agric. Res. Mag. 47(2): 14-17.

McClements, J. and E.A. Decker. 2000. Lipid oxidation in oil water emulsions: Impact of molecular environment on chemical reactions in heterogeneous food system. J. Food Sci. 65: 1270-1282.

Messina, M. J. 1999. Legumes and soybeans: Overview of their nutritional profiles and health effects. Am. J. Clin. Nutr. 70(suppl.):439S-450S.

Miller, J. C., Jr. 1989. Nitrogen fixation in cowpea: The role of the host plant. p. 32-40. In: J.C. Miller, Jr, J.P. Miller, and R.L. Fery (eds.). Cowpea research: A U.S. perspective. Proc. 2nd Southernpea (cowpea) Workshop, Nashville, TN.

Miller, J.C., Jr., K.W. Zary, and G.C.J. Fernandez. 1986. Inheritance of N_2 fixation efficiency in cowpea. Euphytica 35:551-560.

Miller, S.S., L.A.A. Bowman, M. Gijzen, and B.L.A. Miki. 1999. Early development of the seed coat of soybean (*Glycine max*). Ann. Bot. 84:297-304.

Minami, H., M. Kinoshito, Y. Fukuyama, M. Kodoma, T. Yoshizawa, M. Suggiura, K. Nakagawa and H. Taga. 1994. Antioxidant xanthones from *Garcina subellipica*. Phytochemistry 36: 501-506.

Morello, M. J., F. Shahidi, and C. T. Ho. 2002. Free radicals in foods: Chemistry, nutrition, and health effects. ACS Symposium Series 807. Am. Chem. Soc., Washington, DC.

Mukai, K., S. Yokoyama, K. Fukuda, and Y. Uemoto. 1987. Kinetic studies of antioxidant activity of new tocopherol model compounds in solution. Bull. Chem. Soc. Jpn. 60:2163-2167.

Murray, D. R. 1988. Nutrition of the angiosperm embryo. Wiley & Sons, New York.

Namiki, M. 1990. Antioxidants/antimutagens in foods. Crit. Rev. Food Sci. Nutr. 29:273-300.

Narasinga Rao, B.S. 1995. Bioactive phytochemicals in Indian foods. NFI bull. 16(1).

Nienhuis, J. and S. P. Singh. 1986. Combining ability analyses and relationships among yield, yield components, and architectural traits in dry beans. Crop Sci. 26:21-27.

Niki, E. and N. Noguchi. 2000. Evaluation of antioxidant capacity. What capacity is being measured by which method? IUBMB Life 50:323-329.

Nzaramba, N.M., D.C. Scheuring, A.L. Hale, and J.C. Miller, Jr. 2003. Antioxidant activity in cowpea determined on a single-seed basis. HortScience 38:1298. (Abstr.).

Onwuliri, V.A and J.A. Obu. 2002. Lipids and other constituents of *Vigna unguiculata* and *Phaseolus vulgaris* grown in northern Nigeria. Food Chem. 78(1): 1-7.

Osawa, T., N. Ramarathnam, S. Kawakishi, M. Namiki, and T. Tashiro. 1985. Antioxidative defense systems in rice hull against damage caused by oxygen radicals. Agric. Biol. Chem. 49:3085-3087.

Pemberton, I.J., G.R. Smith, and J.C. Miller, Jr. 1990. Inheritance of ineffective nodulation in cowpea. Crop Sci. 30:568-571.

Prakken, R. 1970. Inheritance of colors in *Phaseolus vulgaris* L. II. A critical review. Med. Landbouwhogeschool Wageningen 70-23:1-38.

Prakken, R. 1972. Inheritance of colors in *Phaseolus vulgaris* L. III. On genes for red seedcoat color and a general synthesis. Med. Landbouwhogeschool Wageningen 72-29:1-82.

Prior, R.L. and G. Cao. 1999. *In vivo* total antioxidant capacity: Comparison of different analytical methods. Free Radic. Biol. Med. 27:1173-1181.

Quin, F. M. 1997. Introduction. p. ix-xv. In: B.B. Singh, D.R. Mohan Raj, K.E. Dushiell, and L.E.N. Jackai (eds.). Advances in cowpea research. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria.

Rapola, J. M., J. Virtamo, S. Ripatti, J. K. Huttunen, D. Albanes, P.R. Taylor, and O.P. Heinonen. 1997. Randomized trial of alpha-tocopherol and beta-carotene supplements on the incidence of major coronary events in men with previous myocardial infection. Lancet 349: 1715-1720.

Reddy, N. R., M. D. Pierson, S. K. Sathe, and D. K. Salunkhe. 1995. Dry bean tannins: A review of nutritional implications. J. Am. Oil Chem. Soc. 62:541.

Riedl, K.M., S. Carando, M. H. Alessio, M. McCarty, and E. A. Hagerman. 2002. Antioxidant activity of tannins and tannin-protein complexes: Assessment *in vitro* and *in vivo*, p. 188-200. In: M. J. Morello, F. Shahidi, and C. T. Ho (eds.). Free radicals in food: Chemistry, nutrition, and health effects. Am. Chem. Soc., Washington, DC.

Robards, K., P. D. Prenzler, G. Tucker, P. Swatsitang, and W. Glover. 1999. Phenolic compounds and their role in oxidative processes in fruits. Food Chem. 66:401-436.

Salah, N., N. J. Miller, G. Pagana, L. Tijburg, G. P. Bolwell and C. Rice-Evens. 1995. Polyphenolic flavonols as scavengers of aqueous phase radicals and as chain-breaking antioxidants. Arch. Biochem. Biophys. 2:339-346.

SAS, 1999. SAS software version 8.0. SAS Institute, Inc., Cary, NC.

Saunders, A.R. 1959. Inheritance in cowpea (*Vigna sinensis* Endl.). 1. Color of the seed coat. S. Afr. J. Agric. Sci. 2:285-306

Schmidt, J.S., J.T. Lindstrom, and L.O. Vodkin. 1994. Genetic length polymorphisms create size variation in praline-rich proteins of the cell wall. Plant J. 6:177-186.

Schuler, P. 1990. Natural antioxidants exploited commercially. p.99-170. In: B. J. F. Hudson (ed.). Food antioxidants. Elsevier, London.

Sellschop, J.P.F. 1962. Cowpeas, *Vigna unguiculata*. Review article. Field Crops Abstr. 15:259-266.

Shahidi F. and M. Naczk. 1995. Food phenolics: Sources, chemistry, effects and applications. Technomic Publishing Co., Lancaster, PA.

Shahidi, F. 2002. Antioxidants in plants and oleaginous seeds. p. 162-175. In: M. J. Morello, F. Shahidi and C. T. Ho (eds.). Free radicals in food: Chemistry, nutrition, and health effects. Am. Chem. Soc., Washington, DC.

Shahidi, F. and M. Naczk. 1995. Phenolic compounds in grains. p. 3-39. In: F. Shahidi and M. Naczk (eds). Food phenolics: Sources, chemistry, effects and applications. Technomic Publishing Co., Lancaster, PA.

Shahidi, F., P. K. Janitha, and P. D. Wanasundara. 1992. Phenolic antioxidants. Crit. Rev. Food Sci. Nutr. 32:67-103.

Sherwin, E. R. 1978. Oxidation and antioxidants in fat and oil processing. J. Am. Oil Chem. Soc. 55:809-814.

Shi, H. and E. Niki. 1998. Stoichiometric and kinetic studies on ginkgo biloba extract and related antioxidants. Lipids 33:365-370.

Siddhuraju, P., P. S. Mohan, and K. Becker. 2002. Studies on the antioxidant activity of Indian laburnum (*Cassia fistula* L.): A preliminary assessment of crude extracts from stem bark, leaves, flowers and fruit pulp. Food Chem. 79:61-67.

Simon, W. P. 1997. Plant pigments for color and nutrition. HortScience 32:12-13.

Singh, B. B., O. L. Chambliss, and B. Sharma 1997. Recent advances in cowpea breeding. p. 30-49. In: B.B. Singh, D.R. Mohan Raj, K.E. Dushiell, and L.E.N. Jackai (eds.). Advances in cowpea research. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria.

Smatt, J. 1976. Tropical pulses. Longman, London.

Sokol, M. J. and R. J. Baker. 1977. Evaluation of the assumptions required for the genetic analysis interpretation of diallel experiments in self-pollinated crops. Can. J. Plant Sci. 57: 1185-1191.

Spillman, W. J. 1912. As cited In: R.L. Fery. Genetics of vigna. Hort. Rev. 1980, 2:311-394.

Spillman, W.J. and W.J. Sando. 1930. Mendelian factors in the cowpea (*Vigna* species). Mich. Acad. Sci., Arts Lett. Pap. 11: 249-283.

Stafford, H. A. 1990. Flavonoid metabolism. CRC Press, Boca Raton, FL.

Stampfer, M. J., C. H. Henneekens, J. E. Manson, G. A. Colditz, B. Rosner, and W. C. Willet. 1993. Vitamin E consumption and the risk of coronary disease in women. New Eng. J. Med. 328: 1444-1449.

Stelljes, K. B. 2001. Colorful potatoes offer nutrition, variety. Agric. Res. Mag. 49(10):6.

Summerfield, R.J., Huxley, P.A., and Steelle, W. 1974. Cowpea (*Vigna unguiculata* L. Walp). Field Crops Abstr. 27:301-312.

Thorne, J. H. 1981. Morphology and ultrastructure of maternal seed tissue of soybean in relation to the import of photosynthate. Plant Physiol. 67:1016-1025.

Troszyńska, A., I. Estrella, M.L. López-Amóres, and T. Hernández. 2002. Antioxidant activity of pea (*Pisum sativum* L.) seed coat acetone extract. Lebensm. Wiss. Technol. 35:158-164.

Tsuda. T., Y. Makino, H. Kato, T. Osawa, and S. Kawakishi. 1993. Screening for antioxidantive activity of edible pulses. Biosci. Biotechnol. Biochem. 57:1606-1608.

Van der Veen, J. H. 1959. Test of non-allelic interaction and linkage for quantitative characters in generation derived from two diploid pure lines. Genetica 30:201-232.

Van Wyk, H.P.D. 1955. The nutritive value of South African feeds. Sci. Bull. Depart. Agric. S. Afr. No. 354.

Velioglu, Y. S., G. Mazza, L. Gao, and B. D. Oomah. 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J. Agric. Food Chem. 6:4113-4117.

Wan, L., Q. Xia, X. Qui, and G. Selvaraj. 2002. Early stages of seed development in *Brassica napus*: A seed coat-specific cysteine proteinase associated with programmed cell death of the inner integument. Plant J. 30(1):1-10.

Wang, H., G. Cao, and R. L. Prior. 1996. Total antioxidant capacity of fruits. J. Agric. Food Chem. 44:701-705.

Warrington, R. T., A.L. Hale, D.C. Scheuring, D.W. Whitaker, T. Blessington and J.C. Miller, Jr. 2002. Variability for antioxidant activity in cowpea (*Vigna unguiculata* (L.) Walp.) as influenced by genotype and postharvest rehydration. HortScience 37: 738.

Wassami, N.N., T.G. Isleib and G.L. Hosfield. 1986. Fixed effects genetic analysis of a diallel cross in dry beans (*Phaseolus vulgaris* L.). Theor. Appl. Genet. 72:449-454.

Wheeler, W.A. 1950. Forage and pasture crops. A handbook of information about the grasses and legume grown for forage in the United States. Van Nostrand, New York.

Willet. 1993. Vitamin E consumption and the risk of coronary disease in women. New Eng. J. Med. 328: 1444-1449.

William, L. F. 1950. Structure and genetic characteristics of the soybean. p. 111-134. In: K.S. Markley (ed.). Soybeans and soybean products. Vol. 1, Interscience Publishers, New York.

Winton, A.L. and K.B. Winton. 1932. The structure and composition of foods. Vol. 1. Cereals, starch, oil seeds, nuts, and forage plants. Wiley, New York.

Wobus, S.P. and H. Weber. 1999. Seed maturation: Genetic programmes and control signals. Curr. Opin. Plant Biol. 2:33-38.

Wricke, G. and W.E. Weber. 1986. Quantitative genetics and selection in plant breeding. Walter de Gruyter, Berlin.

Wright, A. J. 1985. Diallel designs, analyses, and reference populations. Heredity 54:307-311.

Yaklich, R.W., E.L. Vigil, E.F. Erbe, and W.P. Wergin. 1992. The fine structure of aleurone cells in the soybean seed coat. Protoplasma 167:108-119.

Yagi, K. 1970. Rapid method for evaluation of autoxidation and antioxidants. Agric. Biol. Chem. 34:142-145.

Yamaguchi, M. 1983. World Vegetables: Principles, production and nutritive values. Ellis Horwood, Chichester, England.

Yen, G. C. and P. D. Duh. 1994. Scavenging effects of methanolic extracts of peanut hulls on free-radical and active-oxygen species. J. Agric. Food Chem. 42:629-632.

Yen, G. C., P. D. Duh, and D. Y. Chuang. 2000. Antioxidant activity of anthraquinones and anthrone. Food Chem. 70: 437-441.

Zary, K. W. 1980. The genetics and heritability of quantitative differences in N_2 fixation in cowpea, *Vigna unguiculata* (L.) Walp. Dissertation, Texas A&M University, College Station.

Zhang, Y., and M. S. Kang. 1997. DIALLEL-SAS: A SAS program for Griffing's diallel analyses. Agron. J. 89:176-182.

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Publication:

Nzaramba N.M., D.C. Scheuring, A.L. Hale and J.C. Miller, Jr. 2003. Antioxidant activity in cowpea determined on a single seed basis. HortScience 38(6):1298. (Abstr).