COLOR RETENTION AND ANTHOCYANIN CONCENTRATION IN CANNED BLACK BEANS

By

Othman Mubarak Al Dossary

A THESIS

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

Crop and Soil Sciences - Master of Science

2016

ABSTRACT

COLOR RETENTION AND ANTHOCYANIN CONCENTRATION IN CANNED BLACK BEANS

By

Othman Mubarak Al Dossary

Common beans (*Phaselous vulgaris* L) represents the most widely cultivated of the *Phaseolus* species. It is a nutritious crop that provides protein, minerals and dietary fiber. Black beans are one of the most popular market classes, with large consumption in the U.S., Mexico, and Central America. Black beans are distinguished from other common beans classes by high anthocyanins in the seed coat which impart the black color. The water soluble anthocyanins make the canning process for black beans challenging. Canning requires several steps, including soaking and boiling. Although the canning process makes beans edible, it also alters the physical and chemical properties of the seeds. In the case of black beans, soaking seeds accelerates anthocyanin loss which lighten the seeds and produce a canned product with poor appearance. Genetic variability for color retention can be exploited to select and incorporate superior genetics for this trait. The objective of this research was to assess the genotypic and phenotypic diversity for a set of 69 black bean breeding lines and cultivars from the major U.S. public bean breeding programs. Each of the lines was grown in field trials in 2013 and 2014. They were evaluated for agronomic, canning characteristics and anthocyanins profile of raw and canned seed. Color retention as determined by a trained sensory panel on a scale of 1 to 5 was highly variable and ranged from 1.4 to 4.5. Delphinidin-3-glucoside was identified as the dominant anthocyanin with the highest concentration among black bean genotypes. The anthocyanin malvidin-3-glucoside was found to be retained after canning more than the other two anthocyanins. Genome wide association analysis was conducted to determine genomic regions responsible for color retention and canning quality in black beans that were genotyped with 5398 SNP markers. A region on Pv05 at 39Mb was associated with color retention and was polymorphic candidates for MAS.

ACKNOWLEDGEMENTS

First of all, I would like to thank my almighty God for honoring me with his grace and mercy, and leading me to what I have achieved. I would like to thank my parents, my wife, and my daughter for their precious patience and infinite support. I will never forget the continued guidance and help from my advisor Dr. Karen Cichy. Many thanks for my committee members Dr. James Kelly and Dr. Kirk Dolan for agreeing to be in my committee. Also, I would like thank my colleagues Dennis Katuramu and Sharon Hooper and all my lab members. Finally, it was a blessing to be a graduate student in one of the best agricultural universities in the world, Michigan State University (MSU).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

KEY TO ABBREVIATIONS

TAC – Total anthocyanin concentration

SAQ – Specific anthocyanin quantification

AR – Anthocyanins retention percentage

CVD - cardiovascular disease

CBB - Common bacterial blight

QTLs - Quantitative trait loci

SNP – Single nucleotide polymorphism

MA - Middle American gene pool

M – Mesoamerica

FAO - The Food and Agriculture Organization

SSA - Sub-Saharan Africa

MAS - Marker assisted selection

RGB - Red, green, blue

HSV - Hue, saturation, and value

UV – Ultraviolet radiation

LD - Linkage disequilibrium

GWAS - Genome-wide association mapping

SSR - Simple sequence repeats marker

SV - Saginaw Valley

MLM - Mixed linear model

ANOVA - Analysis of variance

PCA - Principal Component Analysis

LOD - Logarithm (base 10) of odds

Q-Q - Quantile- quantile plot

LC-MS - Liquid chromatography-mass spectrometry instrument

UPLC - Ultra Performance Liquid Chromatography

ESI - Electrospray ionization mass spectrometry

CV - Coefficient of Variation

PCA – Principal component analysis

CHAPTER 1

INTRODUCTION AND PROBLEM STATEMENT

Dry beans (*Phaseolus vulgaris* L) are the most widely cultivated of the *Phaseolus* species. They are an important crop in many parts of the world including Africa, Latin America, and North America. Dry beans are a nutritious food, rich in protein, fiber, and some essential minerals such as Ca, Fe, and Zn (Ellis, 2007). Including dry beans in a regular diet may contribute to avoidance and treatment of chronic diseases such as, diabetes, cardiovascular disease (CVD) and cancer, which are leading causes of mortality in the U.S. (Hutchins et al., 2012). There are over 14 commercial dry bean classes including dark red kidney, pinto, cranberry, and black (Singh, 2008). The black bean class is one of the most popular market classes, with large consumption in the U.S., Mexico, Central America, and South America.

Like most dry bean types, black beans are considered to be a good source of protein, fiber, folate, and minerals. The uniqueness of this type of beans lies in their black color, can determined by the high amount of anthocyanin compounds in their seed coat. Black beans contain the highest percentage of anthocyanins among all dry bean types (Dzomba et al., 2013). Anthocyanins offer benefits to human health through their anti-oxidant activity, which can help eliminate free radicals (Díaz et al., 2010; La Cruz et al., 2013). The black color is not only related to anthocyanins, but to other phenolic compounds such as tannins, which are concentrated in the internal layer of the seed coat (Cichy et al., 2014). Higher tannin concentrations in conjunction with low anthocyanin content lead to more brownish seeds (Marles et al., 2010). Genetic variability for anthocyanin concentrations exists among black bean genotypes. Selecting genotypes with higher anthocyanins has the potential to increase the health benefits of the beans. In addition, black beans are usually sold as canned products. Canned bean color retention is an important attribute for processors and

consumers. The degree of color retention following canning depends on a cultivar's genetic makeup. Some black bean cultivars such as "Zenith", which show high color retention, have been identified. The optimal black bean seeds for commercial use not only retain more color, but have the ability to expand rapidly and uniformly during soaking, and remain firm during processing (Kelly et al., 2015).

Currently a small scale canning protocol is the standard method used to identify breeding lines with acceptable canning quality and color retention. This method requires at least 200g of seed per genotype and specialized equipment, therefore, it is not feasible for early generation materials neither it is accessible to all breeders. Molecular markers as a tool are extensively used in plant breeding, and compared to traditional breeding, the use of molecular markers reduces time and effort (Sivolap, 2013).

In beans, molecular markers for numerous disease-resistant genes have been integrated into breeding programs. For instance, common bacterial blight (CBB) is a major yield-reducing factor of common bean. In a previous study, two sequence characterized amplified regions (SCARs) BC420 and SU91 were linked to two CBB resistant quantitative trait loci (QTLs) (Miklas et al., 2006). Through the development of candidate gene marker and candidate gene selection, a recent study determined nine and six polymorphic markers in the BC420- and SU91-QTL loci, respectively (Shi et al., 2012). While QTL studies have been conducted for canning quality and color retention in black beans (Wright and Kelly, 2014; Cichy et al., 2014) molecular markers have yet to be developed for these traits and integrated into breeding programs. Molecular markers for these traits would be valuable to bean breeders.

The appearance and color retention of canned black bean products influences the acceptability of the product by consumers. During processing the beans stay whole and intact. Also, consumers

prefer that few ingredients are added to the product and would appear on the label. Therefore, the starting seed is very important in determining the end product quality. While the environmental and handling conditions influence the quality, genetics is also a major determinant of quality. This study aims to identify genomic regions responsible for controlling color retention in 69 black bean breeding lines from the major U.S. bean breeding programs. The objectives of this research are to:

1- Assess genetic variability for color retention in black beans.
2- Assess genetic variability for anthocyanin concentration in black beans.
3- Apply genome wide association analysis (GWAS) to identify genomic region controlling color retention and anthocyanin concentration in black beans.

SNP markers were generated from 69 black bean genotypes. Phenotypic data on, canned bean color, appearance, and anthocyanins concentration were collected over two field experiments. Application of this knowledge to breeding effort will contribute to the end use quality of black beans.

CHAPTER 2

LITERATURE REVIEW

2.1. Seed Quality in Black Bean

2.1.1 Common Bean

Dry bean (*Phaseolus vulgaris* L) is an important crop in many parts of the world including Africa, Latin America, and North America. Dry bean is considered the third most substantial legume crop among all main legumes food after soybean and peanut (Głowacka et al., 2015). Beside beans, cassava and maize are considered the earliest crops in human history in the Americas (Broughton et al., 2003). Together they were the main staple food in the low to mid-latitudes of the Americas (Broughton et al., 2003). Dry beans are known to have originated in the Americas, based on two discoveries of wild dry beans in Argentina and Guatemala, and some archaeologic remains obtained from the Americas (Mamidi et al., 2011). There are five domesticated species and *P. vulgaris* is the oldest species. The other four domesticated species are *P. lunatus*, *P. dumosus, P. coccineus,* and *P. acutifolius* (Tuberosa et al., 2014). *P. dumosus* and *P. coccineus* are the most closely related to *P. vulgaris*, and they are considered partially intercrossable. However, the other two domesticated species (*P. lunatus, P. acutifolius*) are more distantly related (Tuberosa et al., 2014) and only p. acutifolius can be crossed with *P. vulgaris* using embryo rescue technology.

Dry beans include two major gene pools: The Middle American (MA) gene pool, which extends from Mexico to Central America and Colombia, and the Andean gene pool located in Peru, Chile, Bolivia, and Argentina (Mamidi et al., 2011). These two gene pools have parallel wild and domesticated geographical structures, but the MA possess great diversity than the Andean gene pool (Bitocchi et al., 2012). Gene pool division is based on phaseolin seed protein variation, DNA marker diversity, morphology, isozymes, and mitochondrial DNA differences (Mamidi et al.,

2011). Dry beans from the both gene pools are separated into many different market classes that are identified based on growth habit, phonological traits, seed size, shape, color (red, black, white, yellow, and purple), canning and cooking qualities (Singh, 2008). Each gene pool has three races which are characterized based on the variation of plant and seed morphology and adaptation regimes (Singh et al., 1991). The MA gene pool contains the races Mesoamerica (M), Durango, and Jalisco. Black beans are in the (M) race, and are adapted to tropical lowlands (Beebe et al., 2014).

Dry bean is a mainly self-pollinated and annual legume that grows under different environmental conditions such as warm lowlands, dry and humid highlands (Beebe et al., 2014). It is a diploid crop (2n=2x=22) with a small genome ranging between 450-650 Mb and 11 chromosome (Melotto et al., 2005; Schmutz et al., 2014) . Bean plants have a short life cycle (~90 days), and a maximum of three generations can be produced annually (Fageria & Santos, 2016). In 2010, world bean production was around 23 million tons, with almost half of it produced in Latin America and Africa (Creamer, 2014). The Food and Agriculture Organization (FAO) reports that low income countries are the source of half of the world bean production. The other half comes from other countries, such as the U.S., where dry bean is economically valuable with 769,000 hectares planted in 2012, and with a farm gate value of $1.5 billion (USDA-NASS, 2015). Dry bean is more economically valuable than all other legumes such as, lentil, pea, chickpea, and cowpea., these data give an insight into the present and possible future importance of this crop (Porch et al., 2013). Globally, dry beans have the highest amount of direct consumption among all legumes (Akibode and Maredia, 2011). It has been estimated that the consumption of beans in Sub-Saharan Africa (SSA) meets more than 50% of dietary protein needs for households (Beebe et al., 2014). For instance, bean consumption in some regions of Kenya reaches up to 66 kg per

person annually, which is much higher than the amount consumed in Latin America which may reach 17 kg per person annually (Broughton et al., 2003).

Although the major protein sources are of animal origin, they are not affordable to many people especially in low-income areas. Dry bean is a major nutritional dietary legume worldwide, due to its high concentration of plant protein, fiber, and complex carbohydrates (Mamidi et al., 2011). It contains numerous essential and health-promoting compounds. It is a rich source of micronutrients (Ca, Cu, Fe, Mg, Mn, and Zn), calories, and vitamins (folate, thiamin, and B group vitamins) (Acosta-Gallegos & Kelly, 2007). Bean consumption is beneficial to human health and may help in the avoidance of many mineral deficiencies and diseases (Zhang et al., 2014). Indeed, experimental, epidemiological and clinical studies have shown that including dry beans in a regular diet may contribute to the prevention and treatment of chronic diseases such as diabetes, CVD, obesity, and cancer, which are the main causes of the death in the U.S. (Hutchins et al., 2012; Bauer et al., 2011; Oseguera-toledo et al., 2011; Thompson et al., 2009).

Dry beans are a source of polyphenols, antioxidants, and many different phytochemicals (Joseph et al., 2014). Analysis of bean phytochemicals indicated the presence of several bioactive compounds including alkaloids, anthocyanin, catechin, flavonoids, phytic acid, quercetin, and tannins (Nyau et al., 2015). Seeds coats of dry beans contain delphinidin, cyanidin, and phenolic acids such as gallic, vanillic, caffeic, coumaric and ferrulic acids. Several studies have revealed that polyphenols, flavonoids, and antioxidants exist in dry beans which are associated with beneficial health effects such as anti-cancer properties (Sancho et al., 2015; Gebrelibanos et al., 2013; Moreno-jiménez et al., 2015). Polyphenolic components of dry beans function as cancer chemopreventive agents, particularly by their antioxidant characteristics (Gebrelibanos et al., 2013).

A recent study has shown that different dry bean varieties have remarkably different cancer inhibitory activity associated with their genetic heritage (Zhu et al., 2012). Thompson et al., (2009) have evaluated the anticancer activity for six dry beans (*Phaseolus vulgaris L.*) market classes. During their experiment, processed dry bean powder were fed to lab rats in order to determine a possible impact of bean on chemically induced mammary carcinogenesis in comparison to the typical diet in a preclinical model for breast cancer. They found that a bean diet was able to diminish the percentage of cancerous rats by 20%, and different bean market classes varied in terms of cancer inhibitory activity (Thompson et al., 2009). Therefore, characterizing the diversity of dry bean cultivars will lead to more understanding to the chemical composition of dry beans that includes bioactive components which are responsible of anticancer activity.

2.1.2 Black Bean

The consumption of black bean (*Phaseolus vulgaris L*) is widespread, and black beans are a major food in Mexico (Dong et al., 2007). In Brazil, black bean represents 17% of Brazilian bean production with about 490,000 tons produced per year on nearly 370,000 ha (De Faria et al., 2014). Also, it is the second or third most important bean market class in the U.S. with highest production percentages in Michigan and North Dakota (Kelly et al., 2015). Indeed, Michigan is the top producer of black beans in the United States, contributing 57% of the total production. That is attributed to less risk related to cultivating colored seeds types as black beans, and to the increasing consumption of this type of bean due to a better understanding of its health advantages (Kelly et al., 2015). Furthermore, black bean popularity has increased in the U.S. In fact, the yearly consumption per person in the United States was only 4.5 g in 1970, but it dramatically multiplied 68 times by 2008 to 304g per person (Cichy et al., 2014).

The nutritional value of black beans has been known for decades. In recent decades, black bean has been demonstrated to be used in conventional medical practices. For instance, in Japan and Korea, black beans are widely used as a medicinal food since their seed coat is rich in antioxidants activity derived from the anthocyanins. Like most dry bean types, black beans are considered to be a good source of protein, fiber, and folate. Also, they are nutritionally rich in, anthocyanins, and other polyphenols (Jiang et al., 2014). The uniqueness of this type of beans lies in their black color, which is caused by the high level of anthocyanin compounds in their seed coat. Among all dry bean types, black beans contain the highest percentage of anthocyanins including delphinidin 3-glucoside, petunidin 3-glucoside, and malvidin 3-glucoside (Dzomba et al., 2013). Anthocyanins play a role in maintaining human health through their anti-oxidant activity, which helps to capture oxygen free radicals (O_2^-, OH) (Díaz et al. 2010; La Cruz et al. 2013). Those and other reactive oxygen species (H_2O_2; $1O_2$) can stimulate lipid peroxidation, which can cause cell death and tissue damage (Lee et al., 2008). Many studies indicate a correlation between arthritis, cirrhosis, emphysema, atherosclerosis, cancer and the oxidative damage catalyzed by the free radicals. Many researchers have found that including more antioxidants in a regular diet may reduce CVD risk. Therefore, consuming food with high anthocyanin content may diminish oxidative damage and contribute to overall health (Lee et al., 2008).

2.1.3 Canning Quality

Canning is an important process to improve the palatability of beans and to reduce flatulence factors (raffinose oligosaccharides) (Xu & Chang, 2009; Kutoš et al., 2003). During the canning process beans lose many of their anti-nutritional factors (Xu & Chang, 2009). Several studies on canning indicate that food preparation and cooking methods are as important as the food itself to determine nutritional value (Boateng et al., 2008). The canning process includes soaking and

cooking the seeds following several variable protocols, and then transferring them to cans. Furthermore, thermal processing (cooking) is a traditional way to preserve food and to ensure its safety and storability (Homayouni & Azizi, 2015). Usually, beans are thermally processed by boiling, pressure boiling, or steaming before consumption (Xu & Chang, 2009). Many thermal processes successfully reduce anti-nutrients such as lectin and trypsin inhibitors (Boateng et al., 2008).

The canning quality of dry beans is affected by the growing environment, canning procedures, and the interaction between the two factors. Moreover, the canning process changes the physical properties and chemical composition of the bean (Xu & Chang, 2009). The impact of the environment was shown by Khanal et al (2015) to be significant for the texture of the beans. Mostly, bean processors prefer seeds that are well hydrated, rapidly expanded, equally cooked, and do not cluster in the bottom of the can during the canning process (Khanal et al., 2015). Other canning quality traits that are susceptible to alteration after processing are for example, color, texture, flavor, visual appearance, and digestibility. Canning quality traits are important to bean consumers and processors, and improving them will contribute to the profitability of canned bean market (Khanal et al., 2015).

Black beans are usually sold as canned products in the U.S. market. Black bean color and appearance are important attributes for both processors and consumers (Cichy et al., 2014). During the canning process, dry bean types that show darker colors such as black, small red, and red kidney are more likely to lose their color. The high amount of anthocyanin in black beans increases their tendency to leach out during industrial canning processes such as washing and soaking, and cause discoloration of canning liquid (Dzomba et al., 2013). Black bean seed coats do not contain only extractable anthocyanins, but also bound condensed tannins, and bound anthocyanins (Kelly

et al., 2015). The amounts of each compound vary due to genetic variability and environmental aspects such as growing location (Rocha-Guzmán et al., 2007). These compounds could impact black bean canning quality since they're responsible of the seed coat color. For example, higher anthocyanin concentration in the seed coat would express darker (blacker) seed color (Mojica et al., 2015). Also, the co-occurrence of the three compounds in the seed coats has an impact on the postharvest appearance, canning quality, and on the dietary influences of the black bean market class (Marles et al., 2010). Indeed, high tannin concentration in conjunction with low anthocyanin content leads to more brownish seeds (Marles et al., 2010). Diaz, Caldas, and Blair (2010) reported that anthocyanins and tannins are primarily inherited together by the seed color genes, whereas anthocyanins concentration can be adjusted by a limited number of genes. Therefore, selecting bean lines with a high anthocyanin: tannin ratio is complex, but would contribute to enhancement of the black color trait.

Black bean canning quality is also influenced by seed coat luster (Cichy et al., 2014). Mature black bean seeds are divided into either opaque (dull) or shiny (glossy). From an industrial point of view, whether raw or canned these two black bean seed types should be treated independently (Cichy et al., 2014). In fact, there's an industrial preference for opaque seed type since it takes up water during the canning process faster than the shiny seed type and reduces the processing cost. This difference is due to the different amount of wax layer between the two seed coat luster types (Beninger et al., 2000). Consequently, less wax in the opaque seed type may form some cracks that allow quicker water intake (Bushey et al., 2001; Ma et al., 2004). However, the genetic control of seed coat luster is separate from the genetic control of color retention in canned black beans (Cichy et al., 2014).

Color retention of black beans is not only affected by the canning process, but by other factors such as its genetic make-up. There is a significant genetic variability for color retention of black beans following canning (Wright & Kelly, 2011). Cichy and others (2014) in a recent study indicated that color retention trait in black beans they found that quantitative trait loci (QTLs) for color retention and seed anthocyanin concentration following canning in black beans co-localized on chromosome Pv05. They were able to determine a small number of candidate genes for marker assisted selection (MAS) for the two traits on Pv05 and on Pv11 for the color retention trait. In fact, there is significant genetic variability in color retention among black bean genotypes following canning (Wright & Kelly., 2011). A good understanding of the genetic differences in canning quality traits of various black bean market classes will help improve the breeding process.

2.1.4 Canning Quality Evaluation

Black bean canning quality is usually assessed by a well-trained sensory panel (Merwe et al., 2006). Parameters including physical appearance after canning are important (Khanal et al., 2015). The widely used parameters in the evaluation of black beans are color, visual appearance, texture, hydration coefficient, and percent washed–drained solids (Merwe et al., 2006). Indeed, color and appearance are considered essential physiochemical traits of any food, since they mainly correlate with physical, chemical, and sensory indicators of the product quality. Another method of black bean color and appearance evaluation is a machine vision system, which has been considered for automatic assessment of color and appearance in canned black beans. This machine tests a number of color and appearance features such as average, standard deviation, contrast, correlation, energy and homogeneity measurements from red, green, blue (RGB), L*a*b*, and hue, saturation, and value color scales. These images are taken from drained/washed black beans and their brine solution (100 ppm calcium, 5.67 g of salt/454 g of water, 7.09 g of sugar/454 g of water). The

quality ratings for color and appearance images are evaluated with sensory scores using multi-statistical models (Mendoza et al., 2014). Texture readings are mostly obtained by specialized equipment (Kramer Shear press), and it is one of the canning quality parameters that measures either hardness or softness (palatability) of seeds after canning (Balasubramanian et al., 2000). In addition, the hydration coefficient is defined as the ratio of dry bean seeds weight prior and after soaking process (De Lange & Labuschagne, 2001). The coefficient is an important parameter in beans marketing, since bean seeds with a high up-take water will increase the canned yield. The percent washed–drained solids parameter means the mass of the rinsed and drained canned bean seeds (Balasubramanian et al., 2000). Low percentage indicates excessive loss of solids during canning process, and a higher tendency of bean seeds to forming clusters in the bottom of the can after processing and during storage (Khanal et al., 2015).

2.2. Color Retention and Anthocyanin in Black Bean

2.2.1 Impact of Polyphenolics (anthocyanin and tannin) on Black Bean Color Retention After Canning

Black bean color is determined by the existence and concentration of different types of polyphenolic compounds such as flavonol glycosides, anthocyanins, and condensed tannins (proanthocyanidins). The most common groups of flavonoids in beans are proanthocyanidins and anthocyanins, which have been reported to be present only in black and blue-violet beans (Aparicio-Fernandez et al., 2005). Anthocyanins are plant-derived flavonoid compounds that control colors varying from bright pink to red, purple, and deep blue (Wang et al., 2013). They are found in almost all plant parts, mainly flowers and fruits, storage organs, roots, tubers, and stems. They are present in most species in the plant kingdom. Anthocyanins are water-soluble pigments stored in vacuoles. The color expressed by anthocyanins depend on the pH of the vacuole. This pigment is very sensitive to environmental stresses like strong light, ultraviolet (UV) radiation, and high temperature. There are about 500 types of anthocyanins although there are three basic structural categories, pelargonidin, cyanidin, and delphinidin with each being characterized by the number of hydroxyl groups on the B-ring (Gould et al., 2009). Anthocyanins are used broadly as natural food colorants in the food industry as substitutes for artificial colorants (Wu et al., 2006). Black beans anthocyanins are concentrated in the seed coat (Dzomba et al., 2013). Most anthocyanins that have been extracted from different black bean accessions are delphinidin 3-glucoside, petunidin 3-glucoside, malvidin 3-glucoside, and malvidin 3,5-diglucoside (Zhang et al., 2014). Some anthocyanins have been extracted with lower quantities for instance cyanidin 3-glucoside, cyanidin 3,5-diglucoside, pelargonidin 3-glucoside, and pelargonidin 3,5-diglucoside (Takeoka et al., 1997). Among all these anthocyanins, delphinidin 3-glucoside was found to have

high antioxidant activity when it was extracted from the black bean cultivar "Yamashirokurosando" (Takeoka et al., 1997).

Black beans have other phenolic commands such as tannins that also influence color. Tannins are polyphenolic compounds that differ in size, bond type, and concentration among plant species (Díaz et al., 2010). They show different impacts at the ecological and nutritional levels. Tannins in the seed coat play an important role in preventing fungal infection. Nutritionally however, the existence of multiple hydroxyl groups in condensed tannins results in the creation of complexes with proteins, metallic ions like iron, and polysaccharides like starch. The formation of complexes results in reduced bioavailability of the above nutrients (Díaz et al., 2010). Tannins are located in the inner layer of the black bean seed coat, and give canned beans their red–brown color (Marles et al., 2010). Therefore, color retention in black beans after canning is dependent not only on presence of anthocyanins, but also on the presence of tannins (Marles et al., 2010).

2.3. Linkage Disequilibrium Mapping

2.3.1. Association Mapping

In plant genetics, a goal is to understand the natural phenotypic differences that are caused by alterations in DNA sequence. Linkage mapping has been the wide spread method to achieve this goal by making some empirical crosses to generate a family with known relatedness in order to identify co-segregation of genetic markers and phenotypes within the same family (Myles et al., 2009) In contrast, association mapping (linkage disequilibrium, LD) has been employed to conduct genotype-phenotype correlations in unrelated individuals (Myles et al., 2009). Association mapping is a powerful tool used to detect genes controlling the quantitative variation of the complex traits. Such variation is influenced by several QTLs, the environment effect, and the interfacing between QTLs and the environment (Zhu et al., 2008). Association mapping can be divided into two types, the first is candidate-gene association mapping, which identifies polymorphisms in the elite candidate genes, and becomes responsible of controlling phenotypic differences for the trait of interest. The second is genome-wide association mapping (GWAS), which is capable to scan the whole genome for any possible genetic variation with the goal of discovering any indication of an association for complicated traits (Zhu et al., 2008). The ultimate goal of association mapping is to reveal the complexity of the traits of interest and narrow it down to a gene or a nucleotide. To successfully apply association mapping, knowledge of all genetic characteristics related to germplasm is needed. Adequate numbers of unlinked and selectively impartial background markers are required to accomplish genome-wide coverage and extensively describe the genetic structure of the crop. For example, while 140,000 markers are considered sufficient coverage of the size of Arabidopsis genome 125Mb, two millions markers will be needed to cover the size of the grape genome 475Mb (Kim et al., 2007). Simple sequence repeats “SSRs”

and SNPs are the most effective markers in term of assessing population structure (Q) and the relative kinship matrix (K). Also, SNP makers considered to be the best choice for complicated traits, because they have a higher genome density, lower mutation rate, and high-throughput detection platforms (Zhu et al., 2008).

CHAPTER 3

PHENOTYPIC EVALUATION AND CANNING QUALITY IN BLACK BEAN

INTRODUCTION

Black bean, as most dry beans, is an ideal source of protein, fiber, and folate. Black bean production has expanded in the U.S. within recent years. Annually consumption of black beans per person in the U.S. expanded from 4.5g in 1970 to 304g by 2008 (Cichy et al., 2014). Such a dramatic society diet change toward black beans consumption may be explained on the basis of its health advantages (Kelly et al., 2015). Furthermore, polyphenols and anthocyanins specifically are found in the outer layer of black bean seeds, and it is these compounds which reduce the black color. Black beans are the dry bean type with the highest anthocyanin concentration. The major anthocyanins in black beans include delphinidin 3-glucoside, petunidin 3-glucoside, and malvidin 3-glucoside (Jiang et al., 2014; Dzomba et al., 2013). Anthocyanins are important chemicals that maintain human health through their anti-oxidant activity, which helps reduce oxygen free radicals ($O2^{-}$, OH) (Díaz et al., 2010; La Cruz et al., 2013). Black bean canning quality may also be associated with anthocyanin levels. This study was conducted on 69 black bean genotypes that had been obtained from U.S. breeding programs. These lines were grown in 2013 and 2014 in replicated field trials at the Saginaw Valley Research Farm and Extension center in Richville, MI. Upon harvest the samples were canned and evaluated for canned bean appearance and color retention. The 69 lines were genotyped with 3975 SNP markers, and genome wide association analysis was conducted for canned bean appearance and color retention related traits.

MATERIALS AND METHODS

3.1. Plant Materials

A group of 69 different black bean lines were obtained from Michigan State University (MSU), International Center for Tropical Agriculture (CIAT), Colorado State University (CSU), North Dakota State University (NDSU), Universidad de Puerto Rico (UPR), Nebraska (NE), University of Cincinnati (UC), Saskatoon, U.S. Department of Agriculture (USDA-MI, USDA-TARS, and USDA-WA) and includes dull and shiny black bean seed types.

Figure 1. A neighbor joining tree of 69 black bean genotypes developed using 2800 SNP markers.

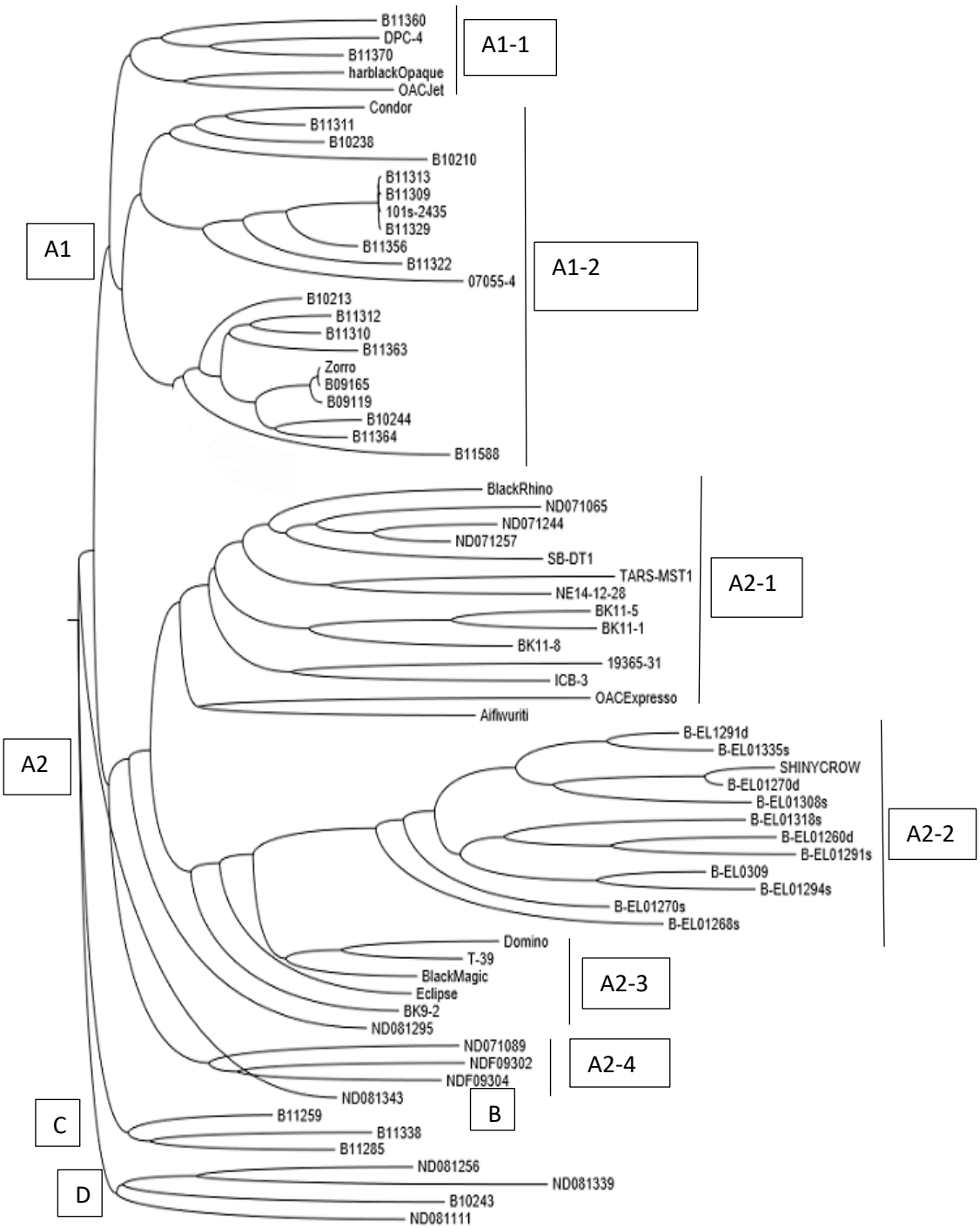


Table 1. A list of the 69 black bean genotypes that were used to evaluate color retention of canned black beans.

No.	Genotype	Shiny / Dull	Origin	Pedigree
1	B11360	Dull	MSU	B04644/B05066
2	Condor	Dull	MSU	Phantom / Black Jack
3	Black Rhino	Dull	CIAT	Negro Tacana/G24424(wild bean)
4	B-EL1291d	Dull	USDA-MI	Black Magic/Shiny Crow
5	B-EL01318s	Shiny	USDA-MI	Black Magic/Shiny Crow
6	B-EL01270s	Shiny	USDA-MI	Black Magic/Shiny Crow
7	B11313	Dull	MSU	B04644//B04349/B05044
8	B10238	Dull	MSU	Zorro/B05055
9	19365-31	Dull	Unknown	Unknown
10	B11356	Dull	MSU	Jaguar/B04644
11	B10213	Dull	MSU	B04587//Zorro/DPC-1
12	SHINYCROW	Shiny	CSU	Unknown
13	ND071065	Dull	NDSU	Unknown
14	B-EL01270d	Dull	USDA-MI	Black Magic/Shiny Crow
15	101s-2435	Dull	Unknown	Unknown
16	TARS-MST1	Dull	USDA-TARS	Ngro Takana/vax6
17	ND081195	Dull	NDSU	Unknown
18	B10210	Dull	MSU	N05324/B04431
19	B11312	Dull	MSU	B04587//B05070/B05044
20	SB-DT1	Dull	USDA-TARS	Unknown
21	Domino	Dull	MSU	Nep2/BTS
22	Zorro	Dull	MSU	B00103*/X00822
23	B-EL01260d	Dull	USDA-MI	Black Magic/Shiny Crow
24	ND081343	Dull	NDSU	Unknown
25	B-EL01268s	Shiny	USDA-MI	Black Magic/Shiny Crow
26	Black Magic	Dull	USDA-MI	Nop-2/black turtle soup
27	BK 11-5	Dull	USDA-WA	Unknown
28	B11363	Dull	MSU	B04644/B07554
29	B11329	Dull	MSU	B04644/B04391
30	ND071089	Dull	NDSU	Unknown
31	ND071244	Dull	NDSU	Unknown
32	ND071257	Dull	NDSU	Unknown
33	DPC-4	Dull	UPR	Arroyo Lorro Negro/Raven
34	Harblack Opaque	Dull	USDA-MI	Unknown
35	B09119	Dull	MSU	B04554/X06127
36	Eclipse	Dull	NDSU	ND9902621-2
37	NE14-12-28	Dull	NE	Unknown
38	B11259	Dull	MSU	N07009//B04349/B05044
39	T-39	Dull	UCDavis	SEL-BTS,T-39
40	B-EL0309	Dull	USDA-MI	Black Magic/Shiny Crow
41	B-EL01294s	Shiny	USDA-MI	Black Magic/Shiny Crow
42	ND081256	Dull	NDSU	Unknown
43	B11588	Shiny	MSU	I82054/B07554
44	B11310	Dull	MSU	B04587//ZORRO/DPC-1

Table 1 (cont'd)

45	BK 11-8	Dull	USDA-WA	Unknown
46	07055-4	Dull	CSU	DOR 500//NAG 210/UI 906(45735) X ARA 13/UI 906//BelMiDak RR2
47	Zenith (B10244)	Dull	MSU	B04644/B07554
48	B11364	Dull	MSU	Unknown
49	B11370	Dull	MSU	B05055/B04265
50	B10243	Dull	MSU	B04610/N05346
51	BK 11-1	Dull	USDA-WA	Unknown
52	B09165	Dull	MSU	B04554/B04587
53	B-EL01335s	Shiny	USDA-MI	Black Magic/Shiny Crow
54	NDFD9302	Dull	NDSU	Unknown
55	B1133	Dull	MSU	N08007//B04349/B05044
56	NDF09304	Dull	NDSU	Unknown
57	OAC Jet	Dull	Saskatoon	OAC Jet
58	OACExpresso	Dull	Saskatoon	Unknown
59	B-EL01291s	Shiny	USDA-MI	Black Magic/Shiny Crow
60	ND081339	Dull	NDSU	Unknown
61	BK9-2	Dull	USDA-WA	Unknown
62	B11285	Dull	MSU	N04152/N05346//N04141/N05317
63	Aifiwuriti	Dull	UPR	Unknown
64	B-EL01308s	Shiny	USDA-MI	Black Magic/Shiny Crow
65	B11311	Dull	MSU	B04587//Zorro/DPC-1
66	ND081111	Dull	NDSU	Unknown
67	B11322	Dull	MSU	B05055/B04644
68	B11309	Dull	MSU	B04587//Zorro/B05055
69	ICB-3	Dull	UPR	Unknown

3.2. Field Description

The 69 genotypes were grown in 2013 and 2014 at the Saginaw Valley (SV), Valley Research Farm and Extension Center (SV) in Richville, MI (Table 1). Materials were planted on June 6th in 2014 and 2013, and harvested September 17th in 2014, and September 15th in 2013.

The growing season in SV, MI is June-September with an average temperature ranging from 9 to 28°C, the soil type is a Tappan- Londo loam with a 2.9% organic matter and an average pH of 7.9 and the altitude is 242 m. Materials were planted in 4-row plots 6.4 m in length with 0.5-m row spacing. Standard management practices were used including fertilizer and irrigation, and farm machinery was involved for these tasks. Seed was direct harvested with a Hege 140 plot. Moisture-adjusted yield per plot was measured on cleaned seed and used to calculate yield per hectare. A neighbor-joining tree, using the Chord distance matrix, was obtained using the TASSEL software (Bradbury et al., 2007).

3.3. DNA Extraction

DNA was extracted from two-wk-old leaves using the hexadecyltrimethyl ammonium bromide method (Rogers and Bendich, 1985). The DNA concentrations were evaluated by quantifying the optical density at A260 and A280 wavelengths with a Nanodrop Spectrophotometer (ND-8000; NanoDrop Products, Wilmington, DE) (Rogers & Bendich, 1985).

3.4. SNP Genotyping

DNA extracts from all 69 black bean genotypes were shipped to the Soybean Genomics and Improvement USDA Laboratory in Beltsville, Maryland, where the DNA extracts were sequenced using an Illumina BARC-Bean 6k-3 beadChip (USDA laboratory, 2014). The whole set of 5398 SNP markers was filtered to remove monomorphic SNPs and those SNPs with a minor allele

frequency of 0.02% or less. After filtering, 2800 SNPs remained for GWAS analysis with a mixed linear model (MLM) (Song et al., 2015).

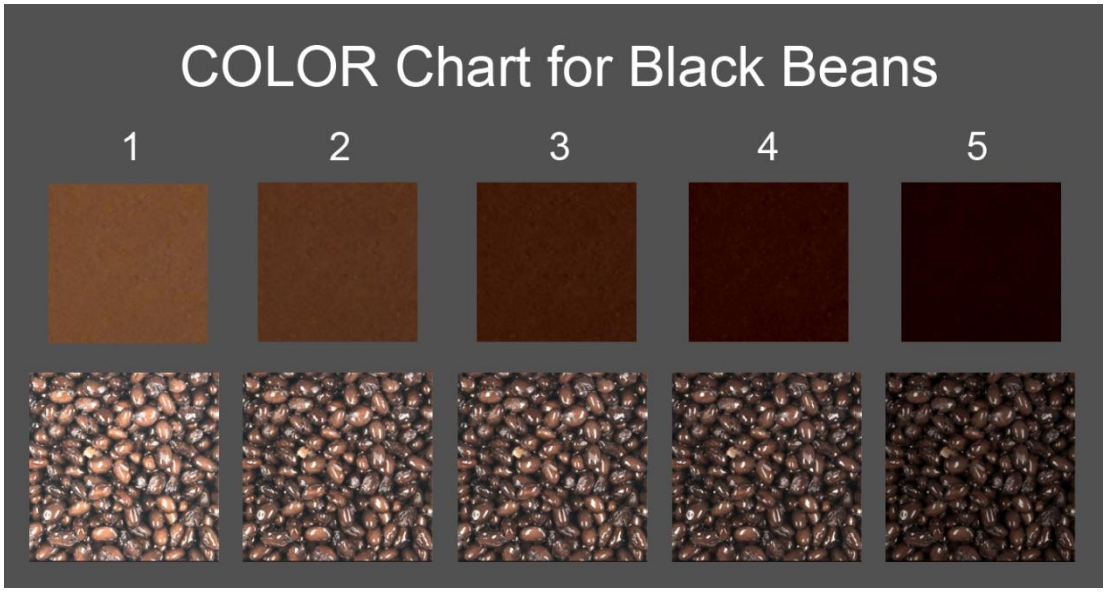
3.5. Canning Process

A 100g of black bean seeds were cleaned weighed and placed in mesh bags. They soaked in a cold solution (25°C) of distilled water with calcium chloride for 30min. The seeds were weighted again and transferred to cans. Then, cans were filled with 98.9°C brine solution (1.5% sucrose, 1.2% sodium chloride, 0.03% calcium chloride, and distilled water). The cans were sealed and processed for 45 min at 115.6°C in a steam retort (National Board No. 813, Loveless Manufacturing, Tulsa, OK). Finally, the cans were stored for two weeks at room temperature (25°C) before evaluation.

3.6. Evaluation Process

An evaluation process was conducted on the canned black bean seeds to assess the quality of the seeds, and was divided into two methods. The first was a sensory evaluation by a group of 14 trained and expert evaluators who visually rated the color and appearance of the beans by comparing them to quality charts for color (Figure 2A), and appearance (Figure 2B) of canned beans. The first category in color chart is very light brown. The second category is slight dark brown or light gray. The third is average brown black. The forth category is dark brown or medium black. The fifth category is very dark.

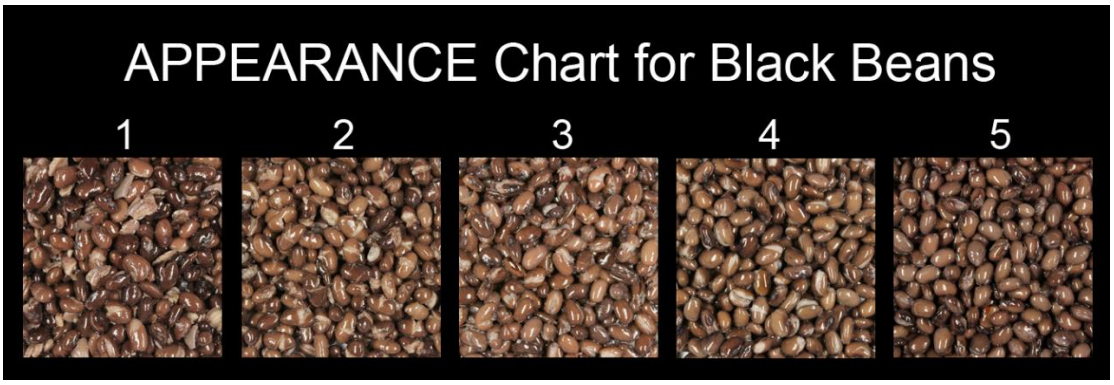
Figure 2A. Color rating chart for black beans.



(Mendoza et al., 2016)

In the appearance chart, the first group is unacceptable, with severely split seeds and burst seeds. The second is poor, with seeds badly split but intact. The third is average, showing 60-69 % of seeds intact. The fourth has 70-89 % of seeds moderately intact, and the fifth has an appearance with >90% of seeds intact.

Figure 2B. Appearance rating chart for black beans.



(Mendoza et al., 2016)

Color was also measured quantitatively to obtain the three color values Lab. In 2013, a colorimeter Hunter Lab scan XE (Hunter Associate Laboratory, Inc., Reston, VA 20190, USA), was used for

extracting color parameters from washed and drained canned beans. The data taken from this machine can be set to any CIE units. However, in 2014 a machinery vision system that was made by (Mendoza et al., 2016), was used to obtain the same three color parameters, L*a*b*.

3.7. Statistical Analysis

Analysis of variance (ANOVA) for the seven traits was conducted with the model:

Yijk= μ + genotype i + year j + genotype i * year j + eijk

using SAS9.4 statistical software package. The GLM procedure for this paper was generated using SAS software, version 9.4 of the SAS System Copyright © 2016 SAS Institute Inc. SAS and all other SAS Institute Inc.

Data means were separated using Fisher's protected LSD at the $\alpha \leq 0.05$ level of significance (Pritchard et al., 2000). Population structure was considered in the model with Principal Component Analysis (PCA) using a correlation matrix in TASSEL 5.2.25 program (Bradbury et al., 2007) and various PCs were accounted. A kinship matrix (K) was also included in the association analysis to account for relatedness. GWAS was conducted using the Mixed Linear Model approach (Pritchard et al., 2000). The quantile- quantile (QQ) plot was generated from the observed and expected (logarithm (base 10) of odds) LOD scores for each trait. Manhattan plot and QQ plot graphics were developed in TASSEL program (Turner, 2014).

The phenotypic data for all the seven traits were averaged across the 2013 and 2014 field seasons prior to GWAS. The traits of seed weight, color, L*, a*, and b* for both years and appearance for 2014 were not normally disrupted and were transformed with the Box-Cox method (Box and Cox, 1964). However, the mentioned traits showed better QQ plots and Manhattan plots with their original data which have been used for GWAS study. The following MLM equation was used: $Y = X\alpha + P\beta + K\mu + e$, where Y is phenotype, X is SNP, p is the PCA (Principle Component Analysis)

matrix and both X and p represent fixed effects, K is the relative kinship matrix value, and e is for residual effects.

RESULTS AND DISSCUSSION

A set of 69 canned black bean accessions were evaluated for traits related to seed quality included yield, seed weight, appearance, color, L*, a*, and b* over two field seasons. The proc mixed procedure was used for analysis of variance with the model including genotype (69 levels) year (2 levels) and rep (2 levels) and the interaction genotype × year. Analysis of variance (ANOVA), (Table 2A-G) indicated that genotypes and year based on ($\alpha \leq 0.05$) as level of significance were significantly different. The interactions between genotype and year for all the traits were also significant based on the same level of significance, and that requires a separation for the data analyses by year (Table 2A-G).

Table 2A. Analysis of variance for yield trait collected on 69 black bean genotypes grown for 2 years (2013 and 2014).

Trait	Source of variation	Degree of freedom	Type III SS	Mean square	F statistic	Pr>F
Yield	Genotype	68	58428662.46	886692.05	3.01	<0.0001
	Year	1	34693849.70	34693849.70	121.65	<0.0001
	Genotype*Year	68	29458632.42	433215.18	1.52	<0.0202

Table 2B. Analysis of variance for seed weight trait collected on 69 black bean genotypes grown for 2 years (2013 and 2014).

Trait	Source of variation	Degree of freedom	Type III SS	Mean square	F statistic	Pr>F
Seed Weight	Genotype	68	773.80	11.38	5.38	<0.0001
	Year	1	250.61	250.61	118.47	<0.0001
	Genotype*Year	68	270.02	3.97	1.88	<0.0010

Table 2C. Analysis of variance for appearance trait collected on 69 black bean genotypes grown for 2 years (2013 and 2014).

Trait	Source of variation	Degree of freedom	Type III SS	Mean square	F statistic	Pr>F
Appearance	Genotype	68	69.67	1.03	3.77	<0.0001
	Year	1	78.70	78.70	289.91	<0.0001
	Genotype*Year	68	26.32	0.39	1.43	0.0417

Table 2D. Analysis of variance for color trait collected on 69 black bean genotypes grown for 2 years (2013 and 2014).

Trait	Source of variation	Degree of freedom	Type III SS	Mean square	F statistic	Pr>F
Color	Genotype	68	195.01	2.86	19.94	<0.0001
	Year	1	63.25	63.39	439.87	<0.0001
	Genotype*Year	68	17.27	0.25	1.77	0.0027

Table 2E. Analysis of variance for L* value trait collected on 69 black bean genotypes grown for 2 years (2013 and 2014).

Trait	Source of variation	Degree of freedom	Type III SS	Mean square	F statistic	Pr>F
L*	Genotype	68	357.07	5.25	3.71	<0.0001
	Year	1	433.45	433.45	306.28	<0.0001
	Genotype*Year	68	1689.50	24.85	17.56	<0.0001

Table 2F. Analysis of variance for a* value trait collected on 69 black bean genotypes grown for 2 years (2013 and 2014).

Trait	Source of variation	Degree of freedom	Type III SS	Mean square	F statistic	Pr>F
a*	Genotype	68	295.20	4.34	23.28	<0.0001
	Year	1	1791.90	1791.90	2610.77	<0.0001
	Genotype*Year	68	40.16	0.59	3.17	<0.0001

Table 2G. Analysis of variance for b* value trait collected on 69 black bean genotypes grown for 2 years (2013 and 2014).

Trait	Source of variation	Degree of freedom	Type III SS	Mean square	F statistic	Pr>F
b*	Genotype	68	463.36	6.81	38.70	<0.0001
	Year	1	381.24	381.24	2165.55	<0.0001
	Genotype*Year	68	67.10	0.99	5.61	<0.0001

The distribution of the data for all the traits including yield, seed weight, appearance, color, L*, a*, and b* of canned black beans for the 2013, and 2014 harvest seasons are shown in Figure 3. Yield was evaluated as kg per hectare. Yield for both years (2013 and 2014) were normally distributed (Figure 3). In 2013, yield ranged from 828 to 3273 kg ha^{-1}, and the average was 2150 kg ha^{-1}, while in 2014 yield ranged from 1649 to 4648 kg ha^{-1}, and the average was 2867 kg ha^{-1} which is much larger than 2013 (Table 3). Seed weight was measured using the weight of 100 seeds. The trait of seed weight was not normally distributed for both years (2013 and 2014) (Figure 3). In 2013, seed weight ranged from 14.9 to 22.6g, and the mean seed weights was 19.1 g. In 2014 the seed weights ranged from 17.6 to 27.6 g, and the mean weight was 21.3g (Table 3). Therefore, the seeds used for canning in 2014 were larger than those in 2013.

The trait of appearance was normally distributed in 2013 with rating values ranged from 1.36 to 3.54, and the ratings average was 2.60 (Table 3). In contrast, in 2014, the trait was not normally distributed and was skewed to higher appearance ratings. The ratings ranged from 2.00 to 4.16, and the ratings mean was 3.27, which is higher than 2013. A commercial standard line was drawn on the rating 3.00 to allow us differentiate between acceptable and non-acceptable genotypes canning quality. Most of the genotypes in 2013 were below the commercial standard with low chances of being in the market. However, most of the genotypes in 2014 were acceptable. The lowest genotypes in both years weren't matching, however the best genotypes (B11311, B-EL01308s, B11313, B-EL01294s) were from the two bean breeding programs MSU and USDA-MI.

Color trait for 2013 as it was determined using the rating system, and rated by a group of trained bean experts showed a binomial distribution, where one group of genotypes appear to have higher color retention than the other (Figure 3). Color rating values ranged from 1.11 to 4.50, and the

average of the color ratings was 2.68 in 2013 as shown in Table 4. In comparison with 2014, the distribution of color measured and was more uniform, but was skewed toward better color ratings. This is due to better environmental factors such as weather effects for canned black beans that occurred in 2014. Color rating value in 2014 ranged from 1.72 to 4.72, and the average of color ratings was 3.26 as shown in Table 3. There is a year advantage of 2014 over 2013 in the trait of color as well as the rest of the previous traits. Seed quality traits are linked to environment and there were superior environmental conditions for the canned black bean in 2014. Also, this express the wide genetic variability among black genotypes specially in the trait of color retention and that may do to fewer bean breeding programs that work on color retention, which are MSU and USDA-MI.

Most of the genotypes in 2014 fell below the commercial standard line, where in 2014 a high percentage of the genotype were felled above the commercial standard. The five highest color-rated genotypes in 2013 are Zenith, B-EL01294s, B11364, B11313, and B-EL01308s, while highest color-rated genotypes in 2014 were B-EL01335s, B-EL0309, B11313, B-EL0126, and Zenith respectively (Table 6A-B). All highest color-rated genotypes came from the two breeding programs of MSU and USDA-MI. Also, this elite group of genotypes belong to the two sup-groups A1-2 and A2-2 in the neighbor joining tree (Figure 1). This result reflects the genetic variability of the black bean genotypes that were obtained from different breeders around the country who may have different genetic background for each black bean line. Also, these two sup-groups were known during this study to have the elite color-retaining genotypes.

A colorimeter was also used to detect color parameters from washed and drained canned beans. Three color measurements: L* a* b* were taken on all the canned black bean genotypes. The value L* is on a scale of 0 to 100 and the closer to 0, the darker, while the closer to 100, the lighter. The

distributions of L* value in both year (2013 and 2014) were skewed toward higher values. In 2013, L* values ranged from 14.16 to 22.53, and the mean of the values was 17.66, where in 2014 the value ranged from 16.23 to 28.30, and the values mean was 19.29. This data, explains colorimeter detection for darker colors in black bean seeds in the year of 2014. The value of a* is on a scale of -127 to +127 and the minus values are greener while positive values are redder. The a* value for both years were skewed with slightly normalized distribution for the year 2013. The values were ranging from 7.36 to 10.98, and the values average was 8.86 in 2013, where in 2014, the values ranged from 1.45 to 7.79, and the values average was 3.75. The value of b* is on a scale of -127 to +127 and the minus values are bluer while positive values are yellower. The b* values for both years were not normally distributed, and 2014 values were right-skewed toward lower b* values. The b* values of 2013 ranged from 2.95 to 10.29 with an average of 6.37, where in 2014 the values were ranging from 2.47 to 7.30 with an average of 3.94. These results indicate that canned black bean seeds in 2014 were greener and bluer (less red and less yellow on the a* and b* scales respectively. Histograms data for each trait are shown in Figure 3. and Table 3.

Table 3. Means and ranges for yield, seed weight, appearance, color, L*, a*, and b* traits for the years 2013 and 2014.

Trait	Year	Mean	Std. Deviation	Minimum	Maximum
Yield (kg ha^{-1})	2013	2150	475	828	3273
Yield (kg ha^{-1})	2014	2867	607	1649	4648
Seed weight (g)	2013	19.1	1.5	14.9	22.6
Seed weight (g)	2014	21.3	2.3	17.6	27.6
Appearance (Rate 1-5)	2013	2.6	0.5	1.4	3.5
Appearance (Rate 1-5)	2014	3.3	0.6	2.0	4.16
Color (Rate 1-5)	2013	2.7	0.9	1.1	4.5
Color (Rate 1-5)	2014	3.3	0.7	1.7	4.7
L*	2013	17.7	2.1	14.2	22.5
L*	2014	19.3	2.7	16.2	28.3
a*	2013	8.9	0.8	7.4	11.0
a*	2014	3.7	1.3	1.5	7.8
b*	2013	6.4	1.7	3.0	10.3
b*	2014	3.9	1.0	2.5	7.3

Figure 3. Histograms for the data sets grown for two years for all the traits (yield, seed weight, appearance, color, L*, a*, and b*) of canned black beans for 2013, and 2014.

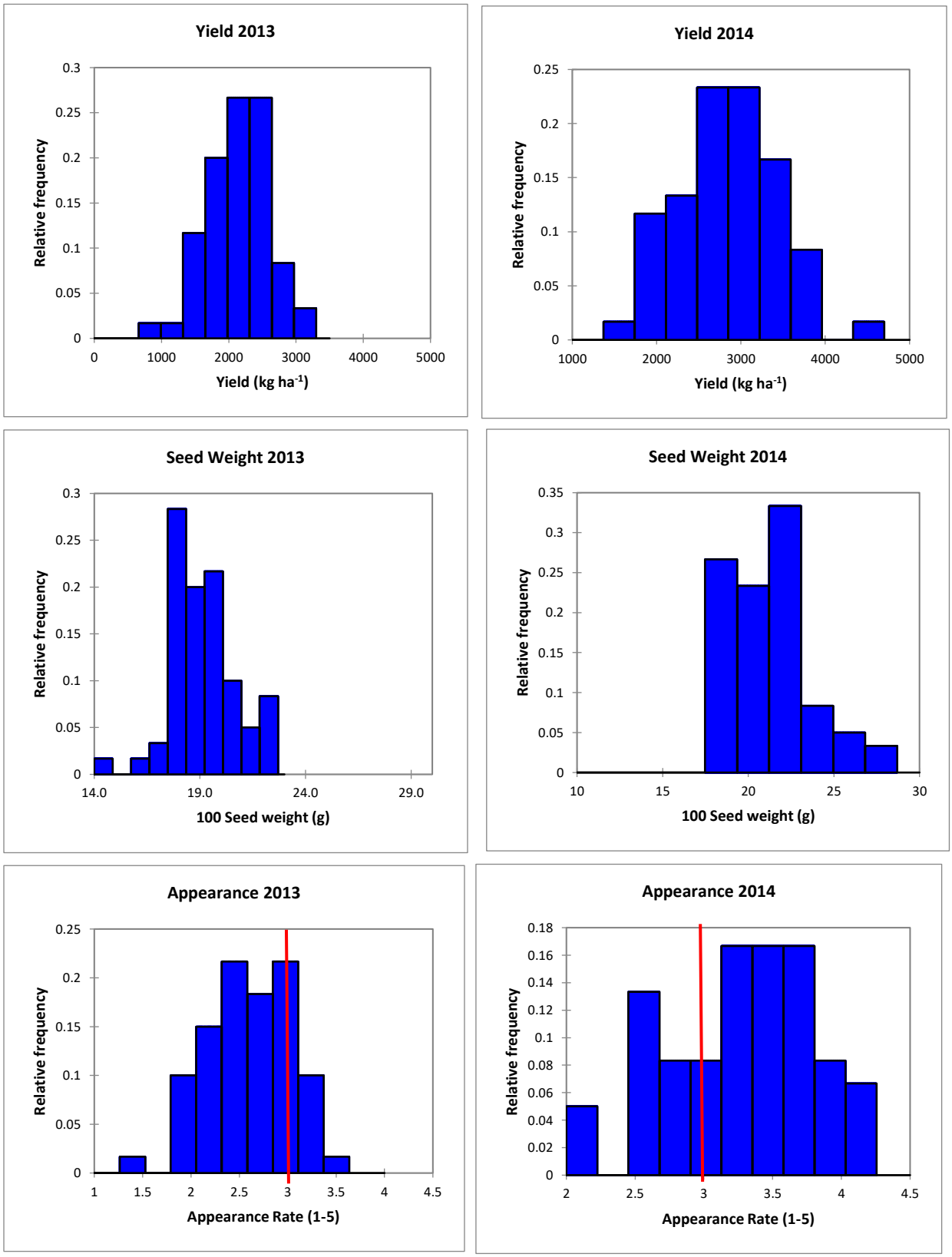


Figure 3 (cont'd)

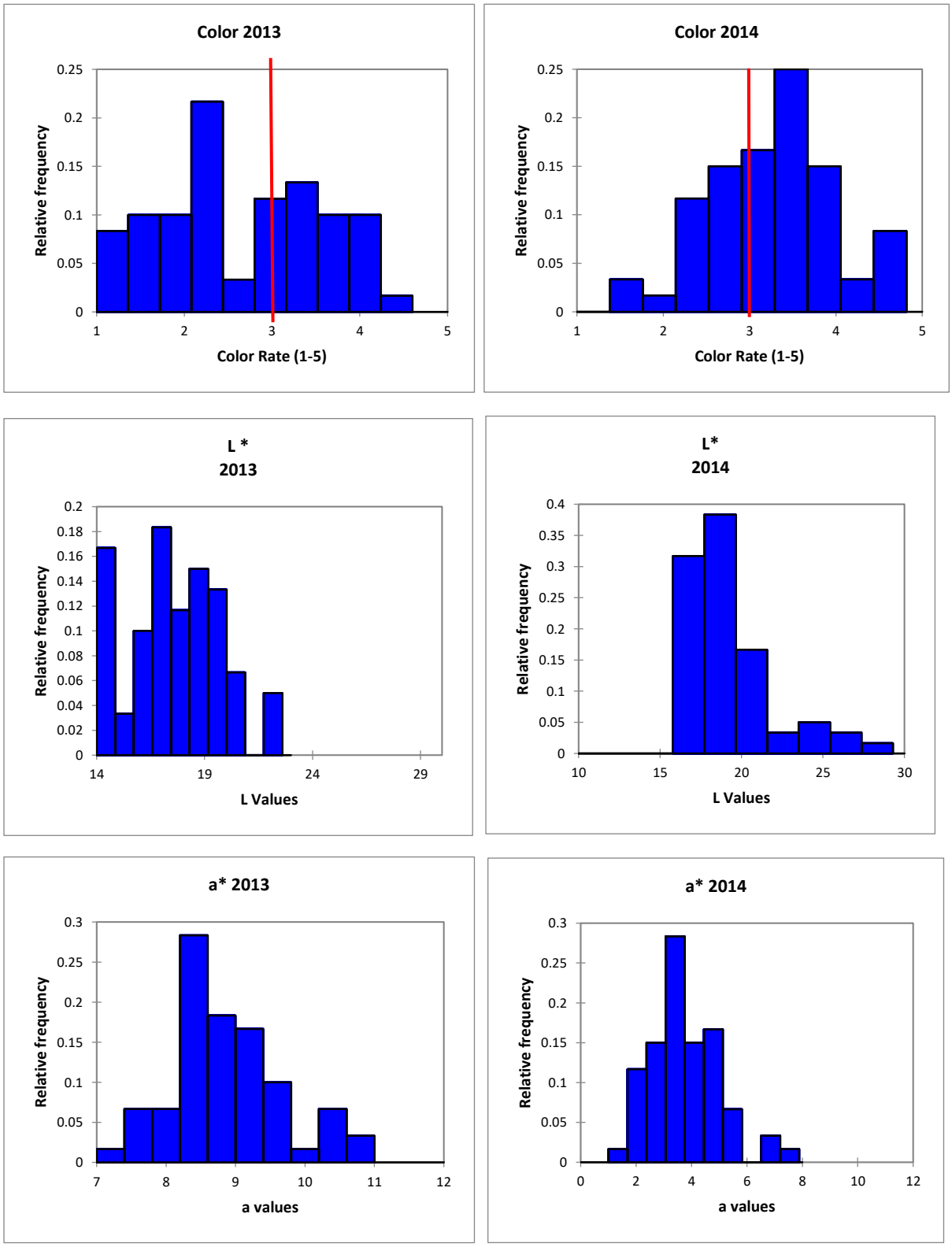
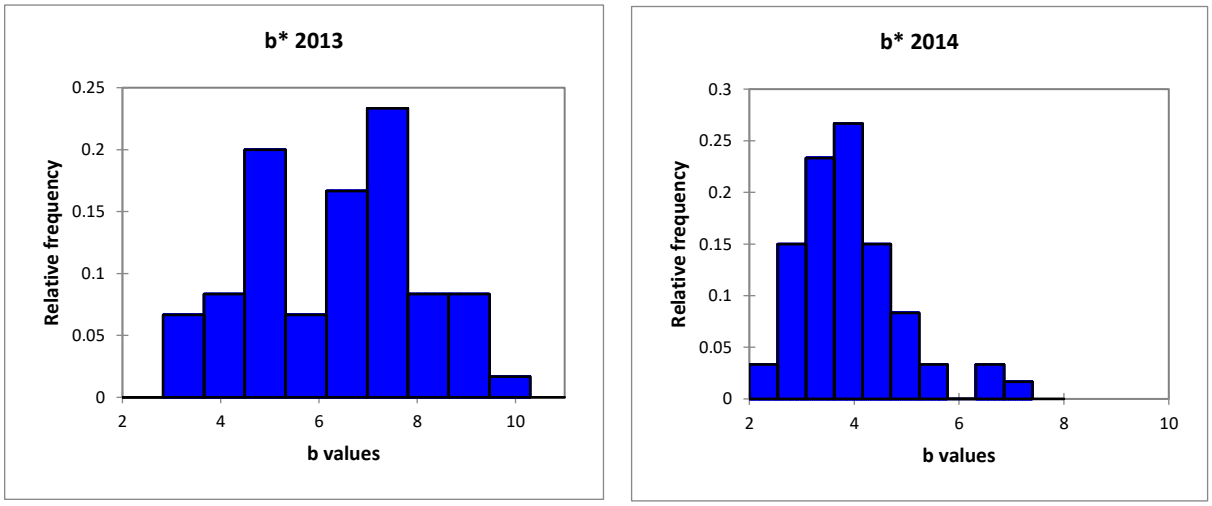


Figure 3 (cont'd)



The CORR procedure was used to determine Pearson's correlation coefficients for each trait of interest (Figure 4). There were several strong correlations detected among the measured traits (Table 4 and Table 5). The trained sensory panel ratings for color 2013 were strongly correlated with L*, a*, and b* values respectively (r=-0.95, p=0.0001, r=-0.79, p=0.0001, and r=0.95, p=0.0001), higher ratings were given for darker lines on the L* scale, and those which tend to be more green and blue on the a* and b* scales respectively. These correlations support findings by Cichy et al (2014) indicating that the trained sensory panel is as efficient and comparable to the Hunter L*a*b* color scans. Color ratings were also strongly correlated with L*, a*, b* values respectively in 2013: (r=-0.95, p=0.0001, r=-0.79, p=0.0001, and r=-0.95, p=0.0001) and 2014: (r=0.81, p=0.0001, r=-0.97, p=0.0001, and r=-0.95, p=0.0001). The L* value in 2014 was positively correlated to color ratings. It is important to note that two different systems for data measurements were used in 2013 and 2014. A colorimeter (HunterLab) was used to obtain L*a*b* values in 2013, while in 2014 a calibrated digital camera was used. A colorimeter is a color measurement machine that is designed for flat surfaces rather than bean seeds which tend to be uneven and have high light reflection. This reflection may interfere with the sample color and results in biased data. Moreover, this machine has a smaller size sensor where it requires several

pictures in order to obtained well representative date for the measured sample. In contrast, machine vision system (calibrated digital camera) has a highly sensitive lens with wider size which is capable of obtaining an image of an entire canned bean sample.

Moderate-weak correlations have been observed between appearance ratings and color traits including color ratings and L*a*b* color parameters in both years (2013 and 2014) (color: r=0.44, p=0.0002, L*: r=-0.44, p=0.0002, a*: r=-0.21, p=0.086, b*: r=-0.43, 0.002) (color: r=0.59, p=0.0001, L*: r=0.47, p=0.0001, a*: r=-0.21, p=0.0001, b*: r=-0.43, p=0.0001). This kind of minor correlation between appearance ratings and color traits including color ratings and L*a*b* color parameters was found because of the bright color of the seed starch that was interfered with lab color parameters detection (Table 4 and Table 5). Canned black beans with poorer appearance tend to split open, therefore the white cotyledons of the seeds will make the overall beans color look whiter than the seed with intact appearance. Also, no correlations were detected between color, L* a* b* values, appearance and the two seed traits for weight and yield. Seed weight was not correlated with yield.

Table 4. Pearson correlation for the seven traits (yield, seed weight, appearance, color, L*, a*, and b*) for year 2013.

	Yield	Seed Weight	Appearance	Color	L*	a*	b*
Yield	.	-0.1966	0.2940	0.2175	-0.1781	0.0132	-0.1899
	.	0.1055	0.0142	0.0726	0.1432	0.9144	0.1181
Seed Weight		.	-0.1520	-0.1455	0.1082	0.0490	0.1128
		.	0.2126	0.2330	0.3763	0.6895	0.3562
Appearance			.	**0.4399**[1]	**-0.4359**	-0.2080	**-0.4340**
			.	0.0002	0.0002	0.0864	0.0002
Color				.	**-0.9465**	**-0.7847**	**-0.9532**
				.	<.0001	<.0001	<.0001
*L					.	**0.8060**	**0.9295**
					.	<.0001	<.0001
*a						.	**0.8239**
						.	<.0001
*b							.
							.

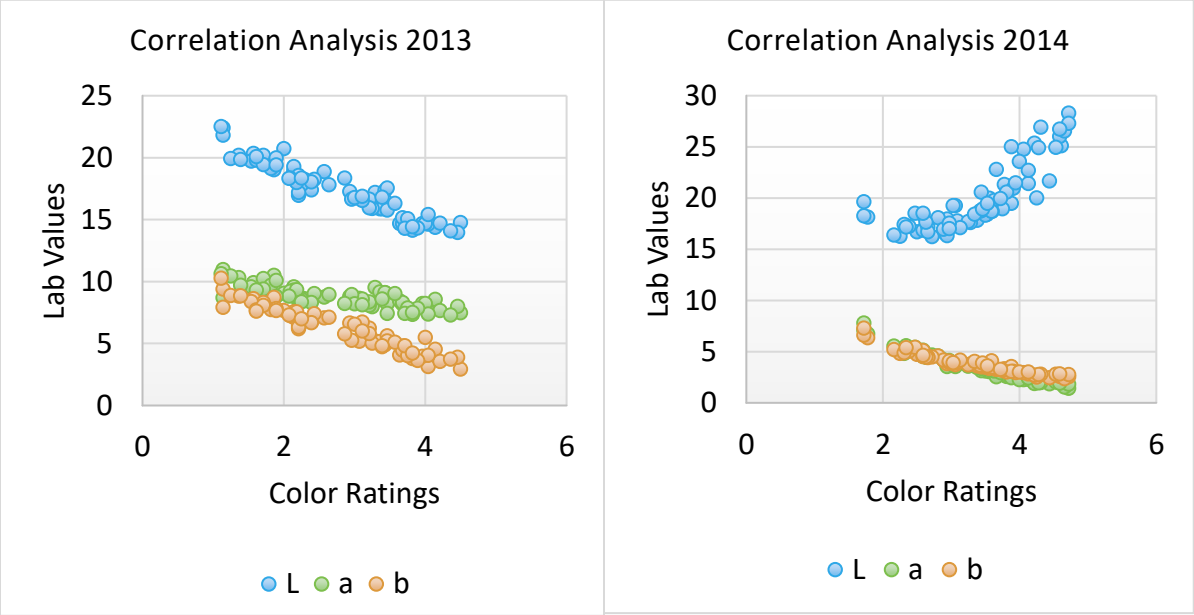
[1]Values with bold have significant p-values

Table 5. Pearson correlation for the seven traits (yield, seed weight, appearance, color, L*, a*, and b*) for year 2014.

	Yield	Seed Weight	Appearance	Color	L*	a*	b*
Yield	.	0.0945	-0.1804	-0.0815	-0.0582	0.0973	0.0878
	.	0.4398	0.1379	0.5056	0.6349	0.4263	0.4732
Seed Weight		.	-0.1520	-0.1519	-0.0977	0.1455	0.1456
		.	0.2126	0.2129	0.4244	0.2330	0.2325
Appearance			.	**0.5860**[1]	**0.4667**	-0.2079	**-0.4340**
			.	<.0001	<.0001	<.0001	<.0001
Color				.	**0.8122**	**-0.9647**	**-0.9455**
				.	<.0001	<.0001	<.0001
L*					.	**-0.7315**	**-0.6595**
					.	<.0001	<.0001
a*						.	**0.9919**
						.	<.0001
b*							.
							.

[1]Values with bold have significant p-values

Figure 4. Pearson correlations for the traits of color, L*, a*, and b* for the 2 years 2013 and 2014.



In a previous study of Cichy et al., (2014) show that there is no correlation between the trait of color retention and seed luster. However, we decided to include eight shiny seed-coated genotypes since we were not only interested in their seed luster effect, but also in their excellent canning quality. Mean separations were obtained for the seven traits for both years (Table 6A-B). The eight black bean genotypes were involved in means separation analysis to conduct their possible trend through the studied phenotypic traits. Mean separation result for the two years of 2013 and 2014 showed a superiority of the eight genotypes in term of color traits including color retention, L*, a*, and b* and also appearance. However, we have decided to exclude the eight shiny seed-coated genotypes from further analysis because of the role of their gloss in confounding the color measurements comparing to the human evaluators that were effective in rating color.

Three major commercial black bean varieties including Eclipse, Zorro, and Zenith were involved in this study. Eclipse is a major black bean variety that was released in 2004 by NDSU, Zorro which is another major black bean variety that was released in 2008 by MSU, and Zenith the most

recent major black bean variety that was released in 2015 by MSU (Kelly et al., 2015). These three verities have different canning properties in term of color, and that is due to genetic and environmental variability. For example, Zenith has a darker color than both of Eclipse and Zorro, which raised the black bean standard color since darker color of black beans is more favorable by consumers. The three genotypes behaved differently for all the seven traits across the two years. The genotype Zenith has an excellent canning quality, and has a high yield potential, where the genotype Eclipse has very poor color retention following canning. The genotype Zorro has a moderate color retention among all the three. Also, the three genotypes have been involved in recent studies that have been conducted by Kelly et al., (2015) and Goffnett et al., (2016).

In 2013, Zenith possessed the highest color rate mean (4.75) and the second highest appearance rate mean (3.25) among all the studied genotypes. Zorro showed moderate rating means for color and appearance (3.00, 2.75) respectively. The variety Eclipse was the lowest among the three with color rating mean (2.50) and appearance rate mean (3.00). In 2014, the variety Zenith was among the highest ten genotype for the color and appearance ratings means (4.00, 4.00). Zorro had a medium color and appearance rate mean (3.50, 3.50), and Eclipse was also the lowest among all the three with color and appearance ratings means (2.75, 2.75). This process supports the results represented by Kelly et al., (2015) and Goffnett et al., (2016) regarding the color differences between the three black bean genotypes. This advantage for the genotype Zenith is also extends to the trait of yield where Zenith had higher yield means over Zorro and Eclipse in both years (2013 and 2014) (2840, 3047 kg ha^{-1}) respectively. The seed quality variation of these three genotypes emphasize a wide genetic variability among the studied population for the seven target traits. One of the reasons why there is a wide genetic variability among black beans is that there are only two programs (USDA-MI and MSU) that work in the improvement of black bean canning quality.

Table 6A. Means of the seven traits (yield, seed weight, appearance, color, L*, a*, and b*) of 66 black bean genotypes that obtained from the year 2013.

Genotype	Yield (kg ha^{-1})	100 Seed Weight (g)	App (1-5)	Color (1-5)	L* (0-100)	a* (+127-127)	b* (+127-127)
Zenith	2840	18.5	3.25	4.75	14.77	7.50	2.95
B-EL01294s	1711	18.6	3.75	4.25	13.98	8.02	3.89
B11364	2164	22.1	3.00	4.25	14.71	7.67	3.56
B11313	2541	17.3	2.75	4.00	14.65	7.59	3.14
B-EL01308s	2457	21.2	3.5	4.00	14.10	7.31	3.74
B-EL01335s	2272	19.1	2.75	4.00	15.42	7.40	4.05
B11309	2178	18.7	1.50	3.75	14.39	8.57	4.55
B-EL0309	1952	18.2	2.00	3.75	14.34	7.84	3.65
B11356	2.085	13.6	2.50	3.50	14.69	8.25	5.51
Condor	2387	17.8	3.25	3.50	15.16	8.32	4.48
B09165	2385	17.3	3.00	3.50	16.55	9.11	5.05
B11360	2335	18.3	2.25	3.50	17.55	8.21	5.63
B-EL01270s	2072	21.3	2.75	3.75	14.69	8.20	4.80
Harblack	2221	18.2	2.00	3.50	16.33	9.05	5.11
B-EL01291s	1681	18.2	2.75	3.50	15.79	7.45	5.21
NE14-12-	2006	20.6	2.75	3.50	14.31	7.43	4.82
DPC-4	1566	18.0	3.00	3.50	15.08	7.87	4.02
SHINYCROW	1858	20.2	3.5	3.50	14.45	7.52	4.23
B-EL01318s	1061	17.4	2.25	3.50	15.97	8.37	5.82
B-EL0126	970	18.4	2.75	3.50	14.16	7.36	3.79

Table 6A (cont'd)

B11363	2735	17.0	3.00	3.25	14.70	8.44	4.08
B10238	2593	18.4	3.50	3.25	16.72	8.65	5.18
B11312	2441	19.7	3.50	3.25	15.89	8.56	4.76
B11310	2353	20.7	3.00	3.25	15.89	9.17	5.16
T-39	2194	17.6	2.50	3.25	16.90	8.14	6.01
B09119	2123	18.0	2.25	3.25	17.19	9.52	5.21
B11311	1819	20.3	3.50	3.25	17.35	9.13	4.90
BlackMag	2619	18.7	2.50	3.00	16.56	8.61	6.73
Zorro	2334	18.0	2.75	3.00	16.94	9.14	5.20
OACExpresso	1652	19.1	1.50	3.00	17.81	8.97	7.13
OACJet	2133	19.2	2.25	2.75	16.80	8.21	6.55
B-EL01268s	2135	17.8	2.75	2.75	16.66	8.08	6.30
B11322	2012	13.6	1.75	2.75	15.91	7.99	5.03
ND071065	2005	20.0	1.50	2.50	18.35	8.83	7.30
Domino	1991	20.8	2.00	2.50	16.65	8.96	5.26
Eclipse	1757	17.8	3.00	2.50	18.43	8.94	7.38
TARS-MST	1724	18.6	1.50	2.50	18.36	8.23	5.79
B-EL1291	2699	19.0	2.00	2.25	17.28	8.81	6.66
19365-31	2275	21.4	1.50	2.25	18.05	8.32	6.70
BK11-8	1797	18.8	3.00	2.25	18.27	9.04	7.41
B10213	2313	17.6	2.25	2.00	19.29	9.33	7.14
AifiWuri	2147	19.7	2.50	2.00	16.98	8.41	6.19

Table 6A (cont'd)

BK11-5	2108	21.1	1.50	2.00	17.83	8.59	6.98
BK11-1	2079	18.9	2.00	2.00	17.41	8.45	6.71
B11370	2149	18.6	2.75	1.75	18.57	8.59	6.50
ND071244	2134	19.9	1.50	1.75	19.13	9.70	7.75
NDF09302	2123	18.2	2.75	1.75	19.43	8.81	7.66
NDF09304	1833	17.6	2.50	1.75	18.36	8.36	7.01
BlackRhi	1669	22.4	2.00	1.75	17.21	8.59	6.34
ND081256	1541	24.1	2.50	1.75	20.00	10.09	7.83
ND081111	1476	18.0	2.00	1.75	19.46	9.41	8.08
ICB-3	1363	22.4	1.75	1.75	17.99	9.35	7.56
B10243	1357	20.5	3.00	1.75	18.26	8.63	6.48
B10210	3136	19.8	2.75	1.50	19.04	10.51	8.74
BK9-2	2452	19.7	2.25	1.50	20.02	9.76	8.59
B11588	2431	21.6	1.75	1.50	20.20	10.25	8.31
ND071089	2282	19.1	1.75	1.50	19.08	8.98	7.73
B11259	2046	16.4	1.75	1.50	20.75	9.07	7.68
ND081295	1290	19.8	2.50	1.50	20.09	9.33	7.63
07055-4	1166	20.2	1.75	1.50	20.36	9.93	8.64
ND071257	2583	20.6	1.50	1.25	19.93	10.46	8.88
B11285	2212	16.7	2.75	1.00	22.41	10.97	9.41
SB-DT1	2026	18.7	1.50	1.00	19.87	9.72	8.85
ND081343	1684	18.6	2.00	1.00	22.53	10.65	10.29
B11338	1383	16.1	2.50	1.00	20.19	10.32	8.83

Table 6A (cont'd)

Mean	**2053**	**19.0**	**2.44**	**2.66**	**17.30**	**8.75**	**6.16**
LSD0.05	**1148**	**2.9**	**1.23**	**0.84**	**1.70**	**1.03**	**1.07**
Coefficient of Variation	**0.21**	**0.1**	**0.25**	**0.37**	**0.12**	**0.10**	**0.28**

**Means for all traits have been ordered based on the means of color trait.*
**Three genotypes were missing across the traits and the two years (101s-2435, B-EL01270d, and ND081339)*

Table 6B. Mean of the seven traits (color, appearance, L*, a*, b*, seed weight, and yield) of 66 black bean genotypes that obtained from the year 2014.

Genotype	Yield (kg ha^{-1})	100 Seed Weight (g)	App (1-5)	Color (1-5)	L* (0-100)	a* (+127-127)	b* (+127-127)
B-EL01335s	2868	22.5	3.5	5.00	24.96	2.07	2.79
B-EL0309	2093	21.5	4.5	5.00	26.74	1.93	2.85
B11313	3503	19.3	3.75	5.00	26.04	1.97	2.58
B-EL0126	2365	20.8	4.00	5.00	28.31	1.45	2.56
Zenith	3047	22.3	4.00	4.75	24.87	1.94	2.59
B-EL01294s	2485	19.1	3.75	4.75	27.31	1.85	2.79
B-EL01270s	2812	26.0	4.25	4.75	26.57	1.54	2.36
B11356	3139	19.2	3.25	4.50	20.97	2.70	3.08
B11363	3861	21.8	4.25	4.50	20.03	2.05	2.53
B11364	3568	21.8	3.50	4.50	21.48	2.63	2.95
B-EL01308s	2460	20.9	4.50	4.50	24.92	1.97	2.76
B11309	3221	18.3	3.25	4.50	21.44	2.30	2.84
B-EL01291s	2649	19.4	3.75	4.50	26.94	1.98	2.82
B11329	3541	18.2	3.75	4.50	21.69	1.86	2.47
B-EL01268s	1345	19.0	2.75	4.25	25.37	1.91	2.71
B-EL01318s	2617	19.1	3.75	4.25	24.77	2.26	2.96
Condor	2624	21.3	3.75	4.25	25.03	2.45	3.08
B11360	3727	20.5	2.50	4.25	20.59	2.60	3.03
NE14-12-	3153	24.2	3.25	4.25	23.59	2.27	3.02

Table 6B (cont'd)

SHINYCROW	2499	22.2	4.00	4.25	22.72	2.44	3.02
BK11-5	2713	22.1	3.50	4.00	18.57	3.61	3.69
B11322	2560	18.1	2.75	4.00	19.49	3.15	3.54
B-EL1291	2592	19.3	3.75	4.00	18.96	2.77	3.19
DPC-4	1209	20.5	3.75	4.00	19.80	3.11	3.44
BlackMagic	3425	19.4	3.50	4.00	22.81	2.56	3.28
OACExpresso	2658	20.0	3.50	3.75	18.84	3.90	3.99
B10243	2567	21.5	3.25	3.75	18.47	3.41	3.54
TARS-MST	2719	21.8	2.00	3.75	19.26	3.69	3.95
B-EL0127	3555	21.5	3.50	3.75	20.59	3.15	3.53
harblack	2910	18.9	3.50	3.50	18.40	3.53	3.58
T-39	3062	20.0	3.50	3.50	19.95	2.96	3.27
B09165	2950	22.6	4.25	3.50	17.83	3.47	3.63
B11370	2625	18.0	3.25	3.50	16.33	3.55	3.83
BlackRhi	3856	24.1	3.00	3.50	17.70	3.58	3.71
B09119	2792	20.5	4.25	3.50	18.43	3.46	3.76
NDF09304	2601	19.8	3.75	3.50	18.95	3.58	3.72
B10238	2409	18.8	4.25	3.50	17.64	3.75	3.81
B11311	2476	18.1	3.75	3.50	18.35	3.13	3.48
OACJet	3403	20.7	4.00	3.50	18.42	3.71	4.04
B11310	2799	22.2	4.00	3.50	19.99	3.05	3.38
Zorro	2324	21.1	3.50	3.25	18.00	3.73	3.86
B10213	2430	19.2	4.00	3.25	17.42	3.97	4.09

Table 6B (cont'd)

BK11-1	2219	21.0	3.75	3.25	19.29	3.57	3.75
19365-31	1416	23.8	3.50	3.25	17.57	3.86	3.95
Eclipse	2773	19.7	2.75	3.25	16.43	4.30	4.26
B11588	2891	24.8	3.00	3.00	18.09	4.59	4.58
NDF09302	2619	18.5	3.25	3.00	18.51	4.59	4.66
Domino	1844	20.2	3.75	3.00	17.13	4.05	4.20
BK11-8	1828	22.0	4.00	3.00	17.77	3.97	3.97
ICB-3	1347	21.3	2.50	3.00	16.87	4.56	4.53
AifiWuri	1750	19.6	2.75	3.00	17.96	3.80	4.08
ND081295	2583	25.5	2.25	3.00	17.65	4.47	4.50
ND071257	3534	21.8	3.50	3.00	16.72	4.68	4.44
ND071065	1884	21.3	3.00	3.00	16.23	4.67	4.48
ND081256	2380	19.8	4.25	3.00	17.04	4.15	4.10
B11259	3345	19.1	3.75	2.75	17.25	5.17	5.07
ND071244	3092	21.3	3.25	2.75	17.18	4.55	4.49
ND081111	3009	22.3	3.00	2.75	18.72	3.80	4.12
B10210	4716	27.4	4.50	2.75	16.72	4.91	4.74
SB-DT1	2814	26.3	2.50	2.75	17.20	5.59	5.41
ND081339	3117	26.4	3.25	2.75	18.53	5.43	5.44
07055-4	1499	23.3	3.25	2.75	18.34	5.15	5.12
B11338	3329	17.3	2.75	2.25	18.27	7.12	6.62
BK9-2	2777	20.0	3.00	2.25	16.39	5.56	5.22
B11285	3430	19.2	3.75	2.00	18.16	6.74	6.37

Table 6B (cont'd)

ND081343	2789	20.4	3.25	1.75	19.65	7.79	7.30
Mean	**2745**	**21.0**	**3.50**	**3.61**	**20.03**	**3.54**	**3.81**
LSD0.05	**983**	**2.9**	**0.82**	**0.67**	**2.91**	**0.63**	**0.52**
Coefficient of Variation	**0.24**	**0.1**	**0.16**	**0.22**	**0.16**	**0.37**	**0.26**

**Means for all traits have been ordered based on the means of color trait.*
**Three genotypes were missing across the traits and the two years (101s-2435, B-EL01270d, and ND081339)*

A neighbor joining tree developed with the data revealed that this study population consists of four major branches A, B, C, and D. The branch A was split into six sub-branches A1-A, A1-2, A2-1, A2-2, A2-3, and A2-4 (Figure 1). All major branches have several genotypes except for the branch B. Analysis of variance was conducted for the neighbor joining tree groups for the two years (2013 and 2014). The means of all the genotypes for the seven traits were averaged for each major and sub branches, and the standard deviation were also obtained.

The sub-branch A2-2 was found to have higher means for the traits of appearance, a*, and b* for both years (2.73, 3.78, 7.85, 2.11, 4.73, and 2.86) respectively (Table 7A-B). The sub-branch A2-2 has all the shiny seed-coated genotypes tested. This result suggests the superiority of shiny seed-coated genotypes over dull seed-coated genotypes, and that is due not only to their seed luster, but to their excellent canning quality that have been chosen based on. All the other traits varied between the two years. For the trait of yield, the sub-branch A1-2 had the higher yielding mean in the year of 2013, while the mean of the major branch C was the highest in 2014 respectively (2323, 3121 kg ha^{-1}). The sub-branch A2-1 had the highest mean of seed weight in 2013 (20.14) with a standard deviation of 1.27, while the major branch D had the highest seed weight mean in 2014 (23.40) with a standard deviation of 2.15. The two traits of color retention and L* have been dominated by the sub-group A2-2 with the highest mean values (3.61, 15.17) and standard

deviation (0.58 and 1.08) respectively. The same two traits were varied in the year of 2014. The sub-group A1-2 had the highest color retention mean (3.68) with a standard deviation of 0.53. This group also had the second highest color retention mean in the year of 2013 (3.06) with standard deviation of 0.96. In 2014, the highest mean of the trait of L* was or the major branch C (17.68) with standard deviation of 0.54 (Table 7 A-B).

Table 7A. Means and standard deviations for all the major and sub branches that obtained from the neighbor joining tree for the seven traits (color, appearance, L*, a*, b*, seed weight, and yield) of 69 black bean genotypes that obtained from the year 2013.

NJ Group	Average/St. Deviation	Yield (kg ha^{-1})	Seed Weight (g)	Appearance (1-5)	Color (1-5)	L* (0-100)	a* (-127+127)	b* (-127+127)
A1-1	Avg	2081	18.4	2.45	3.00	16.87	8.39	5.56
	Std.dev	267	0.4	0.37	0.69	1.17	0.40	0.94
A1-2	Avg	2323	18.5	2.64	3.06	16.50	8.77	5.38
	Std.dev	440	2.4	0.64	0.96	2.05	0.89	1.80
A2-1	Avg	1969	20.1	1.86	2.11	17.96	8.86	7.03
	Std.dev	294	1.3	0.51	0.62	1.32	0.73	1.05
A2-2	Avg	1894	19.2	2.73	3.61	15.17	7.85	4.73
	Std.dev	480	1.3	0.44	0.58	1.08	0.47	1.04
A2-3	Avg	2050	19.0	2.46	2.38	18.11	8.96	6.93
	Std.dev	441	1.1	0.30	0.67	1.51	0.51	1.09
A2-4	Avg	2079	18.3	2.33	1.67	18.96	8.72	7.47
	Std.dev	185	0.6	0.42	0.12	0.45	0.26	0.32
B	Avg	1684	18.6	2.00	1.00	22.53	10.65	10.29
C		1795	18.3	2.38	1.31	20.84	10.11	8.44
	Std.dev	343	3.4	0.38	0.32	0.95	0.68	0.71
D	Avg	1417	19.2	2.50	1.75	18.86	9.02	7.28
	Std.dev	59	1.3	0.50	0.00	0.60	0.39	0.80

Table 7B. Means and standard deviations for all the major and sub branches that obtained from the neighbor joining tree for the seven traits (color, appearance, L*, a*, b*, seed weight, and yield) of 69 black bean genotypes that obtained from the year 2014.

NJ Group	Average/St. Deviation	Yield (kg ha^{-1})	Seed Weight (g)	Appearance (1-5)	Color (1-5)	L* (0-100)	a* (-127+127)	b* (-127+127)
A1-1	Avg	2775.22	19.69	3.40	3.40	18.71	3.30	3.58
	Std.dev	871.05	1.07	0.51	0.51	1.45	0.40	0.35
A1-2	Avg	2984.58	20.95	3.68	3.68	19.91	3.26	3.64
	Std.dev	664.08	2.39	0.53	0.53	2.74	1.03	0.98
A2-1	Avg	2499.21	22.15	3.14	3.14	18.20	4.05	4.10
	Std.dev	750.23	1.74	0.53	0.53	1.75	0.67	0.53
A2-2	Avg	2565.53	20.94	3.78	4.28	24.84	2.11	2.86
	Std.dev	434.90	2.17	0.24	0.38	2.69	0.46	0.29
A2-3	Avg	2744.48	20.78	3.13	3.13	18.39	3.98	4.12
	Std.dev	483.41	2.13	0.52	0.52	2.31	0.99	0.68
A2-4	Avg	2610.10	19.10	3.50	3.50	18.73	4.09	4.19
	Std.dev	8.9	0.65	0.25	0.25	0.22	0.505	0.47
B	Avg	2789.70	20.40	3.25	3.25	19.65	7.79	7.30
C		3121.35	18.83	3.63	3.63	17.68	5.80	5.54
	Std.dev	429.34	0.95	0.54	0.54	0.54	1.20	1.02
D	Avg	2898.10	23.40	3.17	3.17	18.57	4.21	4.37
	Std.dev	238.09	2.15	0.12	0.12	0.11	0.87	0.80

3.8. Genome-Wide Association Mapping

GWAS was conducted for the seven traits (yield, seed weight, canned appearance, color, L*, a*, and b*) of 61 black bean genotypes that were grown for two years 2013 and 2014. Eight genotypes with shiny seed coat were excluded from the original 69 genotypes, because of their luster that interfered with the color of the dull genotypes, which are the dominant group in the study. Including the shiny seed-coated genotypes may confound the final result by influencing L*, a*, and b* scores.

For all traits, there were significant SNP markers detected above LOD 2 cutoff. Even though the size of the studied population was small, it was strong enough to detect some significant SNPs above LOD 6 on Pv07 where the region of ASP gene, which is responsible of seed luster in black bean (Figure 19). For the trait of seed yield, significant SNP marker trait associations were found on Pv04 and Pv06, the significant SNPs mapped to two locations with different effects on yield. (Figure 5 and Figure 12). The three associations on Pv04 for 2013, 2014, and the two-year average (where the seed yield values from the two years were averaged) were the most significant and also explained the most phenotypic variation with R^2 values of 0.26 for all the three associations (Table. 8A). The significant SNPs from 2013 was detected on the physical position ranged from 2.27 to 3.36Mb and had an R2 of 17%. The SNP markers from 2014 were identified on the physical positions ranged (between 5.63 to 5.71Mb) and carried 26% of phenotypic variation. The significant SNPs from the two-year average were detected on the physical position ranged from 2.04 to 2.36Mb and had an R2 ranged between 22-23%. The two association on Pv06 for 2013 and the two-year average explained from 17% to 23% of the phenotypic variation. The SNP markers from 2013 were identified on the physical positions ranged (between 20.43 to 20.57Mb) and carried phenotypic variation ranged from 17-20%. The SNP markers from the two-year average

were identified on the physical position of 20.63Mb and carried 23% of phenotypic variation. We could not find any previous studies matching what was found in this study result for the trait of seed yield.

The significant marker trait associations were found on Pv06 and Pv10 for the trait of seed weight (Figure 6 and Figure 13). The two associations on Pv06 for 2013 and 2014 were significant and explained 15% and 17% phenotypic variation. The significant SNPs for the two years (2013 and 2014) were identified on the physical positions of 24.82 and 20.81 Mb respectively, and mapped to one region with different effects on seed weight. This result agreed with what was discovered in the study of Cichy et al., where they detected some QTLs for the trait of seed weight on Pv06 for two years of 2000 and 2005. The QTL that was found in 2000 for the trait of seed weight explained phenotypic variation ranged from 10-18%, where the one in 2005 had R^2 ranged from 21-27%. One significant SNP marker for 2013 was found associated with seed weight on Pv10 with a physical position of 40.83Mb and explain 17% from the phenotypic variation (Table 8B).

Significant marker trait associations were found on Pv04, Pv07, and Pv10 for the trait of appearance (Figure 7 and Figure 14). The significant associations on Pv04 for 2013 were identified on the physical regions ranged between 41.56 and 42.31Mb, and explained 17-21% of the phenotypic variation. The significant SNPs on Pv07 for the two-year average were detected on the physical locations ranged between 4.23 to 10.56Mb, and explained phenotypic variation ranged between 17-20%. The significant associations on Pv07 for 2013 were identified on the physical regions ranged between 3.92 and 4.19Mb, and had R^2 ranged between 18-23%. The significant SNP on Pv07 was identified on the physical location of 36.47Mb, and had R^2 of 18% (Table 8C). The significant SNP on Pv10 for 2014 was identified on the physical location of 8.25Mb, and had

R^2 of 17%. We were not able to find any previous studies matching what was found in this study result for the trait of appearance.

Significant marker trait associations were found on Pv04, Pv05, Pv06, and Pv09 for the trait of color (Figure 8 and Figure 15). The overall associations were significant and explained 13-23% of the phenotypic variation. The significant markers on Pv04 for 2013 were detected on the physical region ranged between 2.28 and 2.46Mb, and explained phenotypic variation ranged between 13-15%. The significant SNP on Pv04 for the two-year average was detected on the physical region of 10.56Mb, and explained phenotypic variation of 18%. The significant SNPs on Pv05 for 2013 were detected on the physical region ranged between 39.47 and 39.51Mb, and explained phenotypic variation ranged between 39.47 and 39.51Mb, and explained phenotypic variation of 13%. The significant SNP on Pv05 for 2014 was detected on the physical region of 39.47Mb and explained phenotypic variation of 20%. The significant SNP on Pv05 for the two-year average was detected on the physical region of 39.47Mb, and explained phenotypic variation of 22%. In a study conducted by Wright and Kelly, (2011), two QTLs on Pv05 was also found associated with the trait of color with R^2 of 0.10 and 0.13, and carried different effect on the trait. On Pv06 for 2013, significant SNPs were identified on the physical position ranged between 0.03 and 18.33Mb, and explained phenotypic variation ranged between 13-17%. On Pv09 for 2013, significant SNP was also identified on the physical position of 32.56Mb, and explained phenotypic variation of 13%. On the same chromosome for 2014, a significant SNP was found on the physical region of 35.32 and explained phenotypic region of 22%. The significant SNP on Pv09 for the two-year average was detected on the physical region of 35.32Mb, and explained phenotypic region of 23% (Table 8D). In a study by Cichy et al., (2014), QTLs on Pv04 and Pv05 were identified for the trait of color retention which agrees with our finding in this study.

Significant marker trait associations were found on Pv03, Pv04, Pv05, Pv06, and Pv09 for the trait of L^* (Figure 9 and Figure 16). The significant SNPs on Pv03 for 2013 were identified on the physical region ranged between 0.89 and 50.12Mb, and had R^2 ranged between 14-17%. The significant SNP on the same chromosome for 2014 was identified on the physical region of 45.59Mb with R^2 of 19%. The significant SNP markers on Pv04 for 2013 were identified on the physical region ranged between 2.27 to 4.4Mb, with R^2 ranged between 15-20%. The significant SNP markers that were found on Pv05 and were identified on the physical region ranged between 4.81 and 39.47Mb, and had R^2 ranged between 14-16%. On Pv06 for 2013, significant SNPs were identified on the physical region ranged between 18.33 and 22.22Mb, and had R^2 ranged 12-20%. The significant SNP on Pv09 for 2014 was identified on the physical region of 32.95MB, and had R^2 of 13%. On the same chromosome for the two-year average significant SNP markers were identified on the physical region ranged between 20.58 and 25.25Mb, and had R^2 ranged between 14-16% (Figure 18E). In a study by Cichy et al., (2014), a QTL was also detected on Pv04 and Pv05 associated with L^* trait and had R^2 of 3.3 and 9.8 respectively (Table 8E).

Significant SNPs associated with a* were found on Pv02, Pv05 and Pv08 (Figure 10 and Figure 17). On Pv02 for 2013, the significant SNPs mapped to on the physical region ranged between 32.86and 44.91Mb with phenotypic variation ranged between 15-18%. The significant SNP on Pv05 for 2013 were identified on the physical positions of 39.18Mb, and carried 14% of phenotypic variation. The significant SNP on Pv05 for 2014 were identified on the physical positions of 39.47Mb, and carried 23% of phenotypic variation. The significant SNP on Pv05 for the two-year average were identified on the physical positions of 39.47Mb, and carried 25% of phenotypic variation. On Pv08 for 2013, the significant markers were identified on the physical

region ranged between 57.94 and 59.01Mb, and had R^2 ranged 14-15% (Table 8F). We could not find any previous studies matching what was found in this study result for the trait of a*.

For the trait of b^*, significant marker trait associations were found on Pv04, Pv05, Pv06, and Pv07 (Figure 11 and Figure 18). The overall associations were significant and explained 16-29% of the phenotypic variation. The significant markers on Pv04 for 2013 were detected on the physical region ranged between 2.30 and 2.47Mb, and had phenotypic variation ranged between 16-20%. The significant SNPs on the same chromosome for 2014 mapped to the physical location which ranged between 10.56 and 19.99Mb, and had phenotypic variation of 18%. On the same chromosome for the two-year average, significant SNPs were detected in the physical region ranged between 10.56 and 10.99Mb, with phenotypic variation of 17%. On Pv05 for 2013, significant SNPs were detected in the physical region ranged between 39.18 and 39.20Mb, with phenotypic variation of 19%. On the same chromosome for 2014, significant SNPs detected and mapped to the physical region ranged between 39.47 and 39.48Mb, with phenotypic variation of 21%, For the two-year average, significant SNPs were detected in the physical region ranged between 39.47 and 39.51Mb, with R^2 of 29%. The significant SNP markers on Pv06 for 2013 were detected and mapped to the physical region ranged between 18.33 and 18.51Mb, with R^2 of 16%. On the same chromosome for the two-year average, significant SNP markers were detected on the physical location ranged between 18.50 and 18.50Mb. The significant SNPs on Pv07 for 2014 were detected on the physical location of 38.89Mb, and had phenotypic variation of 19%. On the same chromosome for the two-year average significant SNP markers were detected on the physical location ranged between 36.47 and 38.89Mb, with phenotypic variation ranged between 18-19% (Table 8G). In a study by Cichy et al., (2014), their finding was agreed with our result in this study where they identified a QTL on Pv05 that was associated with b^* trait

The significant SNP markers that were found associated with color retention, L^*, a^*, and b^* shared same physical position (39Mb) on the Pv05. This result assured what was found by the study of Cichy et al., (2014), where they identified some significant SNP markers on Pv04 and Pv05 for the traits of color retention, L*, b*, and anthocyanin. However, by comparing the two results, the physical regions that were identified in this study and associated with color traits (color retention, L*, a*, and b*) were novel locations. The traits of color retention and L* were also co-localized on Pv04, Pv05, Pv6, and Pv09 and shared very similar regions. Through GWAS, we have also identified regions on Pv04 with physical locations ranged from (4.43 to 10.56Mb) that were related to the traits of color retention, L* and appearance. These findings indicate an important role for the identified SNP markers that are associated with the traits of color and appearance in black beans. This research serves as an important foundation for further studies to understand the genetic control of color retention after canning in black beans.

Figure 5. Manhattan plot of seed yield values averaged across 2013 of 61 Black beans genotypes with 2800 SNP (>0.02 minor allele frequency) and mapping conducted with multiple linear model (MLM) using a kinship matrix and 3 principal components to account for population structure. The black line is p=0.01 threshold for significance.

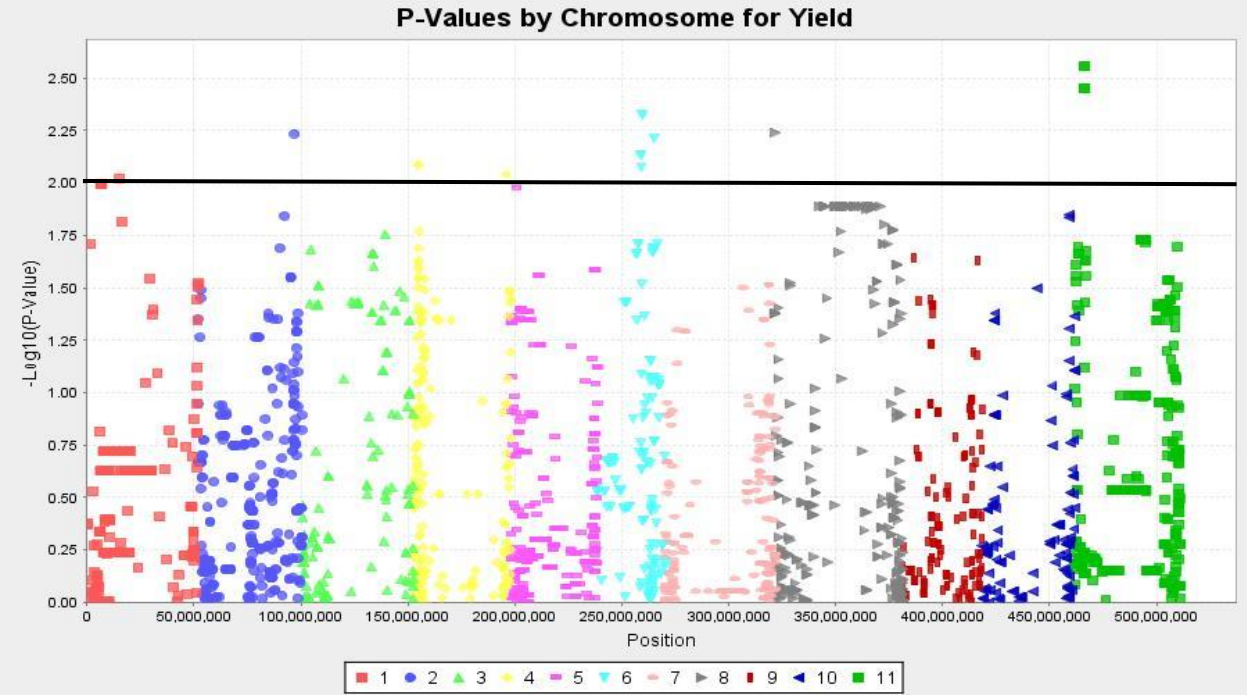


Figure 6. Manhattan plot of seed weight values averaged across 2013 of 61 Black beans genotypes with 2800 SNP (>0.02 minor allele frequency) and mapping conducted with multiple linear model (MLM) using a kinship matrix and 7 principal components to account for population structure. The black line is p=0.01 threshold for significance.

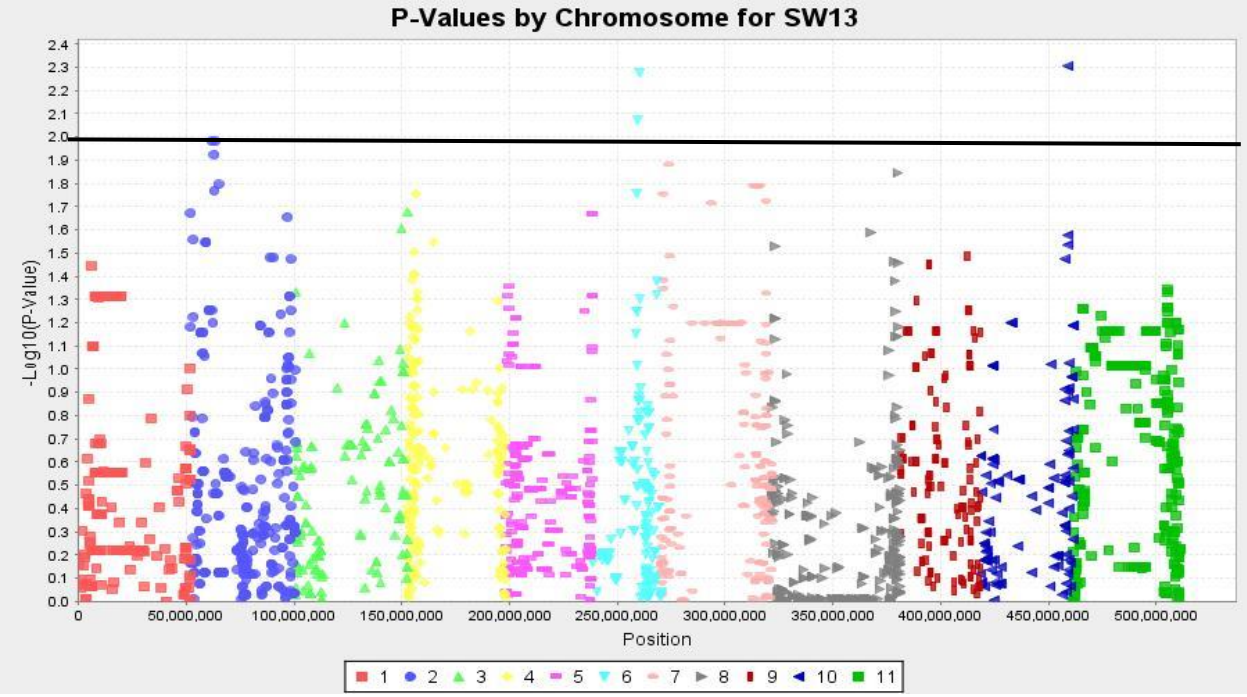


Figure 7. Manhattan plot of appearance rating values averaged across 2013 of 61 Black beans genotypes with 2800 SNP (>0.02 minor allele frequency) and mapping conducted with multiple linear model (MLM) using a kinship matrix and 2 principal components to account for population structure. The black line is p=0.01 threshold for significance, and the red line is p=0.001 threshold for significance.

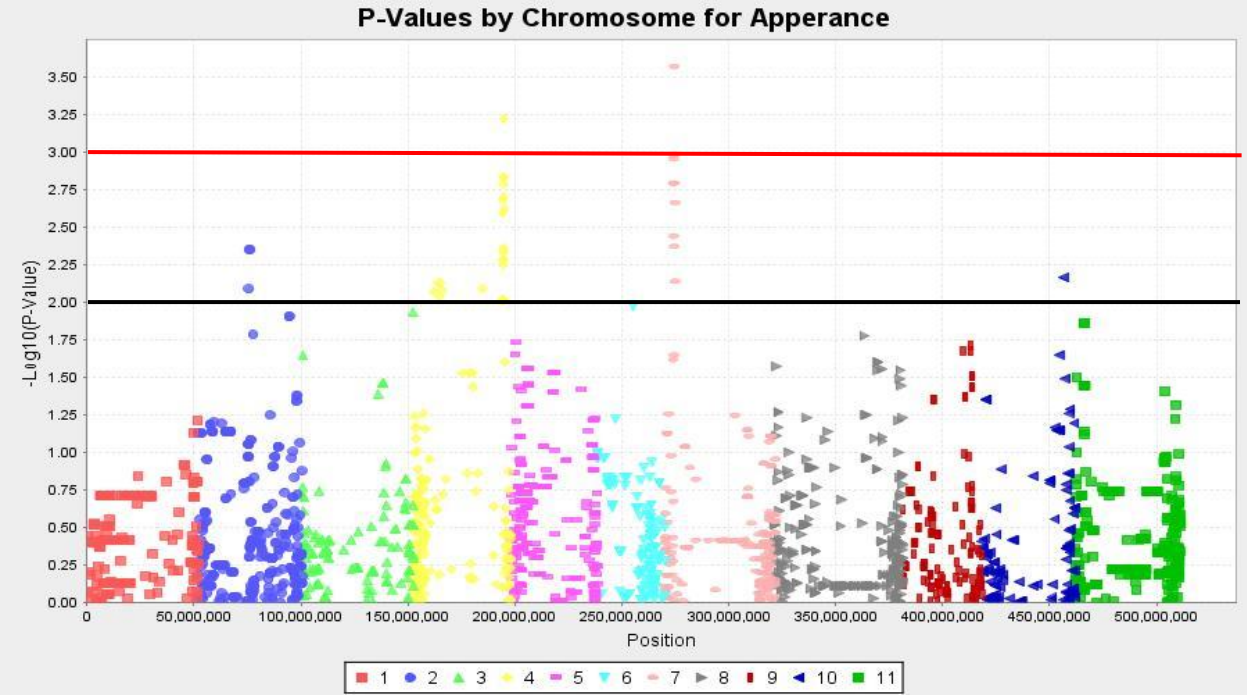


Figure 8. Manhattan plot of color ratings averaged across 2013 of 61 Black beans genotypes with 2800 SNP (>0.02 minor allele frequency) and mapping conducted with multiple linear model (MLM) using a kinship matrix. The black line is p=0.01 threshold for significance.

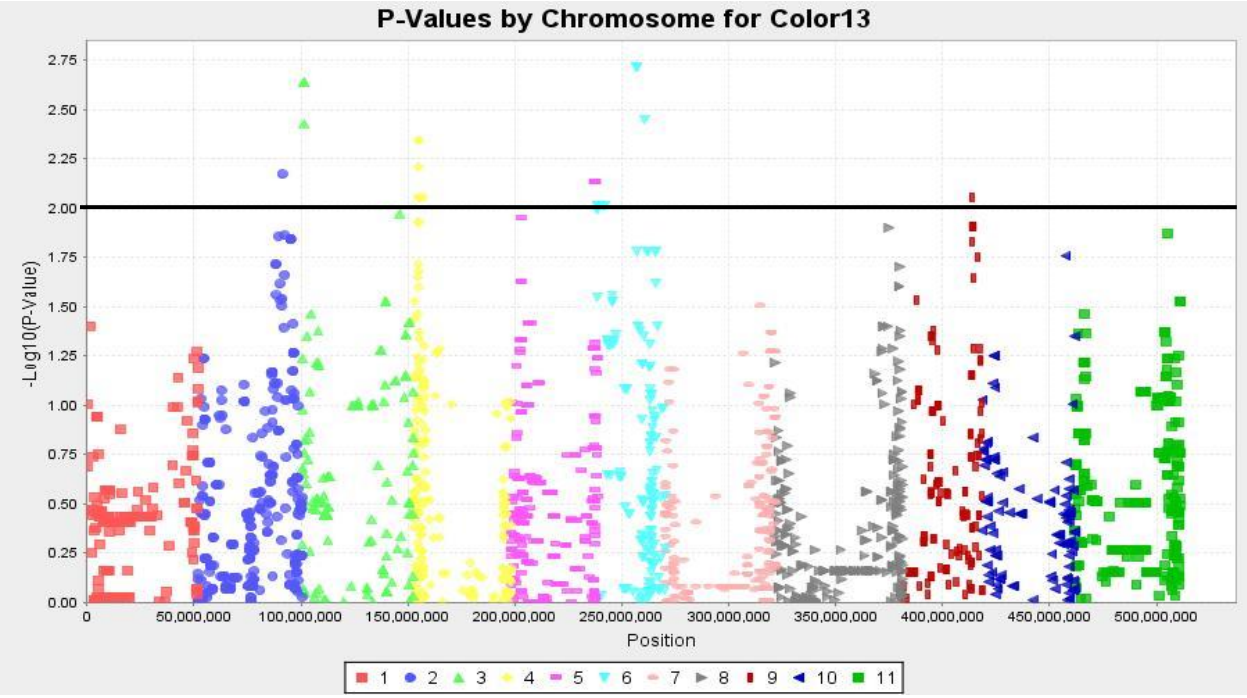


Figure 9. Manhattan plot of the trait of L* values averaged across 2013 of 61 Black beans genotypes with 2800 SNP (>0.02 minor allele frequency) and mapping conducted with multiple linear model (MLM) using a kinship matrix and 7 principal components to account for population structure. The black line is p=0.01 threshold for significance.

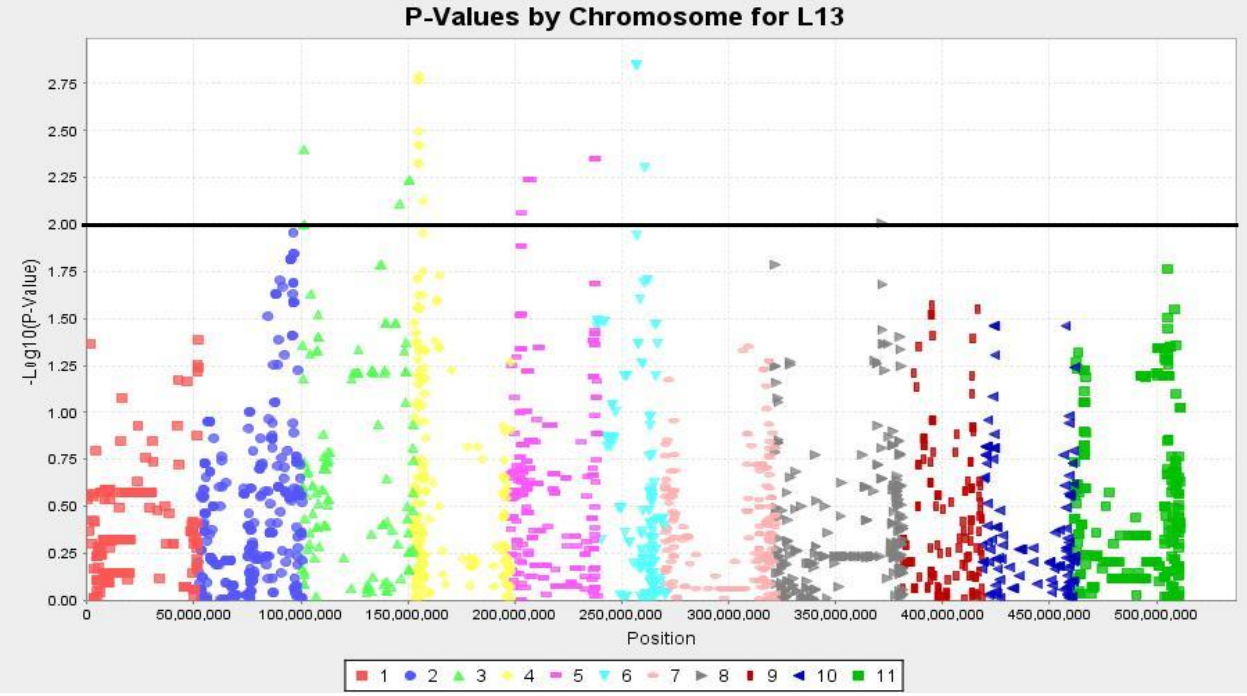


Figure 10. Manhattan plot of the trait of a* values averaged across 2013 of 61 Black beans genotypes with 2800 SNP (>0.02 minor allele frequency) and mapping conducted with multiple linear model (MLM) using a kinship matrix and 3 principal components to account for population structure. The black line is p=0.01 threshold for significance.

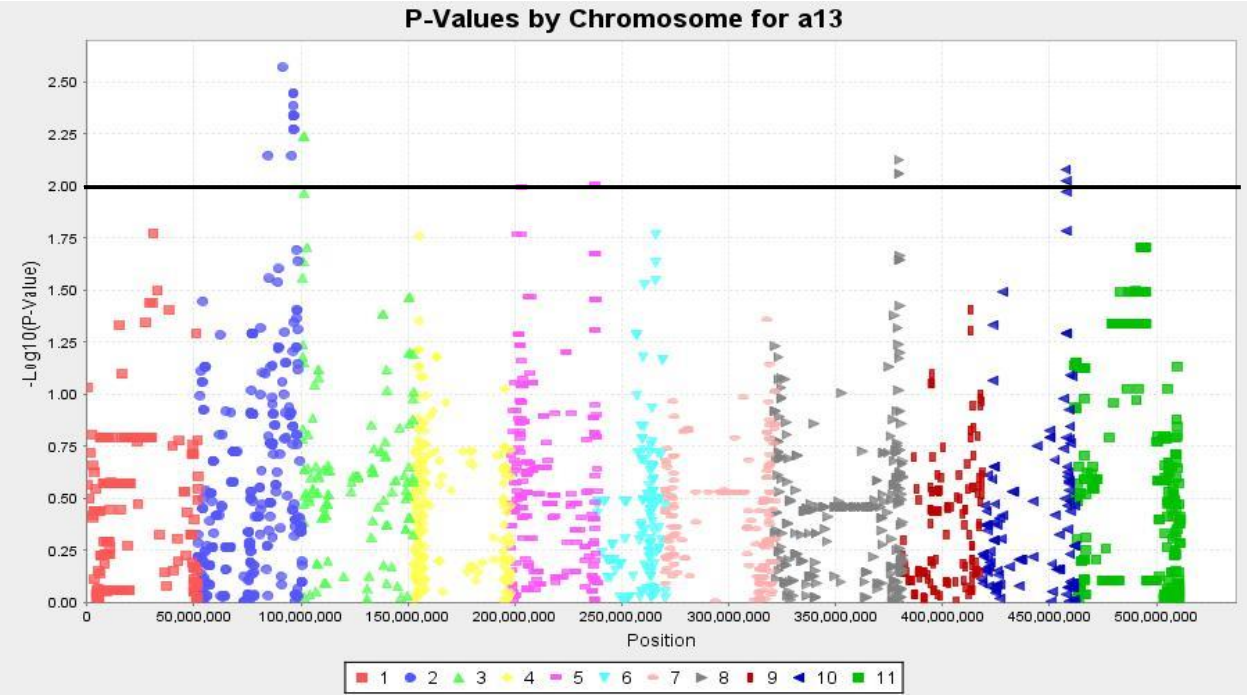


Figure 11. Manhattan plot of the trait of b* values averaged across 2013 of 61 Black beans genotypes with 2800 SNP (>0.02 minor allele frequency) and mapping conducted with multiple linear model (MLM) using a kinship matrix and 3 principal components to account for population structure. The black line is p=0.01 threshold for significance.

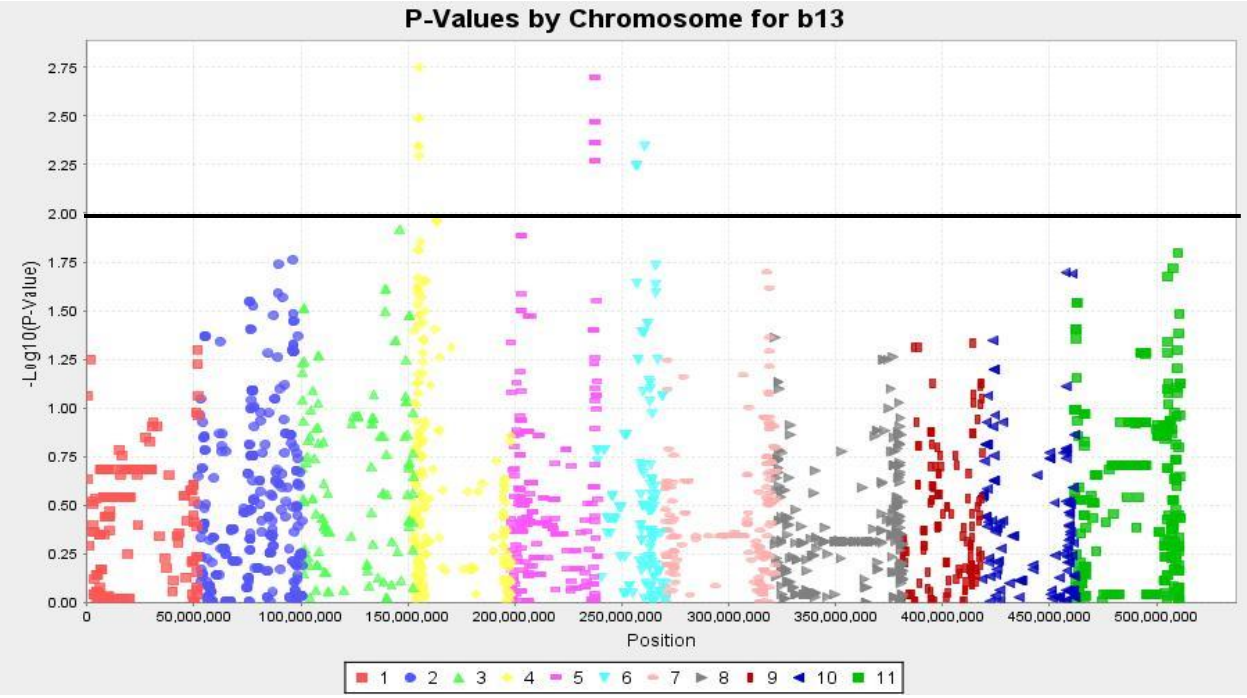
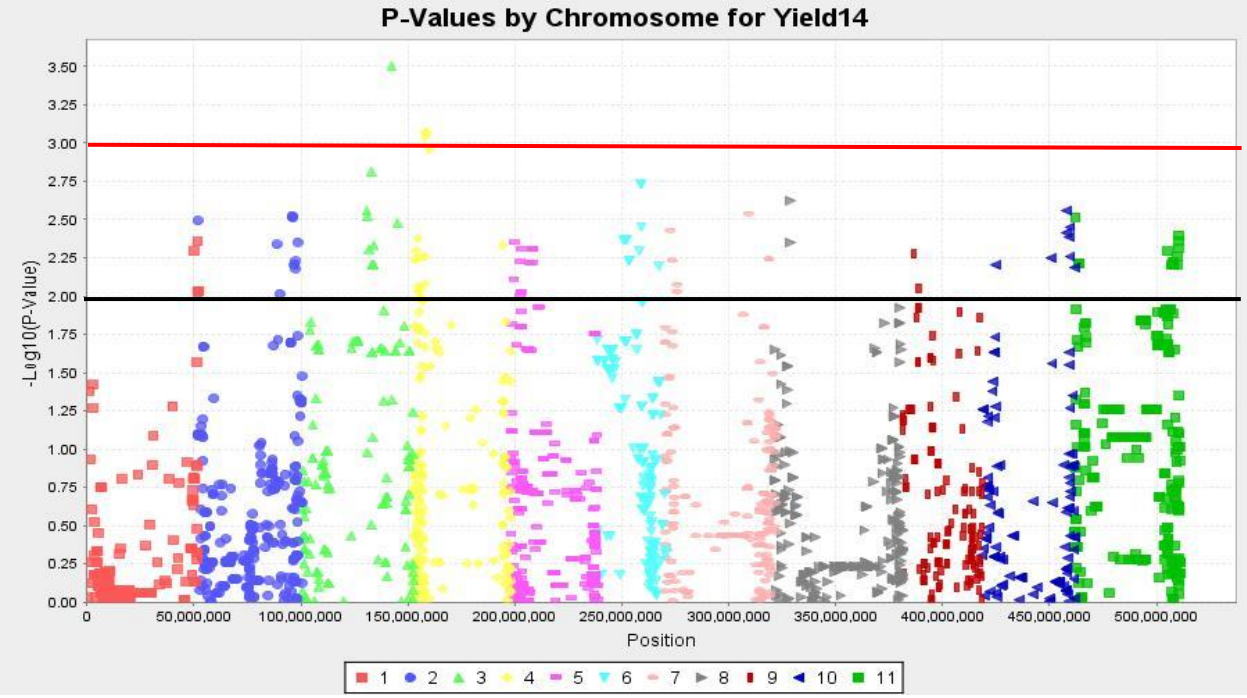


Figure 12. Manhattan plots of seed yield values of 2014 and two-year average for 61 black bean genotypes with 2800 SNP (>0.02 minor allele frequency) and mapping conducted with multiple linear model (MLM) using a kinship matrix. The black line is p=0.01 threshold for significance, and the red line is p=0.001 threshold for significance.



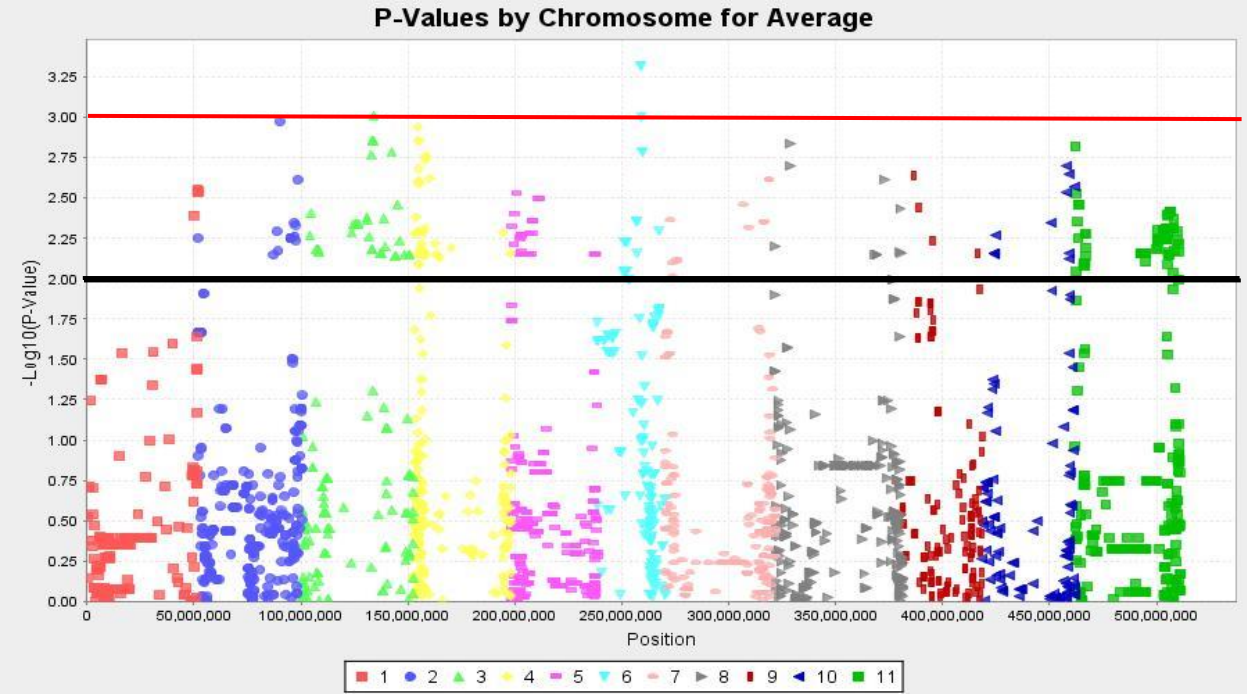


Figure 13. Manhattan plots of seed weight values of 2014 and two-year average for 61 black bean genotypes with 2800 SNP (>0.02 minor allele frequency) and mapping conducted with multiple linear model (MLM) using a kinship matrix and 7 principal components to account for population structure. The black line is p=0.01 threshold for significance.

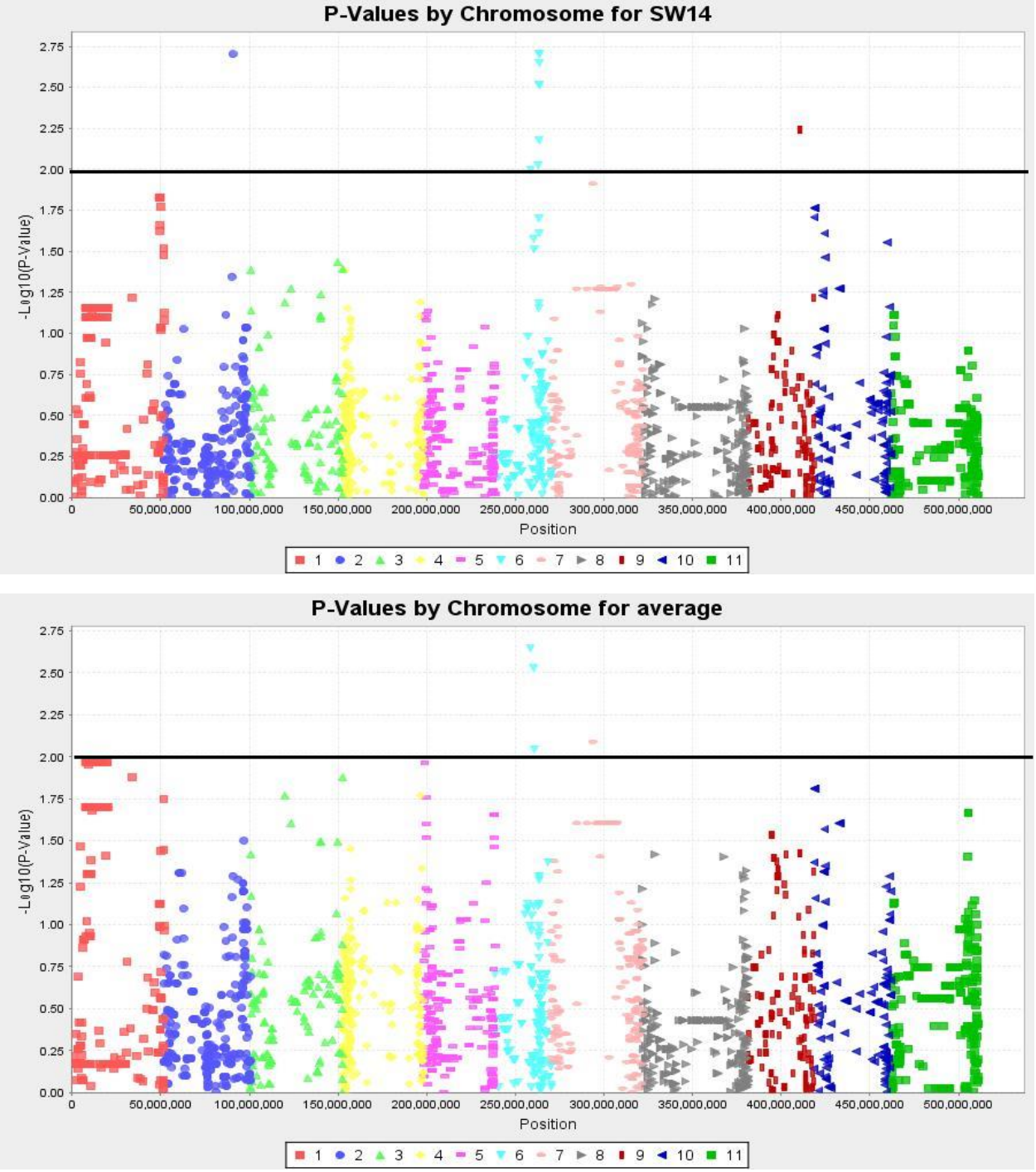
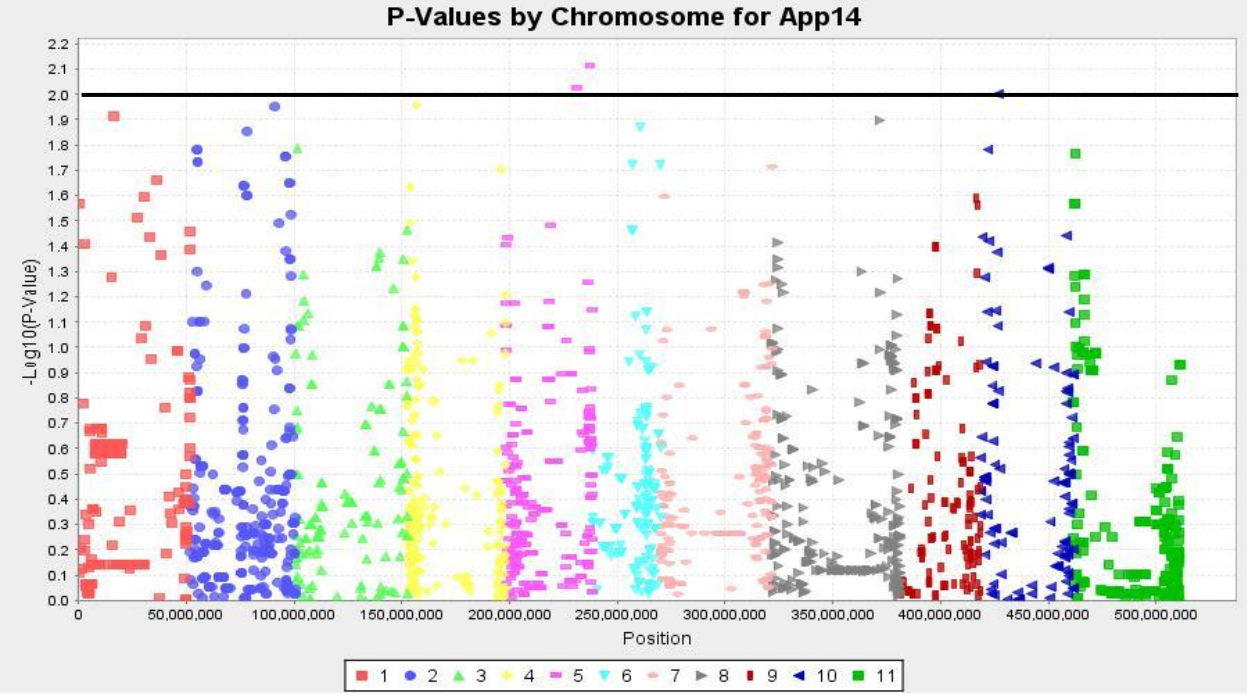


Figure 14. Manhattan plots of appearance ratings of 2014 and two-year average for 61 black bean genotypes with 2800 SNP (>0.02 minor allele frequency) and mapping conducted with multiple linear model (MLM) using a kinship matrix and 2 principal components to account for population structure. The black line is p=0.01 threshold for significance.



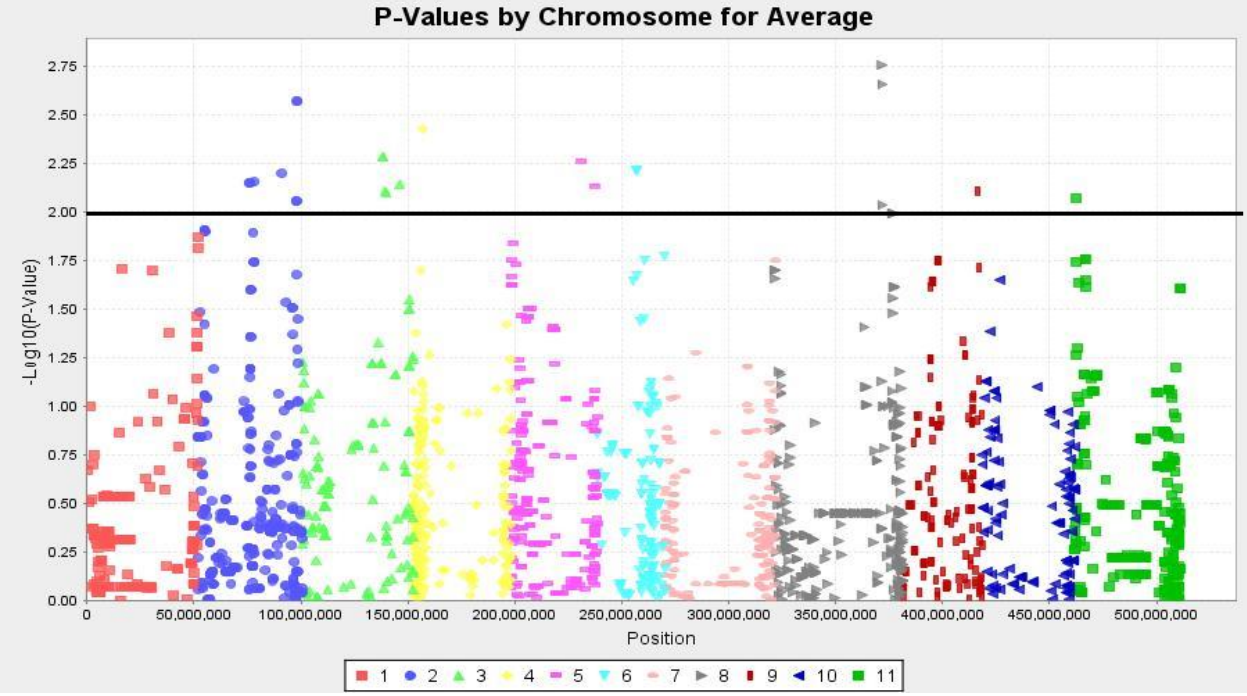
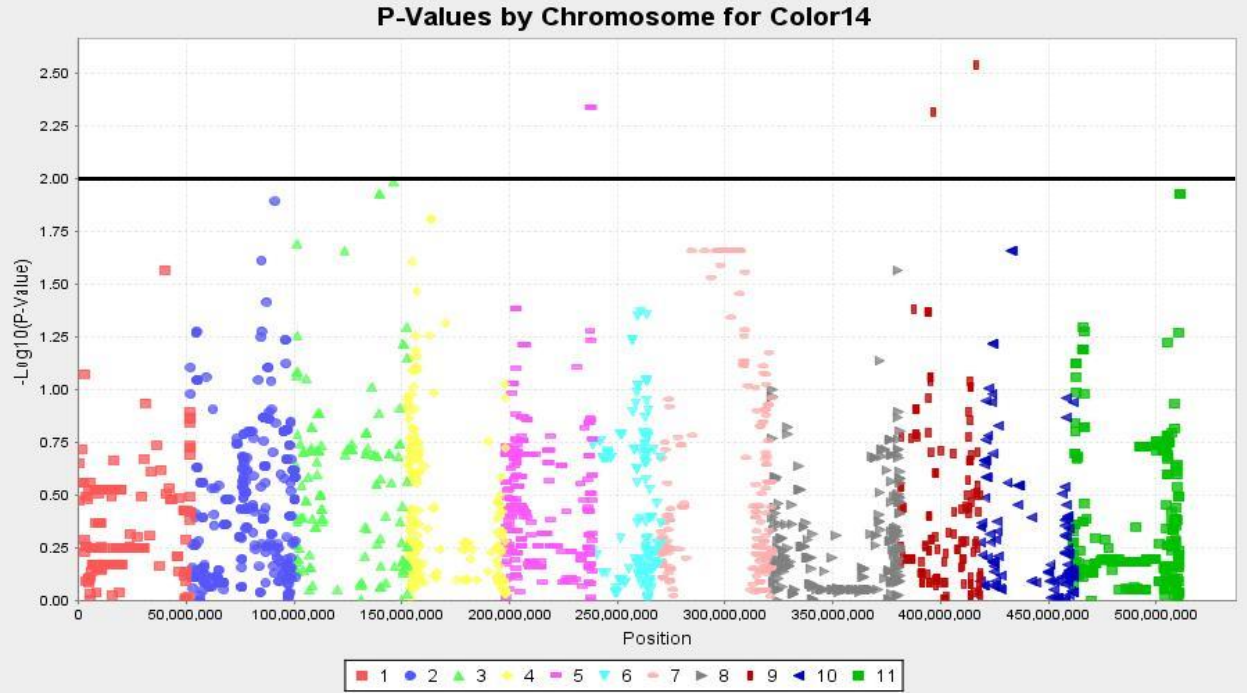


Figure 15. Manhattan plots of color ratings of 2014 and two-year average for 61 black bean genotypes with 2800 SNP (>0.02 minor allele frequency) and mapping conducted with multiple linear model (MLM) using a kinship matrix and 7 principal components to account for population structure. The black line is p=0.01 threshold for significance.



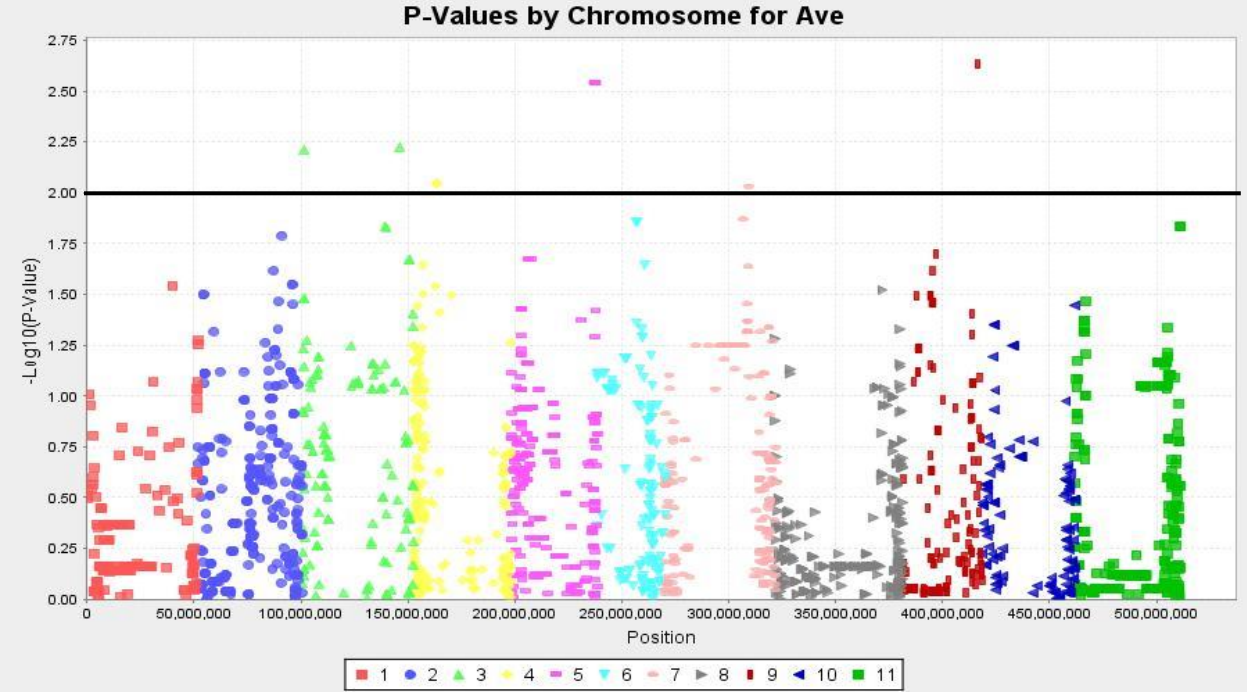
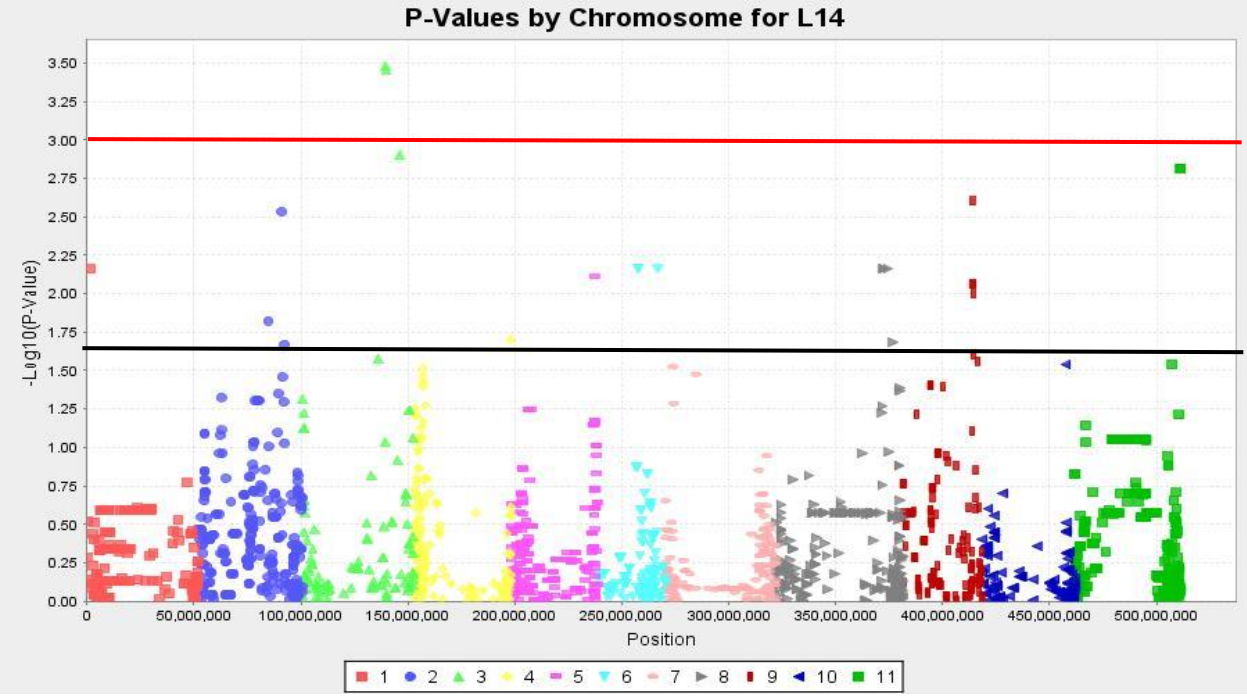


Figure 16. Manhattan plots of the trait of L* of 2014 and two-year average for 61 black bean genotypes with 2800 SNP (>0.02 minor allele frequency) and mapping conducted with multiple linear model (MLM) using a kinship matrix and 5 principal components to account for population structure. The black line is p=0.01 threshold for significance, and the red line is p=0.001 threshold for significance.



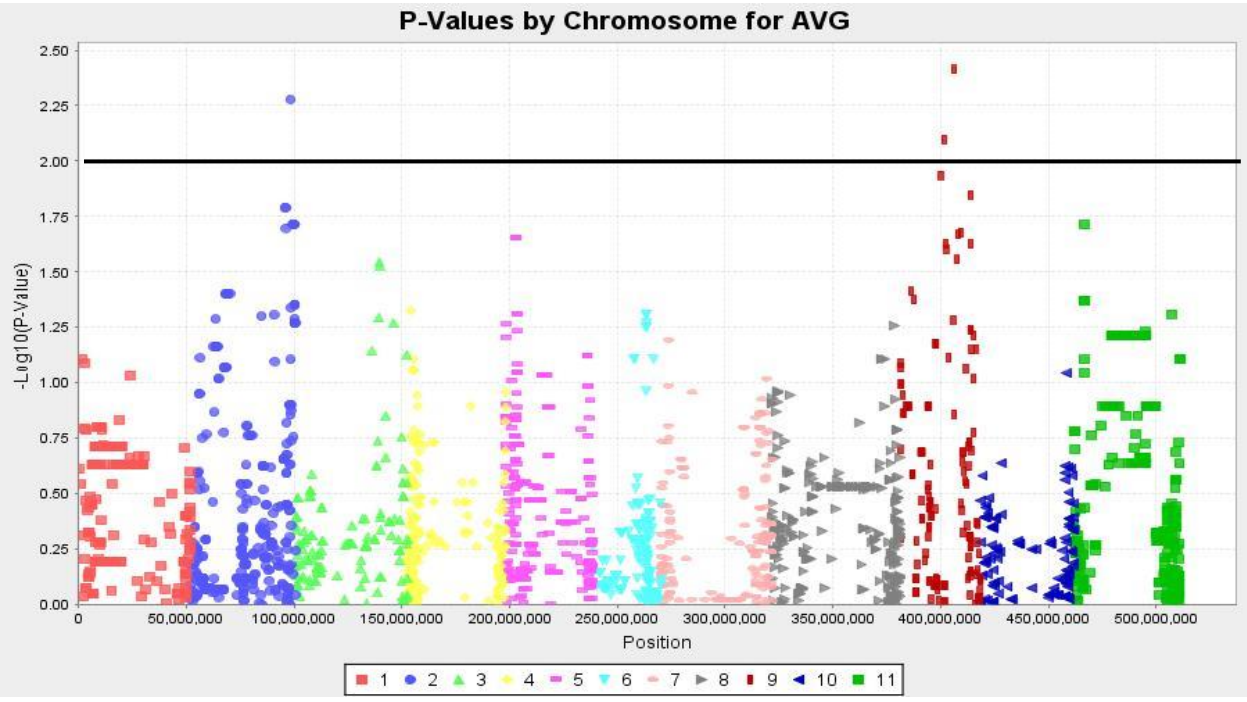
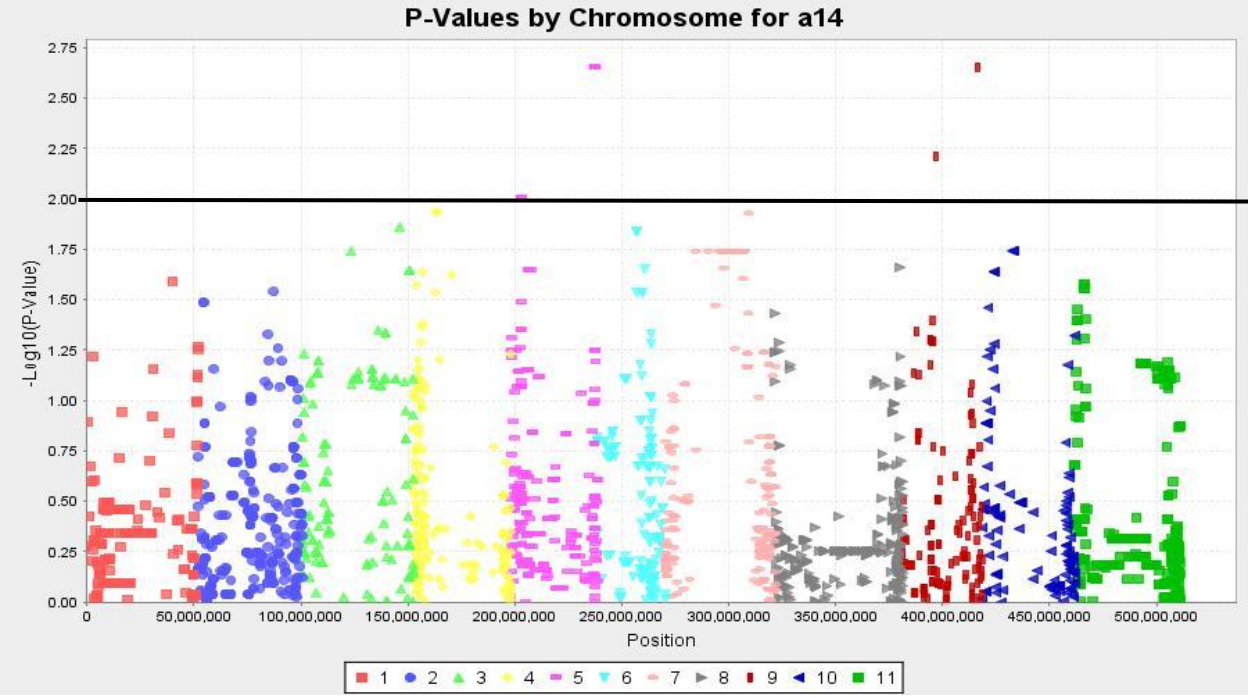


Figure 17. Manhattan plots of the trait of a* of 2014 and two-year average for 61 black bean genotypes with 2800 SNP (>0.02 minor allele frequency) and mapping conducted with multiple linear model (MLM) using a kinship matrix. The black line is p=0.01 threshold for significance.



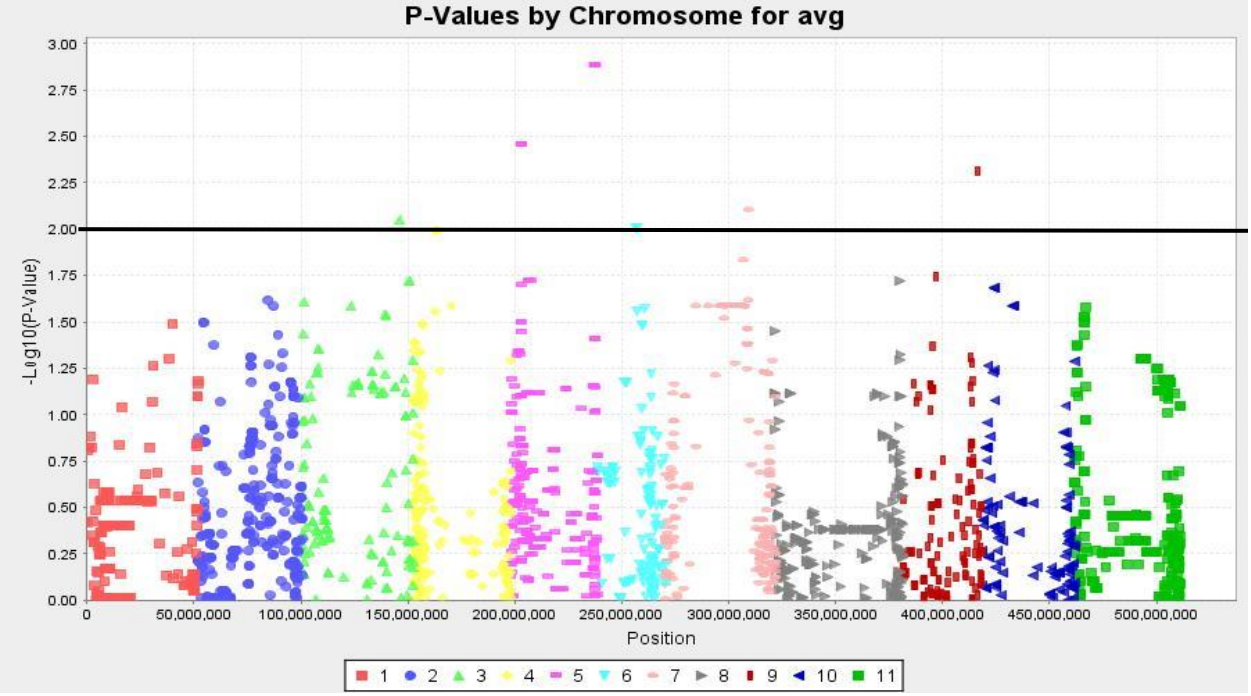
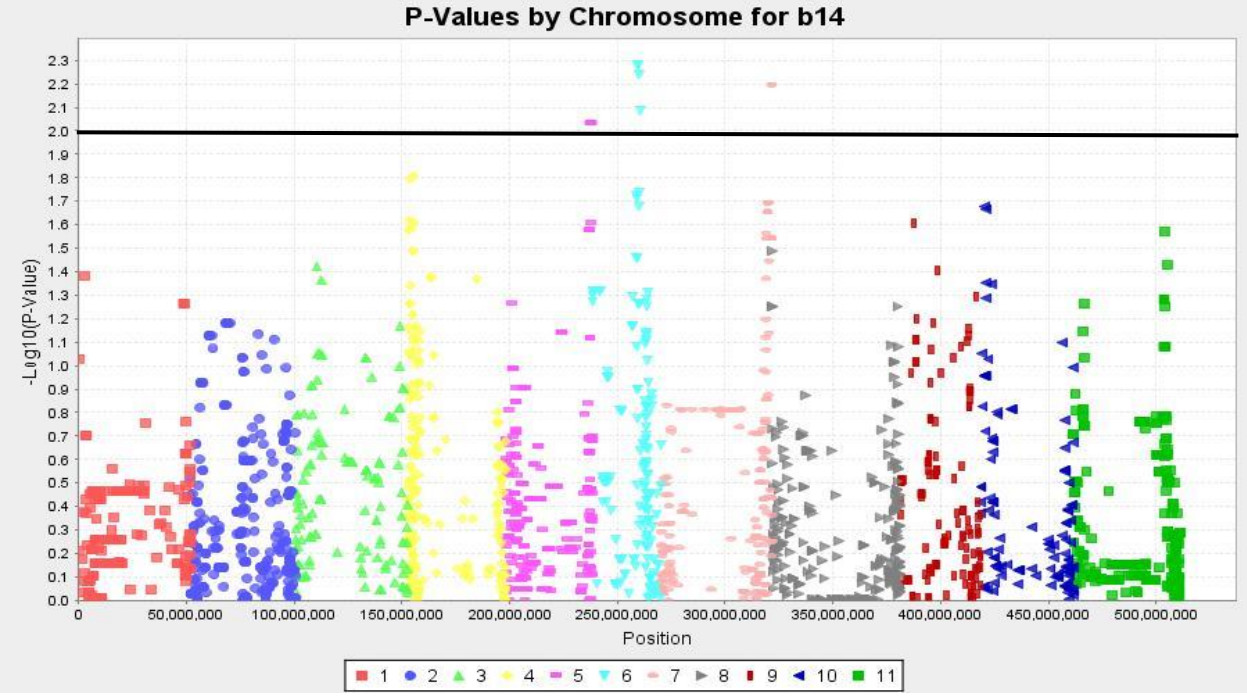


Figure 18. Manhattan plots of the trait of b* of 2014 and two-year average for 61 black bean genotypes with 2800 SNP (>0.02 minor allele frequency) and mapping conducted with multiple linear model (MLM) using a kinship matrix and 3 principal components to account for population structure. The black line is p=0.01 threshold for significance.



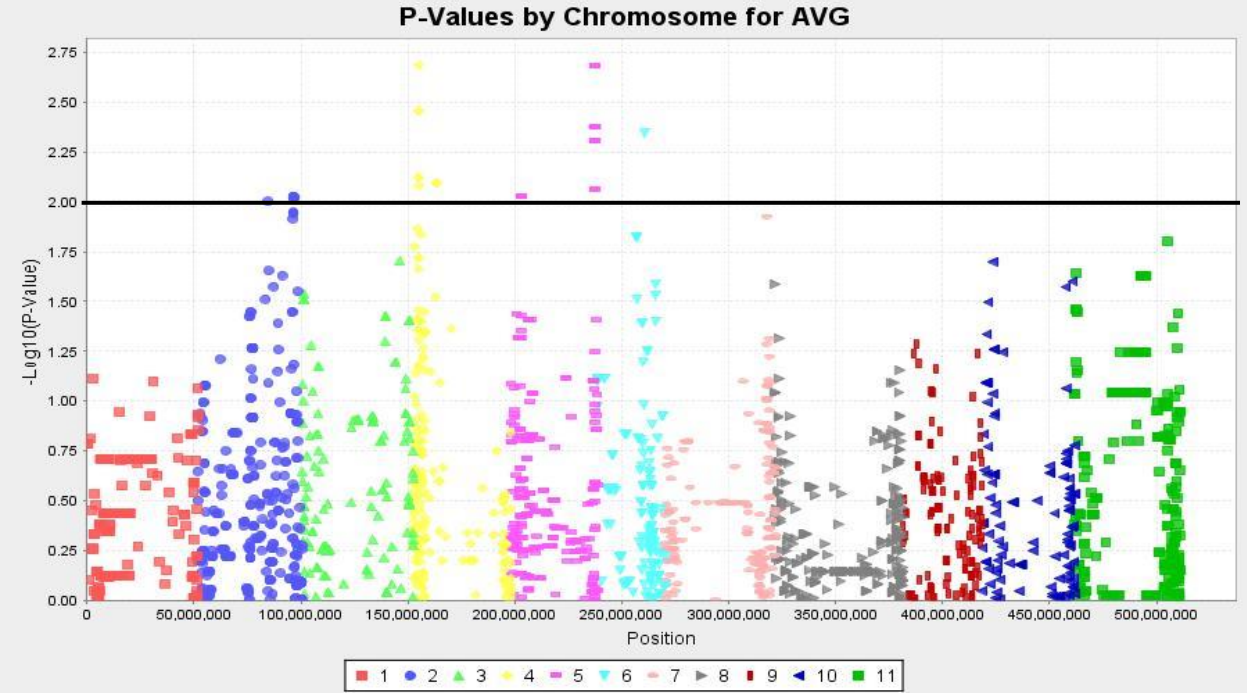


Figure 19. Manhattan plot of the trait of shiny trait of 61 black bean genotypes with 2800 SNPs (>0.02 minor allele frequency) and mapping conducted with multiple linear model (MLM) using a kinship matrix. The black line is p=0.01 threshold for significance. The black line is p=0.01 threshold for significance, and the red line is p=0.001 threshold for significance.

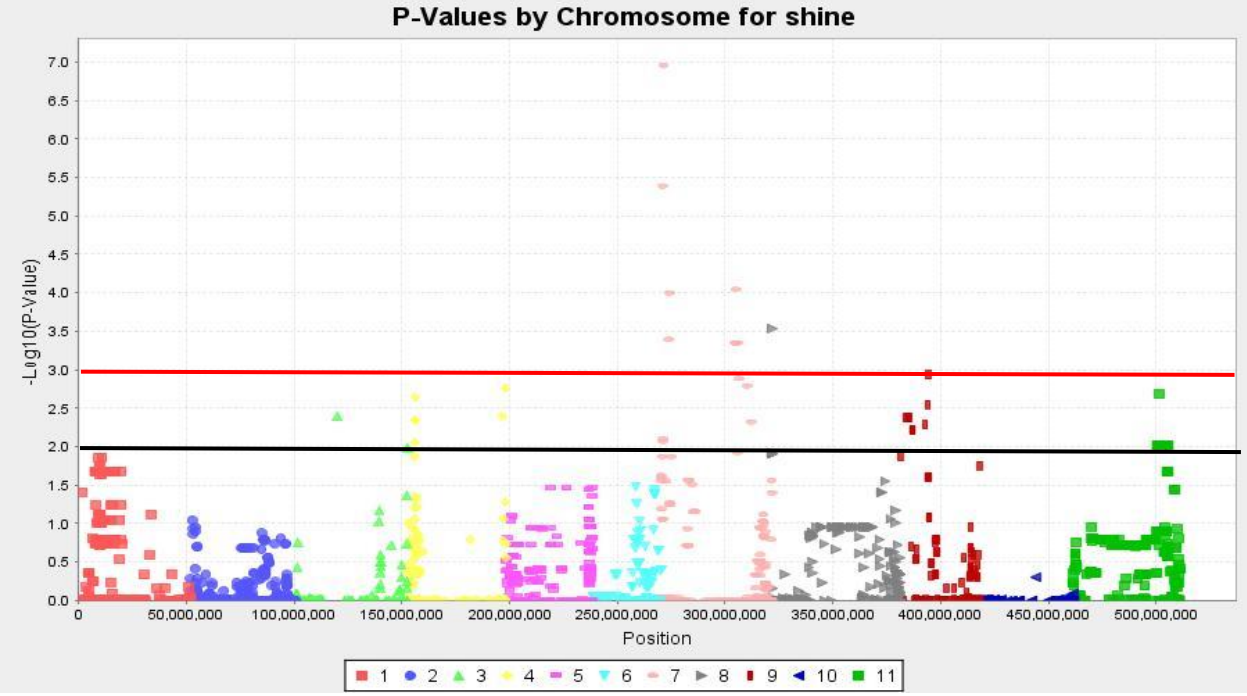


Table 8A. GWAS significant markers, genome position, p-value, R^2, and phenotypic effect associated with the seed yield trait for two-year data (2013 and 2014) and the average of two years.

Yield Year	SNP	CHR[1]	Position (Mb)	P-Value	Add Effect	R^2%
2014	ss715648226	Pv04	5.63	8.60E-04*	-2.48E+02	26
2014	ss715648222	Pv04	5.70	8.60E-04*	248.45886	26
2014	ss715648223	Pv04	5.71	8.60E-04*	248.45886	26
2014	ss715648228	Pv04	5.66	9.03E-04*	247.6965	26
Average	ss715647257	Pv06	20.63	9.89E-04*	-1.98E+02	23
Average	ss715650012	Pv04	2.04	0.00116	-2.60E+02	23
Average	ss715646240	Pv04	2.27	0.00141	145.27562	22
Average	ss715646234	Pv04	2.36	0.00141	-1.45E+02	22
2013	ss715648612	Pv06	20.95	0.00475	185.14331	20
2013	ss715645763	Pv06	26.57	0.00616	392.06839	18
2013	ss715647253	Pv06	20.43	0.00736	199.61102	18
2013	ss715646240	Pv04	2.27	0.00817	158.80195	17
2013	ss715646234	Pv04	2.36	0.00817	-1.59E+02	17
2013	ss715646240	Pv04	2.27	0.00817	158.80195	17
2013	ss715647257	Pv06	20.63	0.00848	-2.07E+02	17
2013	ss715649182	Pv04	43.34	0.00906	-8.32E+01	17

[1]CHR: chromosome.

*SNPs with 3 LOD cutoff.

Table 8B. GWAS significant markers, genome position, p-value, R^2, and phenotypic effect associated with the seed weight trait for two-year data (2013 and 2014) and the average of two years.

Seed Weight Year	SNP	CHR[1]	Position (Mb)	P-Value	Add Effect	R^2%
2013	ss715645537	Pv010	40.83	0.00497	-4.36E-01	17
2014	ss715646411	Pv06	24.82	0.00666	1.97785	17
2013	ss715649416	Pv06	20.81	0.00844	0.60545	15

[1]CHR: chromosome.

Table 8C. GWAS significant markers, genome position, p-value, R^2, and phenotypic effect associated with the appearance trait for two-year data (2013 and 2014) and the average of two-years.

Appearance Year	SNP	CHR[1]	Position (Mb)	P-Value	Add Effect	R^2%
2013	ss715646463	Pv07	4.19	0.00104	0.2241	23
2013	ss715646475	Pv07	4.03	0.00111	0.22083	23
2013	ss715646473	Pv07	4.05	0.00161	-2.19E-01	21
2013	ss715649259	Pv04	42.31	0.00198	0.29723	21
2013	ss715651054	Pv04	41.63	0.00209	0.23837	20
2013	ss715649261	Pv04	42.28	0.0024	0.3073	20
2013	ss715648973	Pv04	41.56	0.00257	0.24427	20
2013	ss715649276	Pv07	3.92	0.00364	-1.96E-01	18
Average	ss715648120	Pv04	4.23	0.00376	1.14E-01	20
2013	ss715646464	Pv07	4.14	0.00425	-1.94E-01	18
2013	ss715648138	Pv04	42.08	0.00435	-3.10E-01	18
2013	ss715649205	Pv04	41.78	0.00468	-3.02E-01	17
Average	ss715647800	Pv07	36.47	0.00807	0.49437	18
Average	ss715650075	Pv04	10.56	0.00854	-3.90E-01	17
2014	ss715649365	Pv10	8.25	0.00995	-0.52951	17

[1]CHR: chromosome.

Table 8D. GWAS significant markers, genome position, p-value, R^2, and phenotypic effect associated with the color trait for two-year data (2013 and 2014) and the average of two years.

Color Year	SNP	CHR[1]	Position (Mb)	P-Value	Add Effect	R^2%
2013	ss715646829	Pv06	18.33	0.00194	0.64152	17
Average	ss715646072	Pv09	35.32	0.00234	-0.35366	23
Average	ss715645369	Pv05	39.47	0.00287	0.35163	22
2014	ss715646072	Pv09	35.32	0.00293	-0.36126	22
2013	ss715646236	Pv04	2.30	0.00455	-0.40351	15
2013	ss715646235	Pv04	2.34	0.00455	-0.40351	15
2014	ss715645369	Pv05	39.47	0.00459	0.34479	20
2013	ss715646238	Pv04	2.28	0.00623	-0.39433	14
2013	ss715645369	Pv05	39.47	0.00737	0.37543	13
2013	ss715645370	Pv05	39.48	0.00737	0.37543	13
2013	ss715645371	Pv05	39.49	0.00737	-0.37543	13
2013	ss715645372	Pv05	39.49	0.00737	-0.37543	13
2013	ss715645375	Pv05	39.51	0.00737	-0.37543	13
2013	ss715645376	Pv05	39.51	0.00737	-0.37543	13
2013	ss715646230	Pv04	2.40	0.00881	-0.41829	13
2013	ss715646229	Pv04	2.43	0.00881	0.41829	13
2013	ss715646227	Pv04	2.46	0.00881	-0.41829	13
2013	ss715645617	Pv09	32.56	0.00883	-0.50188	13
2013	ss715640556	Pv04	4.43	0.00884	-0.23133	13
Average	ss715650075	Pv04	10.56	0.00896	0.27443	18
2013	ss715650985	Pv06	0.03	0.00972	0.41838	13
2013	ss715648431	Pv06	0.18	0.00972	0.41838	13
2013	ss715648429	Pv06	0.20	0.00972	-0.41838	13
2013	ss715648496	Pv06	3.38	0.00972	-0.41838	13
2013	ss715648495	Pv06	3.39	0.00972	-0.41838	13

[1]CHR: chromosome.

Table 8E. GWAS significant markers, genome position, p-value, R^2, and phenotypic effect associated with the L* trait for two-year data (2013 and 2014) and the average of two years.

L* Year	SNP	CHR[1]	Position (Mb)	P-Value	Add Effect	R^2%
2014	ss715639244	Pv03	45.59	0.00125	4.09077	19
2013	ss715646829	Pv06	18.33	0.00142	-1.5125	20
2013	ss715650222	Pv06	18.50	0.00142	-1.51E+00	20
2013	ss715641091	Pv06	18.51	0.00142	-1.51E+00	20
2013	ss715646227	Pv04	2.46	0.00162	1.21984	20
2013	ss715646236	Pv04	2.30	0.0017	1.01947	20
2013	ss715646235	Pv04	2.34	0.0017	1.01947	20
2013	ss715646238	Pv04	2.28	0.00322	0.9469	17
2013	ss715646230	Pv04	2.40	0.00378	1.35858	17
2013	ss715646229	Pv04	2.43	0.00378	-1.36E+00	17
Average	ss715646059	Pv09	25.25	0.00386	0.53827	16
2013	ss715650435	Pv03	0.89	0.00397	-0.85332	17
2013	ss715645369	Pv05	39.47	0.00444	-0.91271	16
2013	ss715646240	Pv04	2.27	0.00477	-0.79655	16
2013	ss715646234	Pv04	2.36	0.00477	0.79655	16
2013	ss715650285	Pv06	22.22	0.00501	NaN	12
2013	ss715645575	Pv03	50.12	0.0058	-2.0857	15
2013	ss715640336	Pv05	8.09	0.0058	2.08575	15
2013	ss715640556	Pv04	4.43	0.00752	0.50605	15
2013	ss715639244	Pv03	45.59	0.00773	-1.6219	14
Average	ss715648756	Pv09	20.58	0.00798	0.52461	14
2014	ss715645638	Pv09	32.95	0.0087	-2.70E+00	13
2013	ss715650411	Pv05	4.81	0.00873	0.63716	14

[1]CHR: chromosome.

Table 8F. GWAS significant markers, genome position, p-value, R^2, and phenotypic effect associated with the a* trait for two-year data (2013 and 2014) and the average of two years.

a* Year	SNP	CHR[1]	Position (Mb)	P-Value	Add Effect	R^2%
Average	ss715645369	Pv05	39.47	0.0013	-0.49187	25
2014	ss715645369	Pv05	39.47	0.0022	-0.56645	23
2013	ss715647408	Pv02	39.66	0.00269	-3.89E-01	18
2013	ss715647243	Pv02	44.72	0.00358	0.40797	17
2013	ss715647244	Pv02	44.74	0.00358	0.40797	17
2013	ss715647242	Pv02	44.68	0.00409	0.40824	17
2013	ss715645959	Pv02	44.80	0.0046	0.39699	16
2013	ss715645968	Pv02	44.80	0.0046	-3.97E-01	16
2013	ss715645958	Pv02	44.81	0.0046	-3.97E-01	16
2013	ss715645970	Pv02	44.89	0.0046	-3.97E-01	16
2013	ss715645955	Pv02	44.91	0.0046	0.39699	16
2013	ss715645963	Pv02	44.84	0.0053	0.3969	16
2013	ss715645956	Pv02	44.92	0.0053	0.3969	16
2013	ss715647099	Pv02	32.86	0.00709	0.37005	15
2013	ss715646101	Pv08	58.01	0.00749	-3.07E-01	15
2013	ss715646094	Pv08	57.94	0.00869	0.30925	14
2013	ss715646095	Pv08	57.94	0.00869	-3.09E-01	14
2013	ss715645338	Pv05	39.18	0.00982	-4.13E-01	14

[1]CHR: chromosome.

Table 8G. GWAS significant markers, genome position, p-value, R^2, and phenotypic effect associated with the b* trait for two-year data (2013 and 2014) and the average of two years.

b* Year	SNP	CHR[1]	Position (BP)	P-Value	Add Effect	R^2%
Average	ss715645370	Pv05	39.48	5.53E-04*	-6.57E-01	29
Average	ss715645371	Pv05	39.49	5.53E-04*	0.6574	29
Average	ss715645372	Pv05	39.49	5.53E-04*	0.6574	29
Average	ss715645375	Pv05	39.51	5.53E-04*	0.6574	29
Average	ss715645376	Pv05	39.51	5.53E-04*	0.6574	29
Average	ss715645369	Pv05	39.47	5.53E-04*	-6.57E-01	29
2013	ss715646227	Pv04	2.46	0.00178	0.83891	20
2013	ss715645338	Pv05	39.18	0.002	-9.60E-01	19
2013	ss715645340	Pv05	39.20	0.002	0.95978	19
Average	ss715650222	Pv06	18.50	0.00258	-6.77E-01	22
Average	ss715641091	Pv06	18.51	0.00258	-6.77E-01	22
2013	ss715646230	Pv04	2.40	0.00323	0.87974	18
2013	ss715646230	Pv04	2.40	0.00323	0.87974	18
2013	ss715646229	Pv04	2.43	0.00323	-8.80E-01	18
2014	ss715645369	Pv05	39.47	0.00323	-3.80E-01	21
2014	ss715645370	Pv05	39.48	0.00323	-3.80E-01	21
2013	ss715646236	Pv04	2.30	0.00448	0.68126	16
2013	ss715646236	Pv04	2.30	0.00448	0.68126	16
2013	ss715646235	Pv04	2.34	0.00448	0.68126	16
Average	ss715649164	Pv07	38.89	0.00502	-4.39E-01	19
2013	ss715646829	Pv06	18.33	0.00569	-9.82E-01	16
2013	ss715646829	Pv06	18.33	0.00569	-9.82E-01	16
2013	ss715650222	Pv06	18.50	0.00569	-9.82E-01	16
2013	ss715641091	Pv06	18.51	0.00569	-9.82E-01	16
2014	ss715649164	Pv07	38.89	0.0057	-3.18E-01	19
2014	ss715646943	Pv04	10.84	0.00747	0.30394	18
2014	ss715646944	Pv04	10.99	0.00747	0.30394	18
2014	ss715650075	Pv04	10.56	0.00747	-3.04E-01	18
Average	ss715647800	Pv07	36.47	0.00807	0.49437	18
Average	ss715646943	Pv04	10.84	0.00854	0.38985	17
Average	ss715646944	Pv04	10.99	0.00854	0.38985	17
Average	ss715650075	Pv04	10.56	0.00854	-3.90E-01	17

[1]CHR: chromosome.

*SNPs with 3 LOD cutoff.

CONCLUSION AND RECOMMENDATION

The appearance and color retention of canned black bean products affects consumer decisions to accept or reject black bean products. In this study, 69 black bean genotypes from the major U.S. bean breeding programs were assessed for their genetic variability for seven quality traits (seed yield, seed weight, canned appearance, color, L*, a*, and b*) over two field experiments grown in 2013 and 2014. A year advantage of 2014 over 2013 was observed in the trait of color retention as well as the rest of the previous traits (yield, seed weight, appearance, L*, a*, and b*). Seed quality traits are linked to environment and there were superior environmental conditions for the canned black bean in 2014. Also, this express the wide genetic variability among black genotypes specially in the trait of color retention and that may do to fewer bean breeding programs that work on color retention, which are MSU and USDA-MI. Strong correlation analysis results were found between color ratings and the traits of L*, a*, b* colors scans in both years 2013 and 2014. This assures that the trained sensory panel was efficient and comparable to the colorimeter that was used to obtain L* a* b* color measurements.

The result of neighbor joining tree revealed that the sub-branch A2-2, which has all the shiny seed-coated genotypes, has higher means for the traits of appearance, a*, and b* for both years (2013 and 2014). That suggests shiny seed-coated genotypes to have superior color retention over opaque seeds, and that is due not only to their seed luster, but to their excellent canning quality that have been chosen based on. All the other traits were varied between the two years. For the trait of yield, the sub-branch A1-2 had the higher yielding mean in the year of 2013, while the mean of the major branch C was the highest in 2014. The two traits of color retention and L* have been dominated by the sub-group A2-2 with the highest mean values (3.61, 15.17) and standard deviation (0.58 and 1.08) respectively. The same two traits were varied in the year of 2014.

GWAS was conducted for the seven traits (seed yield, seed weight, appearance, color, L*, a*, and b*) of 61 black bean genotypes that were obtained from the two years 2013 and 2014. Even though the size of the studied population was small, it was strong enough to detect some significant SNPs above LOD 6 on Pv07 where the region of ASP gene, which is responsible of seed luster in black bean. The significant marker trait associations were found on Pv06 and Pv10 for the trait of seed weight (Figure 6 and Figure 13). The two associations on Pv06 for 2013 and 2014 were significant and explained 15% and 17% phenotypic variation. The significant SNPs for the two years (2013 and 2014) were identified on the physical positions of 24.82 and 20.81 Mb respectively, and mapped to one region with different effects on seed weight. This result agreed with what was discovered in the study of Cichy et al., where they detected some QTLs for the trait of seed weight on Pv06 for two years of 2000 and 2005.

The significant SNP markers that were found associated with color retention, L*, a*, and b* shared same physical position (39Mb) on the Pv05. This result assured what was found by the study of Cichy et al., (2014), where they identified some significant SNP markers on Pv04 and Pv05 for the traits of color retention, L*, b*, and anthocyanin. However, by comparing the two results, the physical regions that were identified in this study and associated with color traits (color retention, L*, a*, and b*) were novel locations. The traits of color retention and L* were also co-localized on Pv04, Pv05, Pv6, and Pv09 and shared very similar regions. Through GWAS, we have also identified regions on Pv04 with physical locations ranged from (4.43 to 10.56Mb) that were related to the traits of color retention, L* and appearance. These findings indicate an important role for the identified SNP markers that are associated with the traits of color and appearance in black beans. This research serves as an important foundation for further studies to understand the genetic control of color retention after canning in black beans.

CHAPTER 4

PHENOTYPIC EVALUATION OF ANTHOCYANIN IN BLACK BEAN

INTRODUCTION

Anthocyanins are water-soluble pigments, and are triggered environmental stresses such as bright light, UV radiation, and high temperature. There are about 500 types of anthocyanins in nature (Gould et al., 2009). Black bean anthocyanins are concentrated in the seed coat (Dzomba et al., 2013). The most common anthocyanins found in black beans are delphinidin 3-glucoside, petunidin 3-glucoside, malvidin 3-glucoside, and malvidin 3,5-diglucoside (Zhang et al., 2014). Some anthocyanin compounds have been extracted in lower quantities for example cyanidin 3-glucoside, cyanidin 3,5-diglucoside, pelargonidin 3-glucoside, and pelargonidin 3,5-diglucoside (Takeoka et al., 1997). Black bean seed coats also contain extractable anthocyanins and bound anthocyanins (Kelly et al., 2015). Anthocyanins are bound either by oxidation or by cross-linking, and the concentration of the bound anthocyanins in some black bean accessions are higher than the extractable ones (Marles et al., 2010). The amounts of each compound vary due to genetic variability and environmental aspects such as the growing location (Rocha-Guzmán et al., 2007). These compounds could impact black bean canning quality since they are responsible for seed coat color. Anthocyanins also play a role in maintaining human health through their anti-oxidant activity, which help to reduce oxygen free radicals (Díaz et al., 2010; La Cruz et al., 2013). In this study, concentrations of three anthocyanins (delphinidin-3-glucoside, petunidin-3-glucoside, and malvidin-3-glucoside) were quantified using liquid chromatography-mass spectrometry (LC-MS). Total anthocyanins were also quantified using an UV assay. Both approaches were carried out on 12 black bean genotypes where raw and canned samples grown for two years (2013 and 2014).

The 12 genotypes were chosen to represent a wide range of the anthocyanin concentration based on color retention of the 69 genotypes described in the third chapter.

MATERIALS AND METHODS

4.1. Materials Selection

A group of 12 genotypes was previously selected based on the canning process evaluation result that obtained earlier in this study (Table 9). The 12 genotypes represent a range of variability in term of color and appearance traits. This group included 96 samples grown for two years (2013 and 2014), two conditions (raw and canned), and two replications, and one duplicate for each replication per sample was involved.

Table 9. A list of the 12 black bean genotypes evaluated traits for total anthocyanins concentrations and the three specific anthocyanins quantification in raw and canned black beans.

No.	Genotype	Shiny / Dull	Origin	Pedigree
1	B-EL1291d	Dull	USDA-MI	Black Magic/Shiny Crow
2	B11313	Dull	MSU	B04644//B04349/B05044
3	B11356	Dull	MSU	Jaguar/B04644
4	ND081343	Dull	NDSU	Unknown
5	Eclipse	Dull	NDSU	ND9902621-2
6	Zenith (B10244)	Dull	MSU	B04644/B07554
7	B11370	Dull	MSU	B05055/B04265
8	B10243	Dull	MSU	B04610/N05346
9	B11338	Dull	MSU	N08007//B04349/B05044
10	NDF09304	Dull	NDSU	Unknown
11	B11285	Dull	MSU	N04152/N05346//N04141/N05317
12	OACExpresso	Dull	Saskatoon	Unknown

4.2. Anthocyanin Extraction and Purification

The seed varieties were separately freeze-dried using Virtis genesis 12el freeze dryer. Dried seeds from each variety of bean was then separately ground in a PX-MFC 90 D mill to yield a 30-40 mesh powder. One gram each of the powdered bean was transferred to 50mL flasks. Total anthocyanins in the bean powder was then extracted by adding a solvent system containing 80 methanol: 20 HPLC H2O: 0.3HCl (20mL) to each flask, shaken for 4h on a Gyrotory to 50 mL tubes and centrifuged (Centrifuge Sorvall RT7 Benchtop Centrifuge) for 10min (270rpm, -10°C). The combined supernatants were then concentrated to 5mL under vacuum using a rotary

evaporator at 35°C (Buchi). The pellets were discorded. The resulting samples were analyzed immediately for total anthocyanin content (Chandra et al., 1992; Bowen-Forbes et al., 2010).

4.3. UV Spectrophotometer Assay

The total anthocyanin was measured using a spectrophotometry (Genesys 10S UV-Vis) for a UV assay. The total anthocyanin was absorbed at anthocyanins maximum wavelengths of 520nm. The calibration curve was made using delphinidin-3-glucoside as a standard (purchased from Cerilliant, TX, USA) that represents the major anthocyanin in black beans (Table 11) (Figure 20) (Takeoka et al., 1997). Several dilution factors have been made based on the preliminary spectrophotometer absorbents for all the samples, and used to obtain a moderate absorbent range of (0.01-1.00) (Table 10). Three different amounts of the extracted stock based on the preliminary samples absorbance were taken to a 15ml tube (300µ/g, 200µ/g, and 100µ/g), and three different amount of the extraction buffer were added to obtain a total volume of 3000µ for each sample (2700µ/g, 2800µ/g, and 2900µ/g). Two duplicates, 1ml each were transferred from the 3000µ-total volume to two 1.5-Cuvettes and were taken for absorbance measurements. Moreover, some sample extracts, especially the raw types, were over the absorbent range (higher than 1.00), and accordingly three dilution factors were obtained (as described) for those over-rang samples 10, 15, and 30. The spectrophotometer was blanked by using distilled water filled in a 1.5-Cuvette and measured at the same wavelength level (Chandra et al., 1992; Bowen-Forbes et al., 2010).

Figure 20. Calibration curve for delphinidin-3-glucoside standard made to calculate the total anthocyanins mass in black bean genotype extracts.

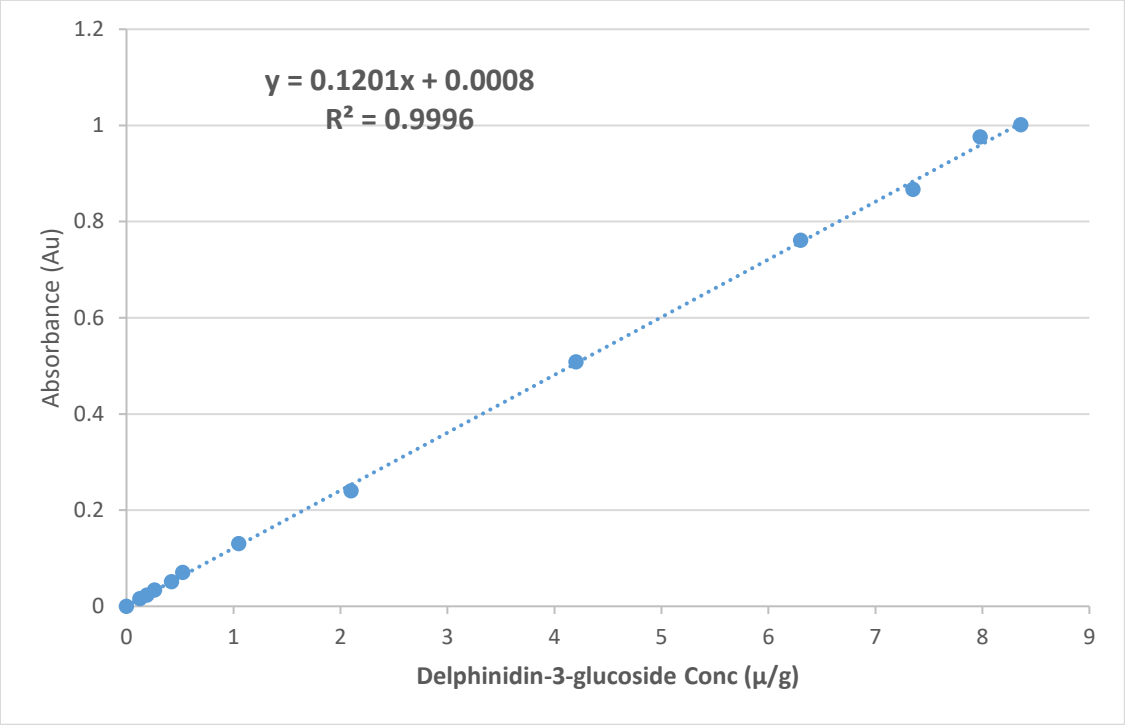
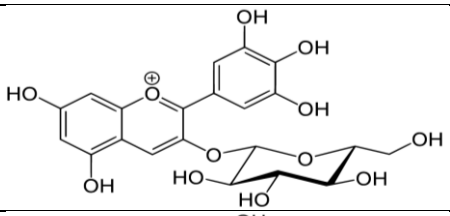
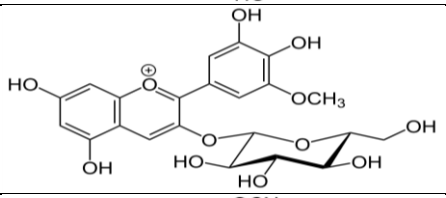
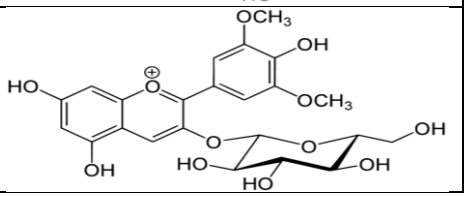


Table 10. Delphinidin-3-glucoside calibration curve points made to calculate the total anthocyanins mass in black bean genotype extracts.

Delphinidin-3-glucoside conc µg/g	Absorbent
0	0
0.126	0.016
0.189	0.023
0.2625	0.034
0.42	0.051
0.525	0.07
1.05	0.13
2.1	0.24
4.2	0.508
6.3	0.761
7.35	0.867
7.98	0.976
8.358	1.001

Table 11. Three major anthocyanins in black beans represent the study standards.

Anthocyanin	Structure
Delphinidin-3-glucoside	
Petunidin-3-glucoside	
Malvidin-3-glucoside	

(Takeoka et al., 1997)

4.4. Anthocyanin Quantification Using Liquid Chromatography–Mass Spectrometry (LC-MS)

High-performance liquid chromatography combined with mass detection (HPLC–MS) is the most commonly used technique for analyzing anthocyanins. HPLC provides powerful and rapid separations of anthocyanins, and MS makes a quick and sensitive detection. HPLC–MS is an important frequently-used instrument for anthocyanin characterization, offering mass spectrum of intact molecular and fragment ions (Acevedo et al., 2012). Anthocyanin extracts for the same chosen genotypes group were analyzed by LC-MS with an Acquity Ultra Performance Liquid Chromatography (UPLC) coupled to a Xevo G2QT of spectrometer (Waters Corp). For LC analysis, 5µL samples of extract was injected and passed through a HSS C18 1.8 mm2.1 x 100mm column (Waters) at 0.4mL/min. The mobile phase contained 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). Anthocyanins were eluted using linear gradients of 97.6 to 80% A in 2.5min, 80 to 60% A in 0.5min, 60 to 48% A in 2min, 48 to 5% A

in 0.5min, 5 to 97.6% A in 1min, and a 0.5min hold at 97.6% A. Electrospray ionization mass spectrometry (ESI) was made in positive VIII ionization mode with a scan speed of 5/s in the mass range from 50 to 1200Da. Lock-mass correction was used, with a fluidic system to deliver LockSpray reference which is leucine enkephalin as the external lock-mass standard. LC-MS data was analyzed using the MassLynx software (Rockenbach et al., 2012).

4.5. Statistical Analysis

Analysis of variance (ANOVA) for the two traits have been obtained using SAS9.4 statistical software package. The GLM procedure was used for analysis of variance (ANOVA) for TAC trait with the model:

$y_{ij} = \mu + \text{Genotypes}_i + \text{Conditions}_j + (\text{Genotypes} * \text{Conditions})_{ij} + \text{Block (Year)}_k + e_{ij}$

including genotype (12 levels) condition (2 level) year (2 levels) and rep (2 levels) and genotype*condition. Data means were separated using POST-HOC Tukey test at $\alpha \leq 0.05$ level of significance.

RESULTS AND DISSCUSSION

A set of 12 black bean genotypes was obtained from the study population based on the result of the trained sensory panel ratings evaluation for canned color and the L* a* b* color parameters. This sub-population represents the range of color ratings including, moderate, and low. The sub-population had been evaluated for total anthocyanin concentration (TAC) and for three specific anthocyanins quantification (SAQ) (delphinidin-3-glucoside, petunidin-3-glucoside, and malvidin-3-glucoside) using spectrophotometer and LC-MS respectively over two field seasons. The result of ANOVA in (Table 12A) indicated that the variation among genotypes was significant based on ($\alpha \leq 0.05$) as a level of significance as well as the difference between the two conditions (raw and canned) based on the same level. This result suggests that canned black beans have considerable total anthocyanins concentrations comparing to the three anthocyanins quantification in canned black beans. The interactions between genotype and condition for total anthocyanin trait was also not significant. The same statistical model was used to obtain ANOVA for the trait of SAQ. The result was found to be significant for genotypes and conditions for all the three anthocyanins used for SAQ trait based on the same level of significance. The interactions between genotypes and conditions were significant for all the quantified anthocyanins except for delphinidin-3-glucoside where it was not significant (Table 12 B-D).

There was genetic variability for TAC in raw black beans in 2013 and 2014. Two genotypes, B-EL1291d and Eclipse (Table 13A) had significantly higher and lowest levels respectively among the other genotypes in both years 2013 and 2014 as follow; B-EL1291d (1.70, 2.024mg/g), Eclipse (0.49, 0.47mg/g) (Table 8C). The TAC in canned black beans was also genetically variable for the both years (2013 and 2014) (Table 13B). Genotype B-EL1291d was significantly higher than the other genotypes in both years, where the genotype ND081343 was the lowest as follow; B-

EL1291d (0.118, 0.169mg/g), Eclipse (0.051, 0.047mg/g) (Table 14B). B-EL1291d was the highest in TAC across all conditions and years. This finding suggests the genotype B-EL1291d has more anthocyanins than the other genotypes in both raw and canned processed products. The genotype B-EL1291d is a black bean accession with dull seeds luster that was obtained from USDA-MI, and was developed as a result of the cross BlackMagic/ShinyCrow (these two genotypes are included in the studied population for seed quality evaluation).

Anthocyanin retention after canning is an important attribute in black beans for the canning industry. Anthocyanin retention percentage for the 12 black bean genotypes were calculated by subtracting the total anthocyanins for a canned genotype from the raw one, and presenting data on a percentage (Table 14). The 12 genotypes varied in the ability to retain anthocyanins. The genotype with the highest total anthocyanin retention percentage in TAC experiment was Eclipse (15.92, 27.10) for both years (2013 and 2014) respectively. Even though the genotype Eclipse had the highest anthocyanin retention value (Table 14), it had a very low total anthocyanin concentration. In 2013, Eclipse had the lowest TAC among all the other genotypes, and it was among the lower TAC-genotypes in 2014. Therefore, higher anthocyanins retention does not necessary mean higher anthocyanins concentration. Two genotypes (B11313 and ND081343) had very similar anthocyanin retention (AR) across the two years, whereas the rest of the genotypes varied for AR in TAC experiment.

Three anthocyanins delphinidin-3-glucoside, petunidin-3-glucoside, and malvidin-3-glucoside were found to have the highest concentration in black beans (Dzomba et al., 2013). In this experiment, the concentrations of these three anthocyanins were quantified in raw and canned beans grown for two years (2013 and 2014). The three anthocyanins standards were used in the LC-MS quantification to identify them in the black bean extracts. The LC-MS chromatograph of

black bean extracts detected the three major anthocyanins in black beans with retention times of 3.73, 3.97, and 4.20 respectively (Figure 21). The electrospray (ES) mass spectrum of the three anthocyanins showed the molecular mass ions at m/z 465.11, m/z 479.12, and m/z 493.14 respectively.

Figure 21. LC-MS chromatograph shows the three anthocyanins peaks have been detected in the studied black bean genotype extracts.

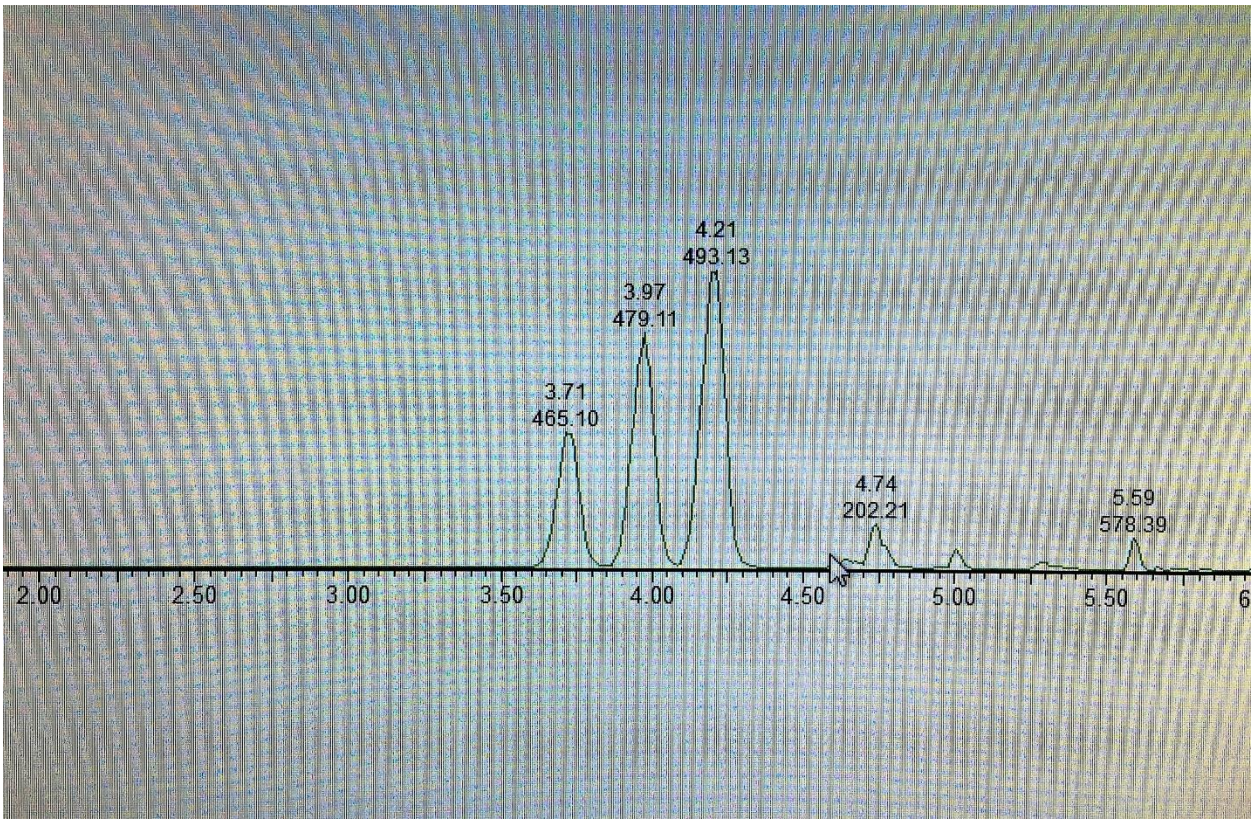


Table 12A. Analysis of variance for total anthocyanins (TA) for 12 black bean genotypes with two levels of conditions (raw and canned), two levels of years (2013 and 2014), and two replications.

Trait	Source of variation	Degree of freedom	Type III SS	Mean square	F statistic	Pr>F
TA	Genotype	11	1.50	0.14	4.06	0.0001
	Condition	1	0.05	0.05	1.34	0.0001
	Year	1	11.70	11.70	348.77	0.2504
	Genotype*Condition	11	0.50	0.11	3.39	0.0008

Table 12B. Analysis of variance for Delphindin-3-glucoside concentration for 12 black bean genotypes with two levels of conditions (raw and canned), two levels of years (2013 and 2014), and two replications.

Trait	Source of variation	Degree of freedom	Type III SS	Mean square	F statistic	Pr>F
Del-3-gluc	Genotype	11	0.38	0.03	1.14	0.034
	Condition	1	24.68	24.68	956.76	<0.0001
	Year	1	0.05	0.05	1.70	0.20
	Genotype*Condition	11	0.36	0.03	1.08	0.39

Table 12C. Analysis of variance for petunidin-3-glucoside concentration for 12 black bean genotypes with two levels of conditions (raw and canned), two levels of years (2013 and 2014), and two replications.

Trait	Source of variation	Degree of freedom	Type III SS	Mean square	F statistic	Pr>F
Pent-3-gluc	Genotype	11	0.47	0.04	2.52	0.0096
	Condition	1	8.63	8.63	508.54	<0.0001
	Year	1	0.001	0.001	0.03	0.001
	Genotype*Condition	11	0.43	0.04	2.31	0.02

Table 12D. Analysis of variance for malvinidin-3-glucoside concentration for 12 black bean genotypes with two levels of conditions (raw and canned), two levels of years (2013 and 2014), and two replications.

Trait	Source of variation	Degree of freedom	Type III SS	Mean square	F statistic	Pr>F
Malv-3-gluc	Genotype	11	0.89	0.08	7.29	0.0001
	Condition	1	4.38	4.38	393.86	<0.0001
	Year	1	0.01	0.01	0.78	0.38
	Genotype*Condition	11	0.79	0.72	6.47	0.001

Table 13A. Tukey test for the trait of total anthocyanins mg/1g total concentration of 12 raw black bean genotypes in 2013 and 2014, with two replications.

Genotype	Year	Mean mg/g			Genotype	Year	Mean			
B-EL1291d	2013	1.697	A		B-EL1291d	2014	2.0235	A		
B11338	2013	1.3505	A	B	B11313	2014	1.4845	A	B	
B11356	2013	1.3125	A	B	OACExpre	2014	1.179	A	B	
NDF09304	2013	1.3115	A	B	B11356	2014	1.177	A	B	
B11285	2013	1.299	A	B	Zenith	2014	1.139	A	B	
B11370	2013	1.2555	A	B	B11370	2014	1.0995	A	B	
Zenith	2013	1.236	A	B	B11285	2014	1.0935	A	B	
OAC Expresso	2013	1.229	A	B	B11338	2014	0.724		B	
B10243	2013	0.9545	A	B	B10243	2014	0.7075		B	
B11313	2013	0.9045	A	B	NDF09304	2014	0.649		B	
ND081343	2013	0.657	A	B	ND081343	2014	0.639		B	
Eclipse	2013	0.493		B	Eclipse	2014	0.4725		B	

[a] *Means followed with the same letter are not significantly different.*

Table 13B. Tukey test for the trait of total anthocyanins mg/1g total concentration of 12 canned black bean genotypes in 2013 and 2014, with two replications.

Genotype	Year	Mean mg/g				Genotype	Year	Mean					
B-EL1291d	2013	0.118	A			B-EL1291d	2014	0.169	A				
Zenith	2013	0.097	A	B		B11313	2014	0.157	A	B			
B11313	2013	0.096	A	B		B11370	2014	0.134	A	B	C		
B11370	2013	0.089	A	B	C	Zenith	2014	0.130	A	B	C	D	
B11338	2013	0.089	A	B	C	Eclipse	2014	0.128	A	B	C	D	
B11356	2013	0.083	A	B	C	OACExpresso	2014	0.117		B	C	D	
OACExpresso	2013	0.082	A	B	C	B11356	2014	0.103			C	D	
Eclipse	2013	0.079	A	B	C	NDF09304	2014	0.103			C	D	
NDF09304	2013	0.076	A	B	C	B11285	2014	0.101			C	D	
B11285	2013	0.070		B	C	B11338	2014	0.095			C	D	
B10243	2013	0.068		B	C	B10243	2014	0.086				D	E
ND081343	2013	0.051			C	ND081343	2014	0.047					E

[a] *Means followed by the same letter are not significantly different.*

Table 14. Anthocyanins Retention percentage for the total anthocyanin data mg/1g total concentration that obtained from 12 black bean genotypes with two years (2013 and 2014).

Genotype	Year	Anthocyanins retention percentage% (AR)	Year	Anthocyanins retention percentage% (AR)
Eclipse	2013	15.92	2014	27.10
B11313	2013	10.61	2014	10.54
Zenith	2013	7.81	2014	11.37
ND081343	2013	7.76	2014	7.28
B11370	2013	7.10	2014	12.19
B10243	2013	7.07	2014	12.16
B-EL1291d	2013	6.92	2014	8.35
OACExpresso	2013	6.63	2014	9.88
B11338	2013	6.59	2014	13.05
B11356	2013	6.29	2014	8.75
NDF09304	2013	5.80	2014	15.87
B11285	2013	5.39	2014	9.24

The mean differences were calculated for each anthocyanin. There were no significant differences among delphinidin-3-glucoside means for either conditions nor years (Table 15A-D). Such a result confirms that delphinidin-3-glucoside is the dominant anthocyanin with the highest concentration in black beans. Means separation for petunidin-3-glucoside in the raw black bean genotypes showed the genotype B11356 to be significantly higher than the rest, while the genotype ND081343 was the lowest (Table 15A-D). The means for petunidin-3-glucoside quantification showed no significant differences in the canned genotypes in 2013, and 2014 for raw and canned beans processed products. The overall concentrations of petunidin-3-glucoside was significantly lower than delphinidin-3-glucoside concentrations among the studied sup-population. Genotype B11356 possessed the highest concentration among all the other genotypes for malvidin-3-glucoside, whereas genotype ND081343 had the lowest for both years and both conditions except for the canned genotypes in 2013 (Table 15A-D). Means separation for malvidin-3-glucoside for the canned genotype in 2013 showed genotype B-EL1291d to be significantly higher than all the other genotypes, while the genotype NDF09304 was the lowest. In 2014, no significant differences were found among raw and canned beans. This result assures the two raw genotypes B11356 and B11370 exhibited superiority for SAQ for all anthocyanins in both years (2013 and 2014). Delphinidin-3-glucoside had the highest concentration among the raw black bean genotypes across the two years, while similar concentrations of delphinidin-3-glucoside, petunidin-3-glucoside, and malvidin-3-glucoside were detected in the canned genotypes. This result suggests the delphinidin-3-glucoside dominance over petunidin-3-glucoside and malvidin-3-glucoside in black beans, and agreed with previous finding of Marles et al., (2010).

Table 15A. Tukey test for the trait of SAQ, for the three anthocyanins (delphindin-3-glucoside, petunidin-3-glucoside, and Malvidin-3-glucoside) concentrations for the 12 raw black bean genotypes in 2013with two replication.

Genotype	Mean D mg/g		Genotype	Mean P mg/g			Genotype	Mean M mg/g			
B11356	1.395	A	B11356	0.950	A		B11356	0.945	A		
NDF09304	1.390	A	Zenith	0.905	A	B	Zenith	0.790	A	B	
B11338	1.310	A	B-EL1291d	0.745	A	B	B11313	0.575	A	B	C
B11285	1.290	A	B11285	0.690	A	B	B-EL1291d	0.480	A	B	C
Zenith	1.225	A	NDF09304	0.640	A	B	B11285	0.395	A	B	C
B10243	1.210	A	B11313	0.625	A	B	NDF09304	0.380	A	B	C
OACExpresso	1.150	A	OACExpresso	0.590	A	B	OACExpresso	0.355	A	B	C
B-EL1291d	1.110	A	B11338	0.550	A	B	B11370	0.320		B	C
B11370	1.085	A	B10243	0.540	A	B	Eclipse	0.310		B	C
B11313	1.060	A	B11370	0.525	A	B	B10243	0.280		B	C
Eclipse	0.995	A	Eclipse	0.520	A	B	B11338	0.235		B	C
ND081343	0.705	A	ND081343	0.205		B	ND081343	0.090			C

[a] *Means followed with the same letter are not significantly different.*
[b]*Mean D, P, and M are the mean values for the anthocyanin delphindin-3-glucoside, petunidin-3-glucoside, and Malvidin-3-glucoside respectively.*

Table 15B. Tukey test for the trait of SAQ, for the three anthocyanins (delphindin-3-glucoside, petunidin-3-glucoside, and Malvidin-3-glucoside) concentrations for the 12 canned black bean genotypes in 2013 with two replications.

Genotype	Mean D mg/g		Genotype	Mean P mg/g		Genotype	Mean M mg/g		
OACExpresso	0.030	A	B-EL1291d	0.025	A	B-EL1291d	0.025	A	
B-EL1291d	0.025	A	Eclipse	0.015	A	B11356	0.015	A	B
Eclipse	0.020	A	OACExpresso	0.015	A	Zenith	0.015	A	B
B11338	0.020	A	Zenith	0.012	A	OACExpresso	0.01	A	B
B11370	0.020	A	B11338	0.010	A	B11313	0.01	A	B
B11313	0.015	A	B11356	0.010	A	Eclipse	0.007	A	B
Zenith	0.015	A	B11370	0.010	A	B11370	0.0065	A	B
ND081343	0.015	A	B11313	0.008	A	B11285	0.0065	A	B
B11285	0.015	A	B11285	0.007	A	B10243	0.0065	A	B
NDF09304	0.010	A	B10243	0.007	A	ND081343	0.0055	A	B
B11356	0.010	A	ND081343	0.006	A	B11338	0.0035	A	B
B10243	0.007	A	NDF09304	0.003	A	NDF09304	0.001		B

[a] *Means followed with the same letter are not significantly different.*
[b]*Mean D, P, and M are the mean values for the anthocyanin delphindin-3-glucoside, petunidin-3-glucoside, and Malvidin-3-glucoside respectively.*

Table 15C. Tukey test for the trait of SAQ, for the three anthocyanins (delphindin-3-glucoside, petunidin-3-glucoside, and Malvidin-3-glucoside) concentrations for the 12 raw black bean genotypes in 2014 with two replications.

Genotype	Mean D		Genotype	Mean P		Genotype	Mean M	
	mg/g			*mg/g*			*mg/g*	
B11370	1.405	A	B11370	0.815	A	B11356	0.780	A
NDF09304	1.170	A	B-EL1291d	0.775	A	B11370	0.635	A
B-EL1291d	1.120	A	B11356	0.700	A	Zenith	0.550	A
OACExpresso	1.110	A	OACExpresso	0.655	A	B11313	0.545	A
B11356	1.095	A	Zenith	0.620	A	B-EL1291d	0.540	A
B11338	1.085	A	NDF09304	0.610	A	B11285	0.450	A
B11285	1.065	A	Eclipse	0.605	A	OACExpresso	0.435	A
ND081343	0.985	A	B11285	0.590	A	Eclipse	0.425	A
Eclipse	0.965	A	B11313	0.565	A	NDF09304	0.375	A
B10243	0.930	A	B11338	0.505	A	B10243	0.315	A
B11313	0.915	A	B10243	0.475	A	B11338	0.245	A
Zenith	0.910	A	ND081343	0.355	A	ND081343	0.170	A

[a] *Means followed with the same letter are not significantly different.*
[b] *Mean D, P, and M are the mean values for the anthocyanin delphindin-3-glucoside, petunidin-3-glucoside, and Malvidin-3-glucoside respectively.*

Table 15D. Tukey test for the trait of SAQ, for the three anthocyanins (delphindin-3-glucoside, petunidin-3-glucoside, and Malvidin-3-glucoside) concentrations for the 12 canned black bean genotypes in 2014 with two replications.

Genotype	Mean D *mg/g*		Genotype	Mean P *mg/g*		Genotype	Mean M *mg/g*	
B10243	0.035	A	OACExpresso	0.025	A	B11356	0.040	A
B11285	0.030	A	B10243	0.025	A	Zenith	0.030	A
OACExpresso	0.030	A	Zenith	0.025	A	B-EL1291d	0.025	A
B11356	0.030	A	B11356	0.025	A	B10243	0.025	A
NDF09304	0.030	A	Eclipse	0.025	A	Eclipse	0.025	A
B11338	0.025	A	B-EL1291d	0.020	A	B11313	0.025	A
Zenith	0.025	A	B11338	0.020	A	B11285	0.020	A
Eclipse	0.025	A	B11285	0.020	A	OACExpresso	0.020	A
B-EL1291d	0.020	A	NDF09304	0.020	A	NDF09304	0.020	A
B11313	0.015	A	B11313	0.015	A	B11338	0.015	A
ND081343	0.010	A	B11370	0.010	A	B11370	0.010	A
B11370	0.010	A	ND081343	0.0035	A	ND081343	0.002	A

[a] *Means followed with the same letter are not significantly different.*
[b] *Mean D, P, and M are the mean values for the anthocyanin delphindin-3-glucoside, petunidin-3-glucoside, and Malvidin-3-glucoside respectively.*

The CORR procedure was used to determine Pearson's correlation coefficients for two groups of traits. The first group is color and L*a*b* values from the previous chapter, and the second is TAC and the three SAQ of the three anthocyanins (delphinidin-3-glucoside, petunidin-3-glucoside, and malvidin-3-glucoside). Two moderate correlations were found between color rating and the two traits TAC and SAQ of malvinidin-3-glucoside for both years 2013 and 2014 (r=-0.58, p=0.0475, r=0.58, p=0.0496, r=0.63 p=0.0287, and r=0.69, p=0.0133) respectively (Table 17A-B). Such correlations suggest an important role of total anthocyanins and the SAQ of malvinidin-3-glucoside in the color concentration of the canned black beans. Also, there were some moderate correlations between the traits of L*a*b* values and the two traits of TAC and the SAQ of malvinidin-3-glucoside. However, the correlations between the above traits were varied across the two years. In 2013, moderate correlations were detected between TAC and the L* a* b* values (r=-0.62, p=0.0313, r=-0.53, p=0.0800, and r=-0.60 p=0.0394) respectively (Figure 22). The correlations between TAC and the a* b* values traits were stronger in 2014 (r=-0.71, p=0.0100 and r=-0.70, p=0.0115) respectively. However, the trait of L* was not correlated to TAC and that is due to the usage of two different machinery systems of obtaining the L*a*b* color values (differences explained earlier in chapter 3). In 2013, the trait of SAQ of malvinidin-3-glucoside was found to be strongly correlated to L* values trait and weakly correlated to a* and b* respectively (r=-0.51, p=0.0935, r=-0.35, p=0.0.2690, and r=-0.42, p=0.1755) (Figure 23). The correlations between the trait of SAQ of malvinidin-3-glucoside and the traits of a* and b* were found to be strong in 2014 (r=-0.69, p=0.0128 and r=-0.70, p=0.0111) respectively. The trait of L* was weakly correlated to SAQ of malvinidin-3-glucoside (r=0.41, p\0.1885). This also assures the impact of TAC and the trait of SAQ of malvinidin-3-glucoside specifically on the color retained by the evaluated canned black bean genotypes.

In a comparison between TAC and the trait of SAQ. In 2013, TAC was strongly correlated to SAQ of pentunidin-3-glucoside and malvinidin-3-glucoside, and weakly correlated to delphinidin-3-glucoside respectively (r=0.72, p=0.0089, r=0.70, p=0.0106, and r=0.43, and p=0.1617). However, in 2014 no correlations were observed between TAC and SAQ of delphinidin-3-glucoside and pentunidin-3-glucoside, while SAQ of malvinidin-3-glucoside was found to be moderately correlated to TAC. There were strong correlations between SAQ of delphinidin-3-glucoside and SAQ pentunidin-3-glucoside across the two years 2013 and 2014 (r=0.71, p=0.0094, r=0.86, p=0.0003) respectively. The trait of SAQ of pentunidin-3-glucoside was to be strongly correlated to SAQ of malvinidin-3-glucoside across the two years 2013 and 2014 (r=0.81, p=0.0016, r=0.80, p=0.0020) respectively. The clear differences observed of the trait of the three SAQ across the two years may suggest a location effect on the concentration of different anthocyanins that involved in black bean color synthesis. This result agrees with the study of Connor et al., (2002c), where significant variances were observed in anthocyanins concentration in blueberries between the same cultivars and different cultivars grown in the same location.

Anthocyanins retention percentage for SAQ experiment showed the superiority of the genotype B10243 across all anthocyanins in 2014 (Table 16). Three different genotypes (OAC Expresso, B-EL1291, and B11356) were capable of retaining their anthocyanins during canning more than the other genotypes in 2013 for all three anthocyanin respectively (delphinidin-3-glucoside, petunidin-3-glucoside, and malvidin-3-glucoside). The anthocyanin malvidin-3-glucoside was found to be retained more than the other two anthocyanins among the genotypes tested. In a comparison between TAC and SAQ anthocyanins retention percentages, TAC has been observed to have higher color retention percentages than SAQ. This finding agreed with several studies results that

other anthocyanins are also involved in the black beans color formation (Zhang et al., 2014; Akond et al., 2011; Takeoka et al., 1997).

It was expected that Zenith would have higher anthocyanins than Eclipse based on color differences (Kelly et al., 2015; Goffnett et al., 2016). This result was proven through the trained rating panel data of the seven traits, although TAC and SAQ data for the two genotypes were interesting. In both experiments (TAC and SAQ) anthocyanins concentrations were higher for Zenith than Eclipse in raw condition, and in some cases the difference was significant. For example, in TAC experiment anthocyanin concentration for Zenith (1.236mg/1g) was significantly higher than Eclipse (0.493mg/1g). However, both genotypes tend to have very similar anthocyanins concentrations in the canned product across all experiments in both years. This finding suggests factors other than anthocyanin content are important for dark black color in Zenith as compared to Eclipse. Anthocyanins degrade thermally during canning process which affects color quality, and may also affect nutritional attributes (Nakitto et al., 2015). Such a stress causes the loss of glycosyl moieties of anthocyanins by hydrolysis of the glycoside bond (Rodrigues and Narciso, 2012). This result in the development of different polyphenolic degradation products based on the stress degree and nature of the heating process (Patras et al., 2010). Nakitto et al., (2015) reported that chemical structure and some organic acids possess a serious impact on anthocyanin degradation mechanism. Markakis, Livingstone, and Fillers (1957) stated that anthocyanins may decompose because of heating into a chalcone structure, and they could even be transformed into a coumarin glucoside derivative with the B-ring being removed. Adams (1973) also mentioned that the aglycon-sugar bond is more unstable. The degradation of anthocyanins leads to color change and ultimately lead to brown products, especially in the presence of oxygen. As in other polyphenols, anthocyanins could be enzymatically degraded in the presence of PPO.

Lower temperature degree may act as an inactivating factor for PPO enzyme which may lead to more anthocyanins retention (Rodrigues and Narciso, 2012)

Table 16. Anthocyanins retention percentage for three anthocyanins concentration mg/1g (delphinidin-3-glucoside, pentuinidin-3-glucoside, and malvinidin-3-glucoside) that obtained from 12 black bean genotypes with two years (2013 and 2014).

	Del-3-gluc 2013	Pent-3-gluc 2013	Malv-3-gluc 2013	Del-3-gluc 2014	Pent-3-gluc 2014	Malv-3-gluc 2014
Genotype	AR%	AR%	AR%	AR%	AR%	AR%
OACExpresso	2.61	2.54	1.83	3.76	5.26	7.94
B-EL1291d	2.25	3.36	2.08	2.82	3.39	4.44
ND081343	2.13	2.68	1.11	2.75	4.03	5.46
Eclipse	2.01	2.89	2.10	2.74	3.57	5.13
B11370	1.84	1.91	2.03	2.70	3.82	4.60
B11338	1.53	1.82	1.49	2.59	4.13	5.88
B11313	1.42	1.20	2.61	2.56	3.28	5.33
Zenith	1.23	1.33	1.90	2.30	3.96	6.12
B11285	1.16	1.02	2.53	1.79	5.58	4.63
NDF09304	0.72	0.47	1.84	1.64	2.66	4.59
B11356	0.72	1.05	2.65	1.02	0.99	1.18
B10243	0.58	1.20	1.96	0.71	1.23	1.58

[a]AR% is the percent of anthocyanins retention.

[b] *Mean Del-3-gluc, Pent-3-gluc, and Malv-3-gluc are the three anthocyanins delphindin-3-glucoside, petunidin-3-glucoside, and Malvidin-3-glucoside respectively.*

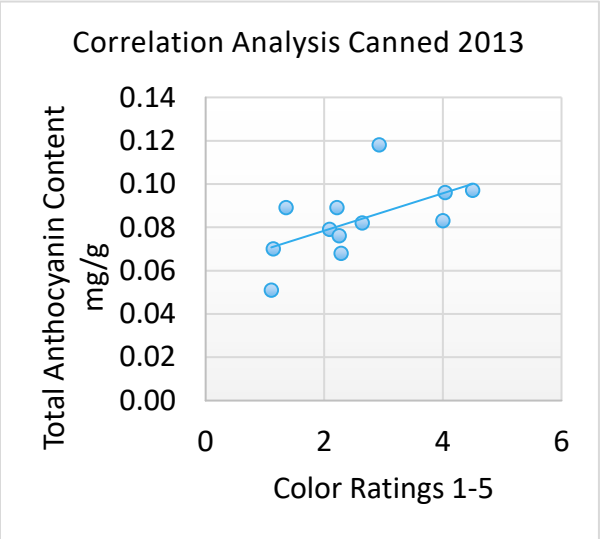
Table 17A. Pearson Correlation for the eight traits (TAC, Delephinidin-3-glucoside, Pentuendin-3-glucoside, Malvinidin-3-glucoside, color, L*, a*, and b*) of the year 2013.

	TAC	Del-3-gluc	Pent-3-gluc	Malv-3-gluc	Color	L*	a*	b*
TAC	.	0.4312	0.7151	0.7039	0.5812	-0.6205	-0.5245	-0.5995
	.	0.1617	0.0089	0.0106	0.0475	0.0313	0.0800	0.0394
Del-3-gluc		.	0.7121	0.3173	-0.1013	0.0761	0.1647	0.1236
		.	0.0094	0.3150	0.7541	0.8141	0.6089	0.7020
Pent-3-gluc			.	0.8049	0.2469	-0.2718	-0.1270	-0.1576
			.	0.0016	0.4392	0.3928	0.6941	0.6247
Malv-3-gluc				.	0.5767	-0.5057	-0.3471	-0.4188
				.	0.0496	0.0935	0.2690	0.1755
Color					.	-0.9661	-0.8937	-0.9480
					.	<.0001	<.0001	<.0001
*L						.	0.9324	0.9407
						.	<.0001	<.0001
*a							.	0.9367
							.	<.0001
*b								.
								.

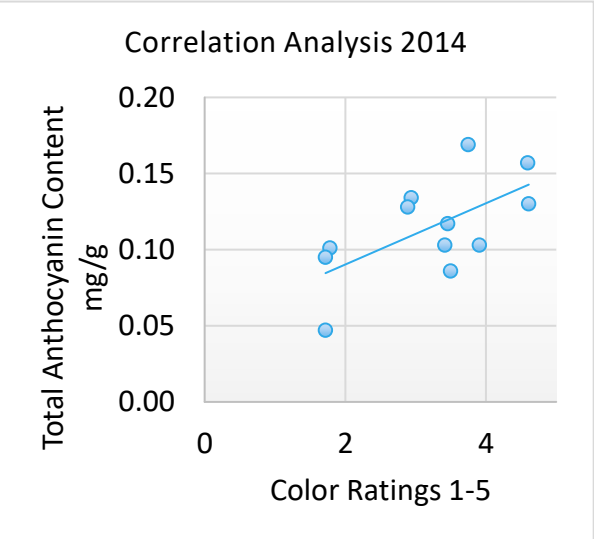
Table 17B. Pearson Correlation for the eight traits (TAC, Delephinidin-3-glucoside, Pentuendin-3-glucoside, Malvinidin-3-glucoside, color, L*, a*, and b*) of the year 2014.

	TAC	Del-3-gluc	Pent-3-gluc	Malv-3-gluc	Color	L*	a*	b*
TAC	.	-0.1466	0.2580	0.4197	0.6283	0.2715	-0.7081	-0.6985
	.	0.6494	0.4182	0.1744	0.0287	0.3933	0.0100	0.0115
Del-3-gluc		.	0.8634	0.5864	0.1192	-0.1118	-0.1293	-0.1671
		.	0.0003	0.0451	0.7121	0.7295	0.6888	0.6037
Pent-3-gluc			.	0.7960	0.4091	0.0568	-0.4367	-0.4678
			.	0.0020	0.1867	0.8609	0.1558	0.1251
Malv-3-gluc				.	0.6885	0.4076	-0.6910	-0.7009
				.	0.0133	0.1885	0.0128	0.0111
Color					.	0.6639	-0.9665	-0.9596
					.	0.0186	<.0001	<.0001
*L						.	-0.4770	-0.4473
						.	0.1169	0.1449
*a							.	0.9985
							.	<.0001
*b								.
								.

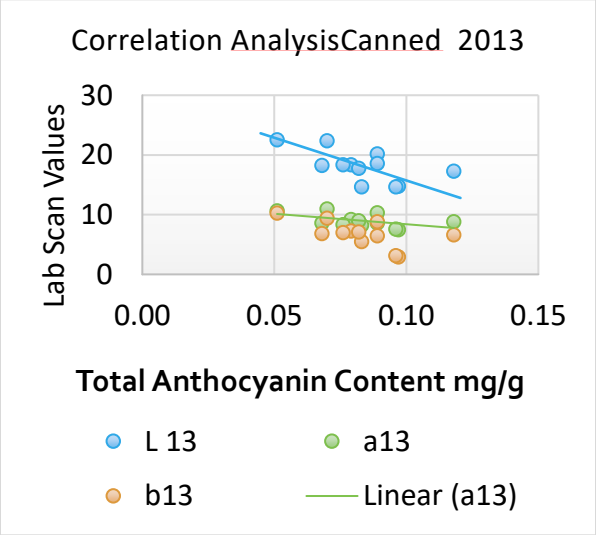
Figure 22. Pearson correlations for the trait of total anthocyanin and the two traits of color and L*, a*, and b* color scans for the two years 2013 and 2014.



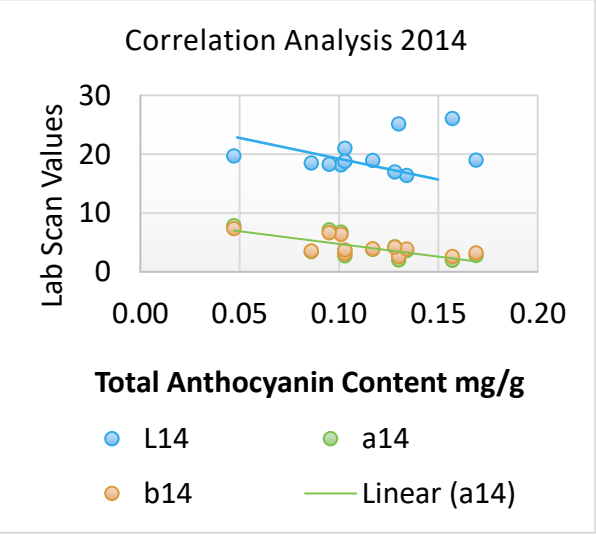
r=0.58
p-value=0.05



r=0.63
p-value=0.03

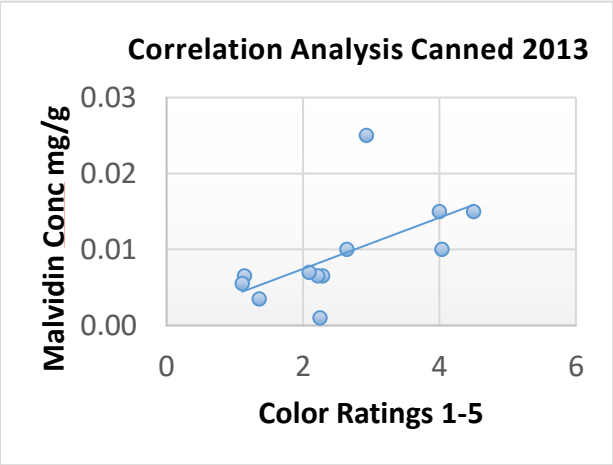


TAC vs. L* r=-0.62, p-value=0.03
TAC vs. a* r=-0.53, p-value=0.08
TAC vs. b* r=-0.60, p-value=0.04

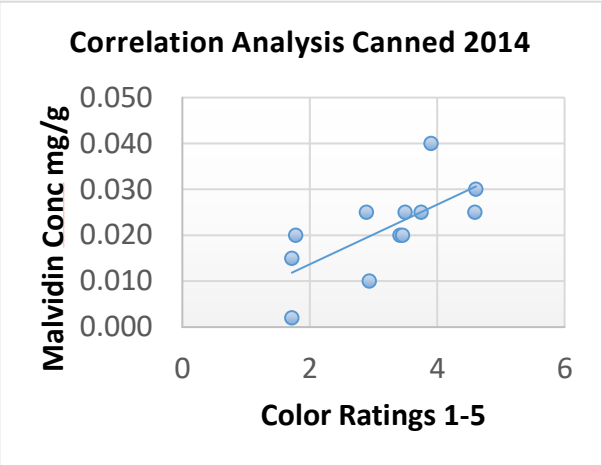


TAC vs. L* r=-0.27, p-value=0.39
TAC vs. a* r=-0.71, p-value=0.01
TAC vs. b* r=-0.70, p-value=0.01

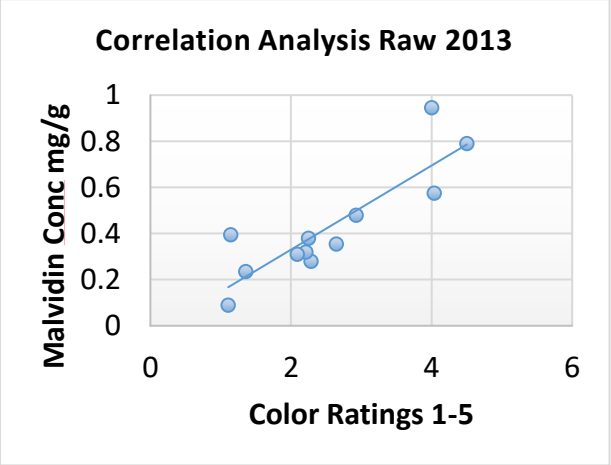
Figure 23. Pearson correlations for the trait of color and malvidin-3-glucoside concentration for the raw and canned genotypes grown for two years 2013 and 2014.



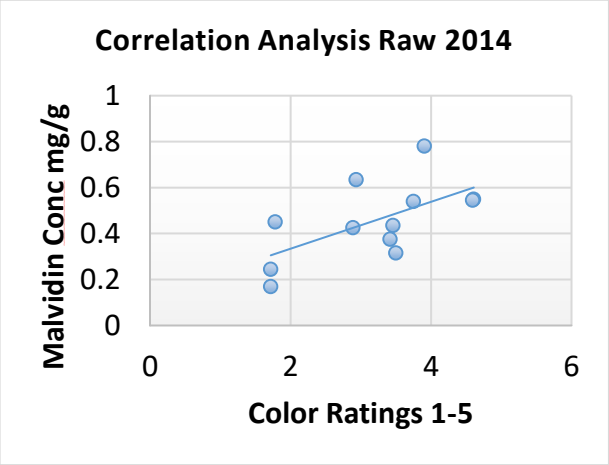
r=0.58
p-value=0.05



r=0.69
p-value=0.01



r=0.87
p-value=0.0003



r=0.62
p-value=0.03

CONCLUSION AND RECOMMENDATION

In this study, concentrations of three anthocyanins (delphinidin-3-glucoside, petunidin-3-glucoside, and malvidin-3-glucoside) were quantified using liquid chromatography-mass spectrometry. Total anthocyanins were also quantified using a UV assay. Both approaches were carried out for 12 genotypes of raw and canned black beans in two years (2013 and 2014). The result of TAC was greater than the SAQ in canned black beans. TAC experiment suggested that the genotype B-EL1291d as the highest in TAC across all the conditions and years. There were two genotypes (B11313 and ND081343) had constant AR across the two years, where the rest of the genotypes were varied for AR in TAC experiment. In the experiment of the three SAQ, the superiority of delphinidin-3-glucoside was conformed as the dominant anthocyanin with the highest concentration in black beans. Two moderate correlations were found between color rating and the two traits TAC and SAQ of malvinidin-3-glucoside for both years 2013 and 2014 ($r=-0.58$, $p=0.0475$, $r=0.58$, $p=0.0496$, $r=0.63$ $p=0.0287$, and $r=0.69$, $p=0.0133$) respectively. Such correlations suggest an important role of total anthocyanins and the SAQ of malvinidin-3-glucoside specifically in the color concentration of the canned black beans. The anthocyanin malvidin-3-glucoside was found to be retained more than the other two anthocyanins among all the sup-population genotypes. In both experiments (TAC and SAQ) anthocyanins concentrations were higher for Zenith than Eclipse in raw condition, and in some cases the difference was significant. However, both genotypes tend to have very similar anthocyanins concentrations in canned condition across all experiments including the two years. This finding suggests different factors other than anthocyanin content are important for dark black color in Zenith as compared to canned color of Eclipse.

APPENDIX

APPENDIX

Table 18. A list of the 69 black bean genotypes that were involved in this study, and each genotype is combined with a picture which has been taken for the canned genotypes in the year of 2014.

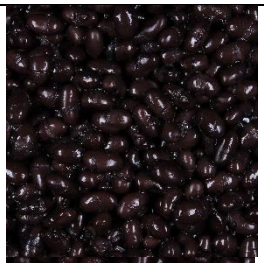
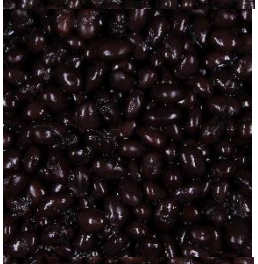
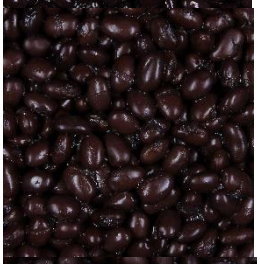
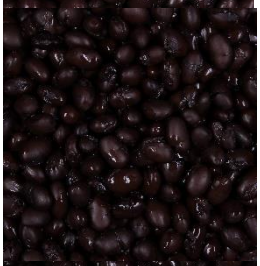
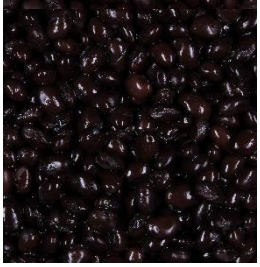
No.	Genotype	
1	B11360	
2	Condor	
3	Black Rhino	
4	B-EL1291d	
5	B-EL01318s	

Table 18 (cont'd)

6	B-EL01270s	
7	B11313	
8	B10238	
9	19365-31	
10	B11356	
11	B10213	

Table 18 (cont'd)

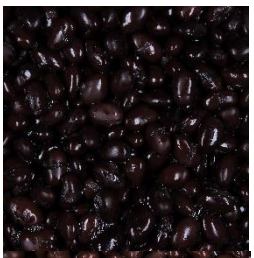
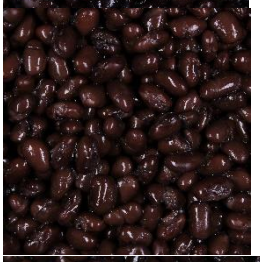
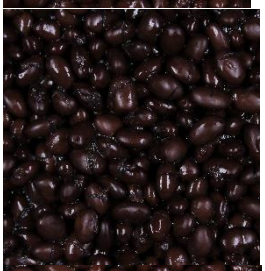
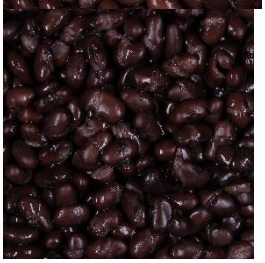
12	SHINYCROW	
13	ND071065	
14	B-EL01270d	
15	101s-2435	
16	TARS-MST1	
17	ND081195	-
18	B10210	

Table 18 (cont'd)

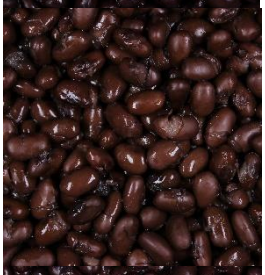
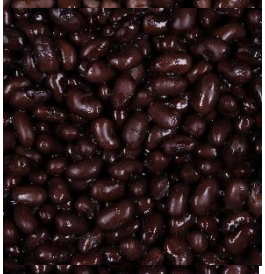
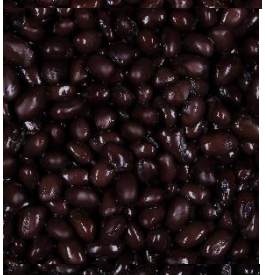
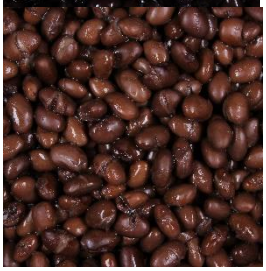
19	B11312	
20	SB-DT1	
21	Domino	
22	Zorro	
23	B-EL01260d	
24	ND081343	

Table 18 (cont'd)

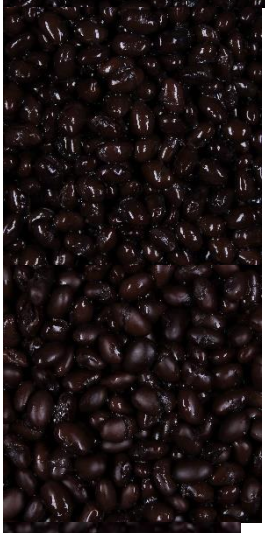
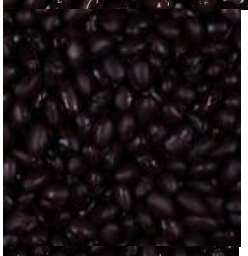
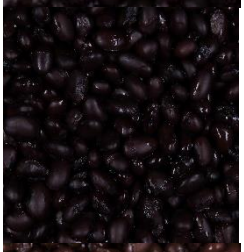
25	B-EL01268s	
26	Black Magic	
27	BK 11-5	
28	B11363	
29	B11329	
30	ND071089	

Table 18 (cont'd)

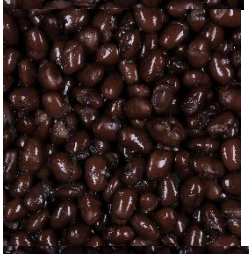
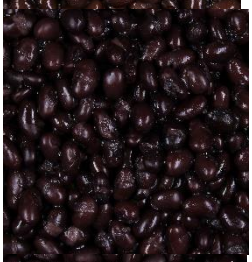
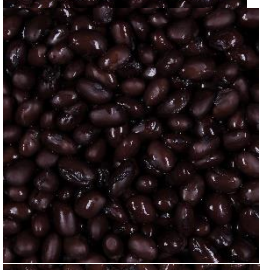
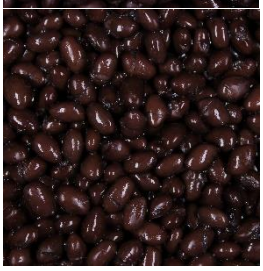
31	ND071244	
32	ND071257	
33	DPC-4	
34	Harblack Opaque	
35	B09119	
36	Eclipse	

Table 18 (cont'd)

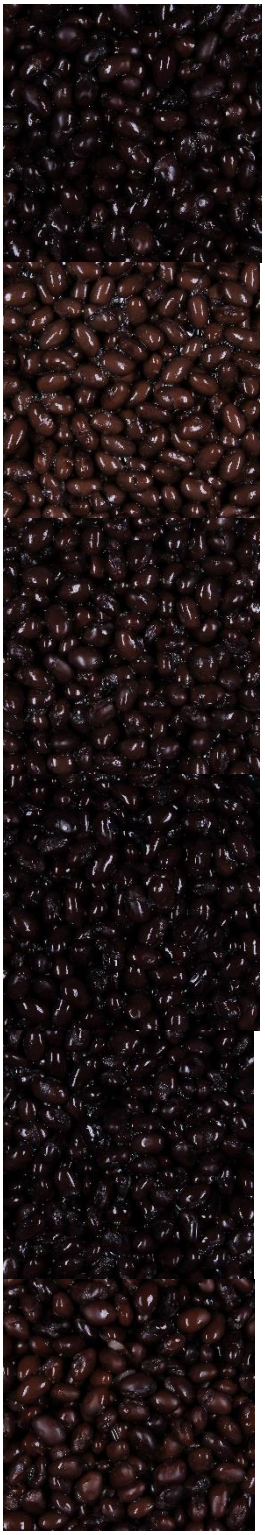
37	NE14-12-28	
38	B11259	
39	T-39	
40	B-EL0309	
41	B-EL01294s	
42	ND081256	

Table 18 (cont'd)

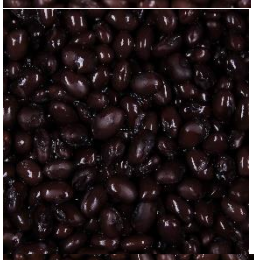
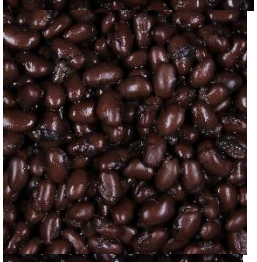
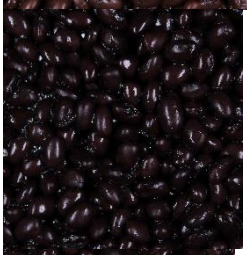
43	B11588	
44	B11310	
45	BK 11-8	
46	07055-4	
47	B10244 (Zenith)	
48	B11364	

Table 18 (cont'd)

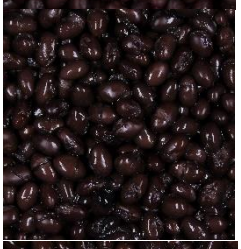
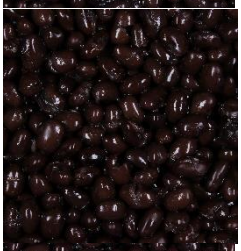
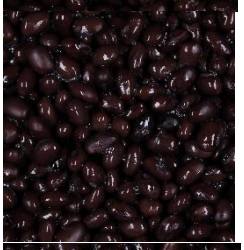
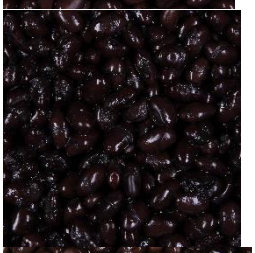
49	B11370	
50	B10243	
51	BK 11-1	
52	B09165	
53	B-EL01335s	
54	NDFD9302	

Table 18 (cont'd)

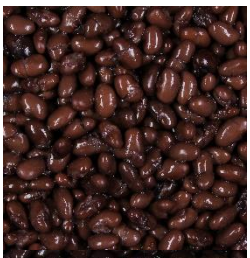
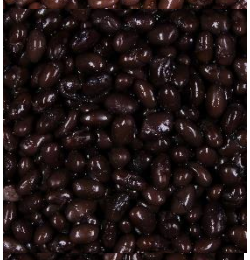
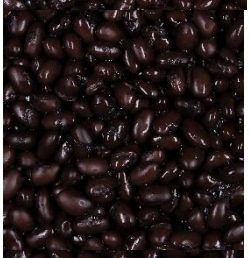
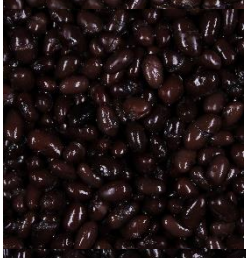
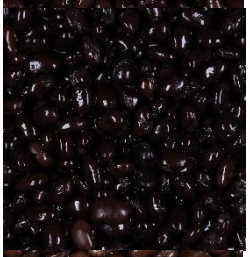
55	B11338	
56	NDF09304	
57	OAC Jet	
58	OACExpresso	
59	B-EL01291s	
60	ND081339	

Table 18 (cont'd)

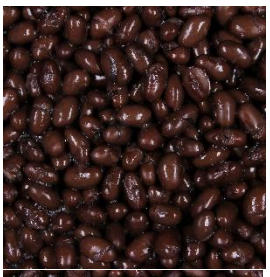
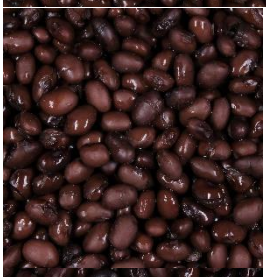
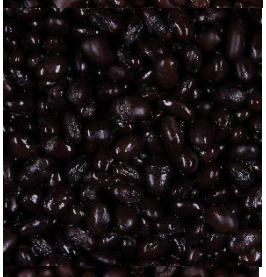
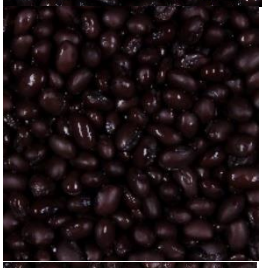
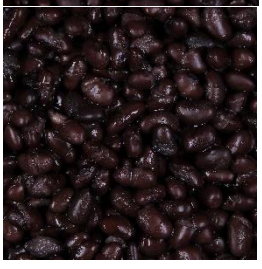
61	BK9-2	
62	B11285	
63	Aifiwuriti	
64	B-EL01308s	
65	B11311	
66	ND081111	

Table 18 (cont'd)

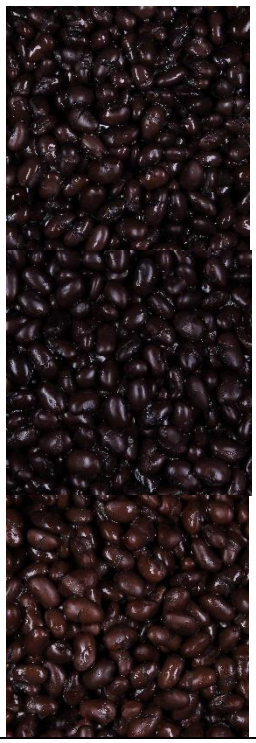
67	B11322	
68	B11309	
69	ICB-3	

Table 19A. Means and standard deviations for all the major and sub branches (including genotypes averages) that obtained from the neighbor joining tree for the seven traits (seed yield, seed weight, appearance, color, L*, a*, and b*) of 69 black bean genotypes that obtained from the year 2013.

NJ Group	Genotypes 2013	Yield	Seed Weight	Appearance	Color	L*	a*	b*
A1-1	B11360	2335	18.3	2.25	3.50	17.55	8.21	5.63
	DPC-4	1566	18.0	3.00	3.50	15.08	7.87	4.02
	B11370	2149	18.6	2.75	1.75	18.57	8.59	6.50
	HarblackOpaque	2221	18.2	2.00	3.50	16.33	9.05	5.11
	OAC Jet	2133	19.2	2.25	2.75	16.80	8.21	6.55
	Average	2081	18.4	2.45	3.00	16.87	8.39	5.56
	Standard Deviation	267	0.4	0.37	0.69	1.17	0.40	0.94
A1-2	Condor	2387	17.8	3.25	3.50	15.16	8.32	4.48
	B11311	1819	20.3	3.50	3.25	17.35	9.13	4.90
	B10210	3136	19.8	2.75	1.50	19.04	10.51	8.74
	B11313	2541	17.3	2.75	4.00	14.65	7.59	3.14
	B11356	2.085	13.6	2.50	3.50	14.69	8.25	5.51
	Zenith	2840	18.5	3.25	4.75	14.77	7.50	2.95
	B11363	2735	17.0	3.00	3.25	14.70	8.44	4.08
	B11312	2441	19.7	3.50	3.25	15.89	8.56	4.76
	B11588	2431	21.6	1.75	1.50	20.20	10.25	8.31
	B11310	2353	20.7	3.00	3.25	15.89	9.17	5.16
	Zorro	2334	18.0	2.75	3.00	16.94	9.14	5.20
	B10213	2313	17.6	2.25	2.00	19.29	9.33	7.14
	B11309	2178	18.7	1.50	3.75	14.39	8.57	4.55
	B11364	2164	22.1	3.00	4.25	14.71	7.67	3.56
	B11322	2012	13.6	1.75	2.75	15.91	7.99	5.03
	07055-4	1166	20.2	1.75	1.50	20.36	9.93	8.64
	Average	2323	18.5	2.64	3.06	16.50	8.77	5.38
	Standard Deviation	440	2.4	0.64	0.96	2.05	0.89	1.80
A2-1	ND071257	2583	20.6	1.50	1.25	19.93	10.46	8.88
	19365-31	2275	21.4	1.50	2.25	18.05	8.32	6.70
	AifiWuriti	2147	19.7	2.50	2.00	16.98	8.41	6.19
	ND071244	2134	19.9	1.50	1.75	19.13	9.70	7.75
	BK11-5	2108	21.1	1.50	2.00	17.83	8.59	6.98
	BK11-1	2079	18.9	2.00	2.00	17.41	8.45	6.71
	SB-DT1	2026	18.7	1.50	1	19.87	9.72	8.85
	NE14-12-	2006	20.6	2.75	3.50	14.31	7.43	4.82
	ND071065	2005	20.0	1.50	2.50	18.35	8.83	7.30
	BK11-8	1797	18.8	3.00	2.25	18.27	9.04	7.41
	TARS-MST	1724	18.6	1.50	2.50	18.36	8.23	5.79
	Black Rhino	1669	22.4	2.00	1.75	17.21	8.59	6.34

Table 19A (cont'd)

	OAC Expresso	1652	19.1	1.50	3.00	17.81	8.97	7.13
	ICB-3	1363	22.4	1.75	1.75	17.99	9.35	7.56
	Average	1969	20.1	1.86	2.11	17.96	8.86	7.03
	Standard Deviation	294	1.3	0.51	0.62	1.32	0.73	1.05
A2-2	B-EL01268s	2084	17.8	2.61	3.21	16.66	8.08	6.25
	B-EL01270s	1992	20.6	3.11	3.68	14.69	8.20	4.80
	B-EL01291s	1797	18.5	2.93	3.46	15.79	7.45	5.21
	B-EL01294s	1747	18.8	3.07	4.46	13.98	8.02	3.89
	B-EL01308s	2451	20.3	3.46	4.36	14.10	7.31	3.74
	B-EL01318s	1179	17.8	2.46	3.21	15.97	8.37	5.82
	B-EL01335s	2225	19.7	2.61	4.04	15.42	7.40	4.05
	SHINYCROW	1734	22.0	3.04	3.82	14.45	7.52	4.23
	B-EL1291d	2699	19.0	2.00	2.25	17.28	8.81	6.66
	B-EL0309	1952	18.2	2.00	3.75	14.34	7.84	3.65
	B-EL01260d	970	18.4	2.75	3.50	14.16	7.36	3.79
	Average	1894	19.2	2.73	3.61	15.17	7.85	4.73
	Standard Deviation	480	1.3	0.44	0.58	1.08	0.47	1.04
A2-3	Black Magic	2619	18.7	2.50	3.00	16.56	8.61	6.73
	BK9-2	2452	19.7	2.25	1.50	20.02	9.76	8.59
	T-39	2194	17.6	2.50	3.25	16.90	8.14	6.01
	Domino	1991	20.8	2.00	2.50	16.65	8.96	5.26
	Eclipse	1757	17.8	3.00	2.50	18.43	8.94	7.38
	ND081295	1290	19.8	2.50	1.50	20.09	9.33	7.63
	Average	2050	19.0	2.46	2.38	18.11	8.96	6.93
	Standard Deviation	441	1.1	0.30	0.67	1.51	0.51	1.09
A2-4	ND071089	2282	19.1	1.75	1.50	19.08	8.98	7.73
	NDF09302	2123	18.2	2.75	1.75	19.43	8.81	7.66
	NDF09304	1833	17.6	2.50	1.75	18.36	8.36	7.01
	Average	2079	18.3	2.33	1.67	18.96	8.72	7.47
	Standard Deviation	185	0.6	0.42	0.12	0.45	0.26	0.32
B	ND081343	1684	18.6	2.00	1.00	22.53	10.65	10.29
C	B11285	2212	16.7	2.75	1.00	22.41	10.97	9.41
	B11259	2046	16.4	1.75	1.50	20.75	9.07	7.68
	ND081256	1541	24.1	2.50	1.75	20.00	10.09	7.83
	B11338	1383	16.1	2.50	1.00	20.19	10.32	8.83
	Average	1795	18.3	2.38	1.31	20.84	10.11	8.44
	Standard Deviation	343	3.4	0.38	0.32	0.95	0.68	0.71
D	ND081111	1476	18.0	2.00	1.75	19.46	9.41	8.08

Table 19A (cont'd)

B10243	1357	20.5	3.00	1.75	18.26	8.63	6.48
Average	1417	19.2	2.50	1.75	18.86	9.02	7.28
Standard Deviation	59	1.3	0.50	0.00	0.60	0.39	0.80

Table 19B. Means and standard deviations for all the major and sub branches (including genotypes averages) that obtained from the neighbor joining tree for the seven traits (seed yield, seed weight, appearance, color, L*, a*, and b*) of 69 black bean genotypes that obtained from the year 2014.

NJ Group	Genotypes 2014	Yield	Seed Weight	Appearance	Color	L*	a*	b*
A1-1	B11360	3727	20.5	2.50	2.50	20.59	2.60	3.03
	OACJet	3403	20.7	4.00	4.00	18.42	3.71	4.04
	Harblack	2910	18.9	3.50	3.50	18.40	3.53	3.58
	B11370	2625	18.0	3.25	3.25	16.33	3.55	3.83
	DPC-4	1209	20.5	3.75	3.75	19.80	3.11	3.44
	Average	2775	19.7	3.40	3.40	18.71	3.30	3.58
	Standard Deviation	871	1.1	0.51	0.51	1.45	0.40	0.35
A1-2	B10210	4716	27.4	4.50	4.50	16.72	4.91	4.74
	B11363	3861	21.8	4.25	4.25	20.03	2.05	2.53
	B11364	3568	21.8	3.50	3.50	21.48	2.63	2.95
	B11329	3541	18.2	3.75	3.75	21.69	1.86	2.47
	B11313	3503	19.3	3.75	3.75	26.04	1.97	2.58
	101s-243	3331	21.3	2.50	2.50	16.29	4.98	4.83
	B11309	3221	18.3	3.25	3.25	21.44	2.30	2.84
	B11356	3139	19.2	3.25	3.25	20.97	2.70	3.08
	Zenith	3047	22.3	4.00	4.00	24.87	1.94	2.59
	B09165	2950	22.6	4.25	4.25	17.83	3.47	3.63
	B11588	2891	24.9	3.00	3.00	18.09	4.59	6.37
	B11310	2799	22.2	4.00	4.00	19.99	3.10	3.38
	B09119	2792	20.5	4.25	4.25	18.43	3.46	3.76
	Condor	2624	21.3	3.75	3.75	25.03	2.45	3.08
	B11322	2560	18.1	2.75	2.75	19.49	3.15	3.54
	B11311	2476	18.1	3.75	3.75	18.35	3.13	3.48
	B10213	2430	19.2	4.00	4.00	17.42	3.97	4.09
	B10238	2409	18.8	4.25	4.25	17.64	3.75	3.81
	Zorro	2324	21.1	3.50	3.50	18.00	3.73	3.86
	07055-4	1499	23.3	3.25	3.25	18.34	5.15	5.12
	Average	2984	21.0	3.68	3.68	19.91	3.26	3.64
	Standard Deviation	664	2.4	0.53	0.53	2.74	1.03	0.98
A2-1	BlackRhi	3856	24.1	3.00	3.00	17.70	3.58	3.71
	ND071257	3534	21.8	3.50	3.50	16.72	4.68	4.44
	NE14-12-	3153	24.2	3.25	3.25	23.59	2.72	3.02
	ND071244	3092	21.3	3.25	3.25	17.18	4.55	4.49
	SB-DT1	2814	26.3	2.50	2.50	17.20	5.59	5.41
	TARS-MST	2719	21.8	2.00	2.00	19.26	3.69	3.95
	BK11-5	2713	22.1	3.50	3.50	18.57	3.61	3.69
	OACExpre	2658	20.0	3.50	3.50	18.84	3.90	3.99
	BK11-1	2219	21.0	3.75	3.75	19.29	3.57	3.75
	ND071065	1884	21.3	3.00	3.00	16.23	4.67	4.48

Table 19B (cont'd)

	BK11-8	1828	22.0	4.00	4.00	17.77	3.97	3.97
	AifiWuri	1750	19.6	2.75	2.75	17.96	3.80	4.08
	19365-31	1416	23.8	3.50	3.50	17.57	3.86	3.95
	ICB-3	1347	21.3	2.50	2.50	16.87	4.56	4.53
	Average	2499	22.2	3.14	3.14	18.20	4.05	4.10
	Standard Deviation	750	1.7	0.53	0.53	1.75	0.67	0.53
A2-2	B-EL01260d	2420	20.7	4.06	4.72	28.30	1.44	2.55
	B-EL01268s	1839	18.7	3.72	4.22	25.37	1.91	2.71
	B-EL01270d	3636	22.7	3.34	3.44	20.59	3.15	3.53
	B-EL01270s	2874	26.2	3.94	4.66	26.56	1.54	2.36
	B-EL01291s	2686	19.2	3.69	4.31	26.94	1.98	2.82
	B-EL01294s	2582	19.3	3.94	4.72	27.31	1.85	2.78
	B-EL01308s	2489	20.9	3.94	4.28	24.92	1.97	2.76
	B-EL01318s	2430	18.8	4.06	4.06	24.77	2.26	2.96
	B-EL01335s	2858	22.7	3.34	4.53	24.95	2.06	2.79
	B-EL0309	2027	21.4	3.91	4.59	26.73	1.93	2.85
	B-EL1291d	2623	18.6	3.63	3.75	18.96	2.76	3.19
	SHINYCROW	2316	22.1	3.81	4.13	22.72	2.44	3.01
	Average	2565	20.9	3.78	4.28	24.84	2.11	2.86
	Standard Deviation	434	2.2	0.24	0.38	2.69	0.46	0.29
A2-3	BlackMag	3425	19.4	3.50	3.50	22.81	2.56	3.28
	T-39	3062	20.0	3.50	3.50	19.95	2.96	3.27
	BK9-2	2777	20.0	3.00	3.00	16.39	5.56	5.22
	Eclipse	2773	19.7	2.75	2.75	16.43	4.30	4.26
	ND081295	2583	25.5	2.25	2.25	17.65	4.47	4.50
	Domino	1844	20.2	3.75	3.75	17.13	4.05	4.20
	Average	2744	20.8	3.13	3.13	18.39	3.98	4.12
	Standard Deviation	483	2.1	0.52	0.52	2.31	0.99	0.68
A2-4	NDF09302	2619	18.5	3.25	3.25	18.51	4.59	4.66
	NDF09304	2601	19.8	3.75	3.75	18.95	3.58	3.72
	Average	2610	19.1	3.50	3.50	18.73	4.09	4.19
	Standard Deviation	8	0.7	0.25	0.25	0.22	0.505	0.47
B	ND081343	2789	20.4	3.25	3.25	19.65	7.79	7.30
C	B11285	3430	19.2	3.75	3.75	18.16	6.74	6.37
	B11259	3345	19.1	3.75	3.75	17.25	5.17	5.07
	B11338	3329	17.3	2.75	2.75	18.27	7.12	6.62
	ND081256	2380	19.8	4.25	4.25	17.04	4.15	4.10
	Average	3121	18.8	3.63	3.63	17.68	5.80	5.54
	Standard Deviation	429	1.0	0.54	0.54	0.54	1.20	1.02
D	ND081339	3117	26.4	3.25	3.25	18.53	5.43	5.44

Table 19B (cont'd)

ND081111	3009	22.3	3.00	3.00	18.72	3.80	4.12
B10243	2567	21.5	3.25	3.25	18.47	3.41	3.54
Average	2898	23.4	3.17	3.17	18.57	4.21	4.37
Standard Deviation	238	2.2	0.12	0.12	0.11	0.87	0.80

Figure 24. QQ plot for the trait of seed yield values averaged across 2013 of 61 black bean genotypes with 2800 SNP (>0.02 minor allele frequency) and mapping conducted with multiple linear model (MLM) using a kinship matrix and 3 principal components to account for population structure.

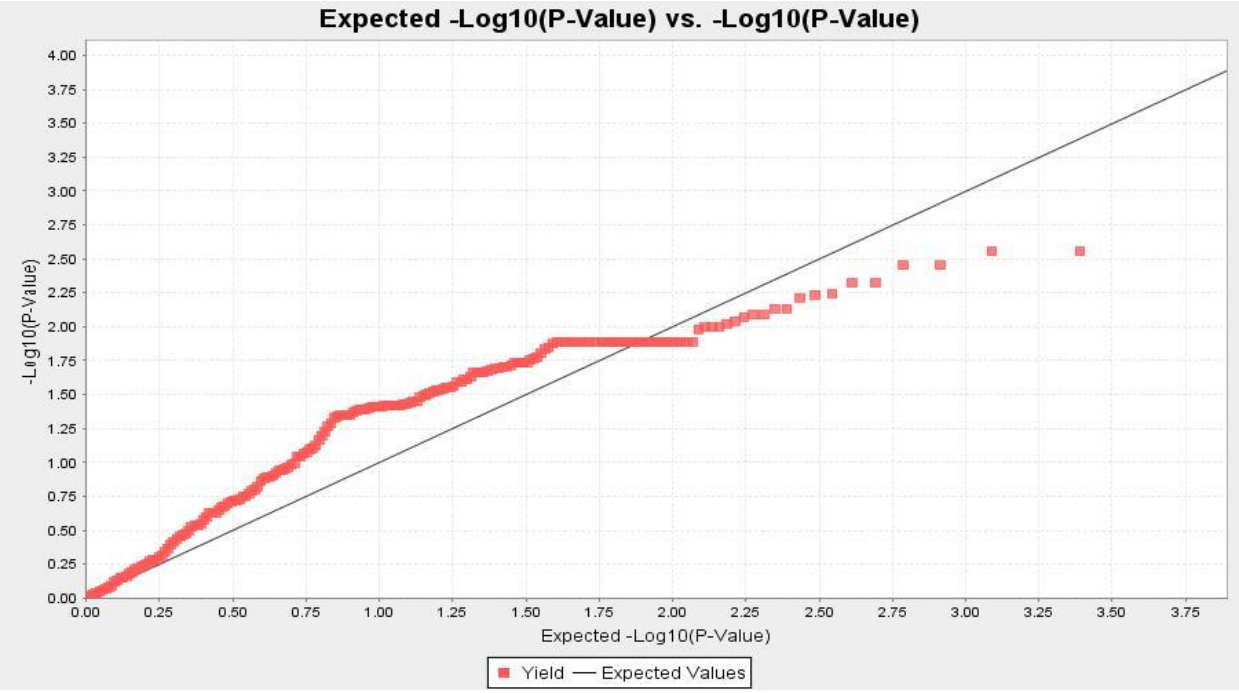


Figure 25. QQ plot for the trait of seed weight values averaged across 2013 of 61 black bean genotypes with 2800 SNP (>0.02 minor allele frequency) and mapping conducted with multiple linear model (MLM) using a kinship matrix and 7 principal components to account for population structure.

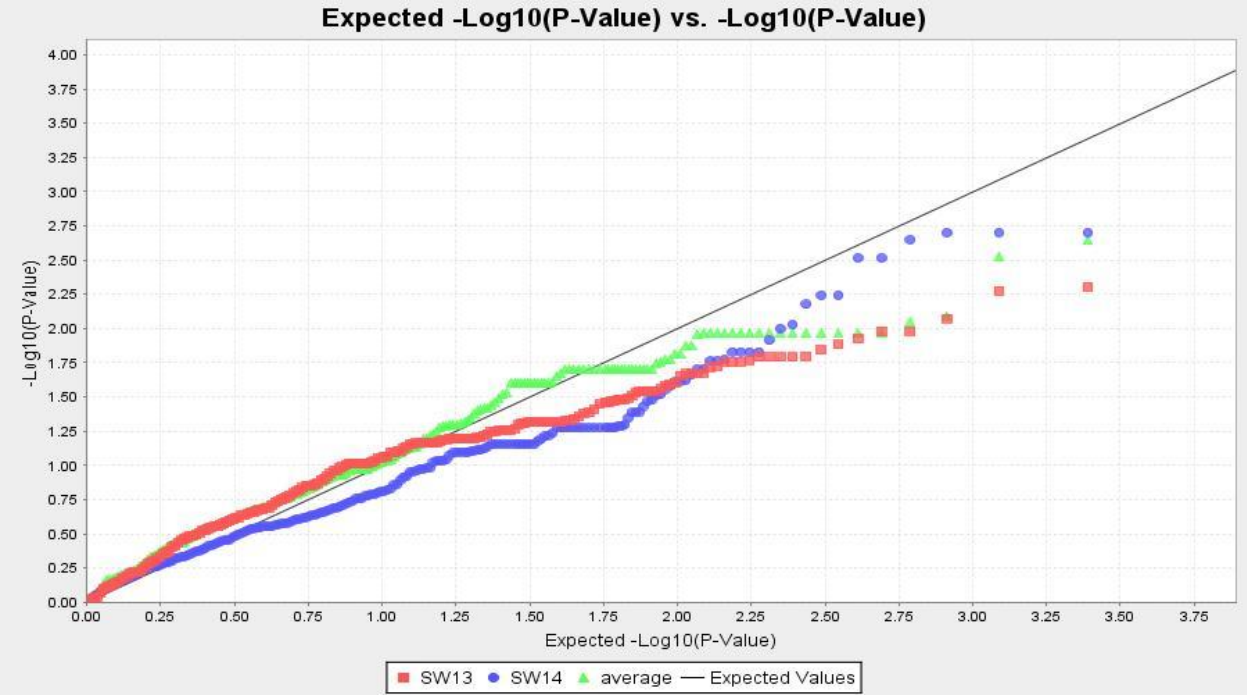


Figure 26. QQ plot for the trait of appearance ratings averaged across 2013 of 61 black bean genotypes with 2800 SNP (>0.02 minor allele frequency) and mapping conducted with multiple linear model (MLM) using a kinship matrix and 2 principal components to account for population structure.

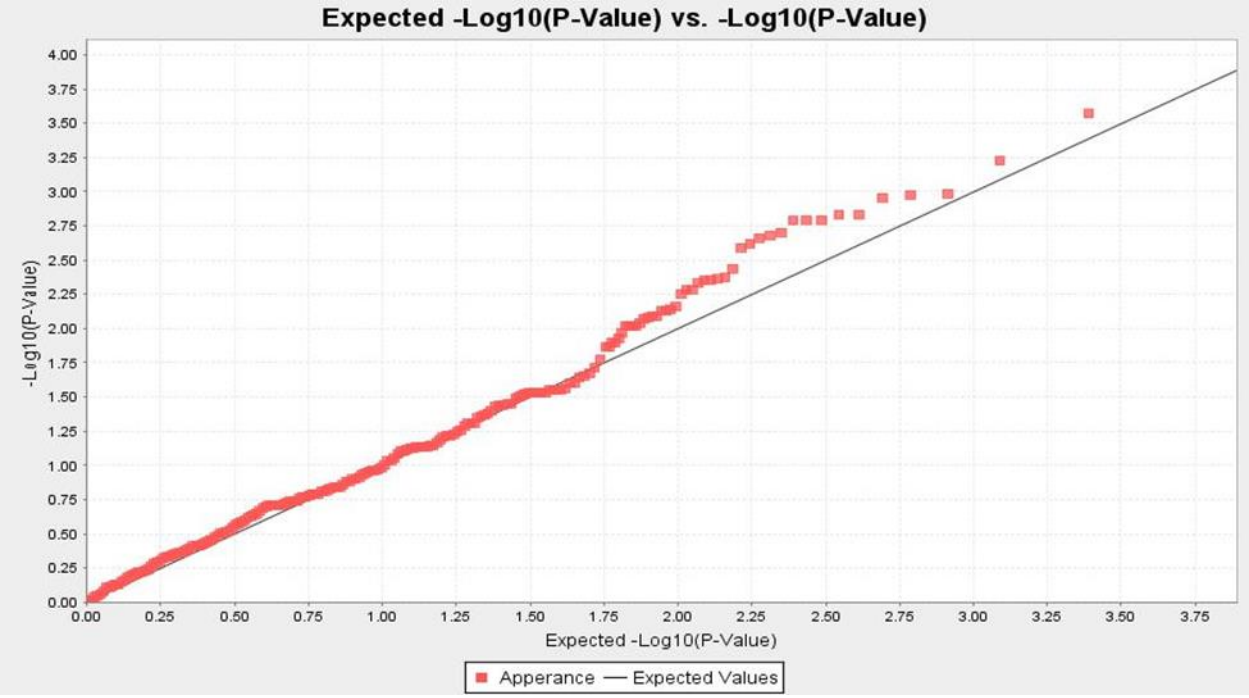


Figure 27. QQ plot for the trait of color ratings averaged across 2013 of 61 black bean genotypes with 2800 SNP (>0.02 minor allele frequency) and mapping conducted with multiple linear model (MLM) using a kinship matrix.

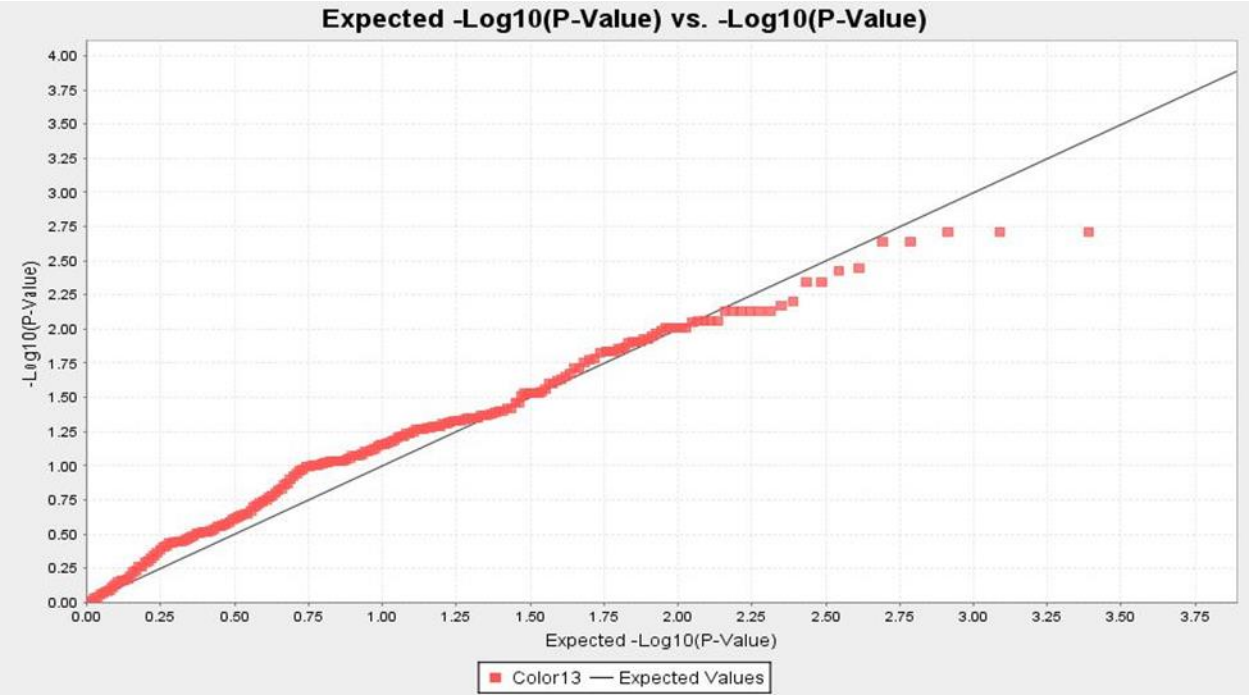


Figure 28. QQ plot for the trait of L* values averaged across 2013 of 61 black bean genotypes with 2800 SNP (>0.02 minor allele frequency) and mapping conducted with multiple linear model (MLM) using a kinship matrix and 7 principal components to account for population structure.

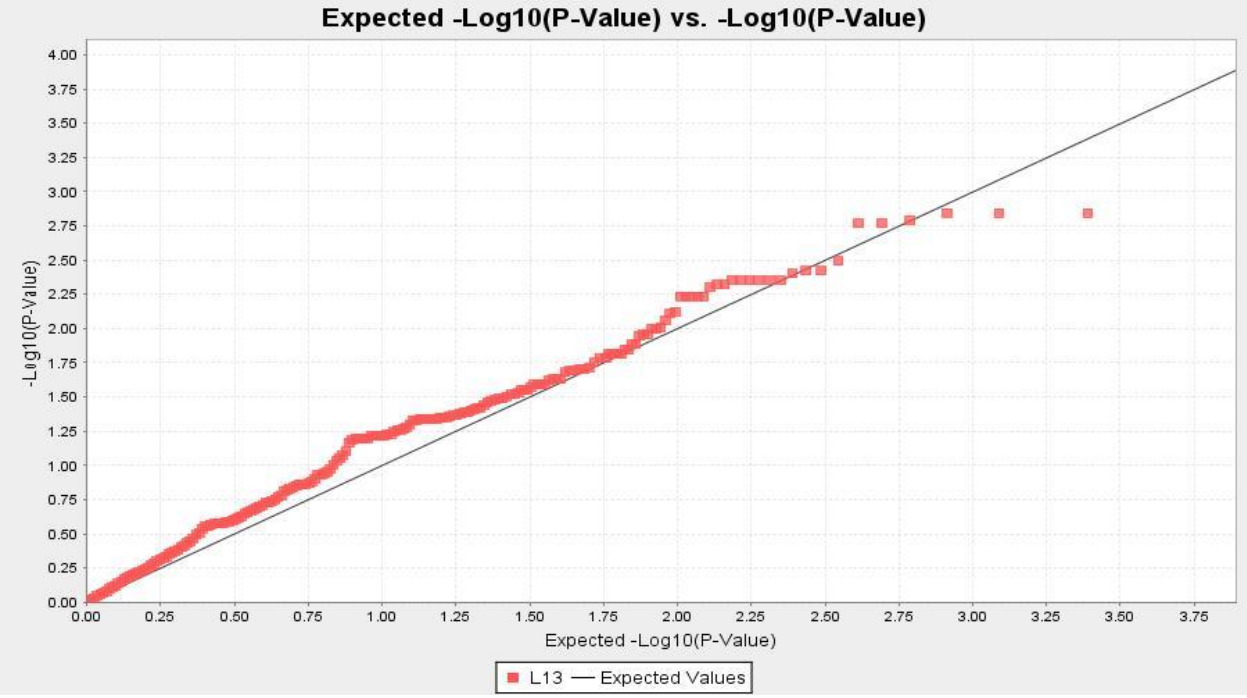


Figure 29. QQ plot for the trait of a* values averaged across 2013 of 61 black bean genotypes with 2800 SNP (>0.02 minor allele frequency) and mapping conducted with multiple linear model (MLM) using a kinship matrix and 3 principal components to account for population structure.

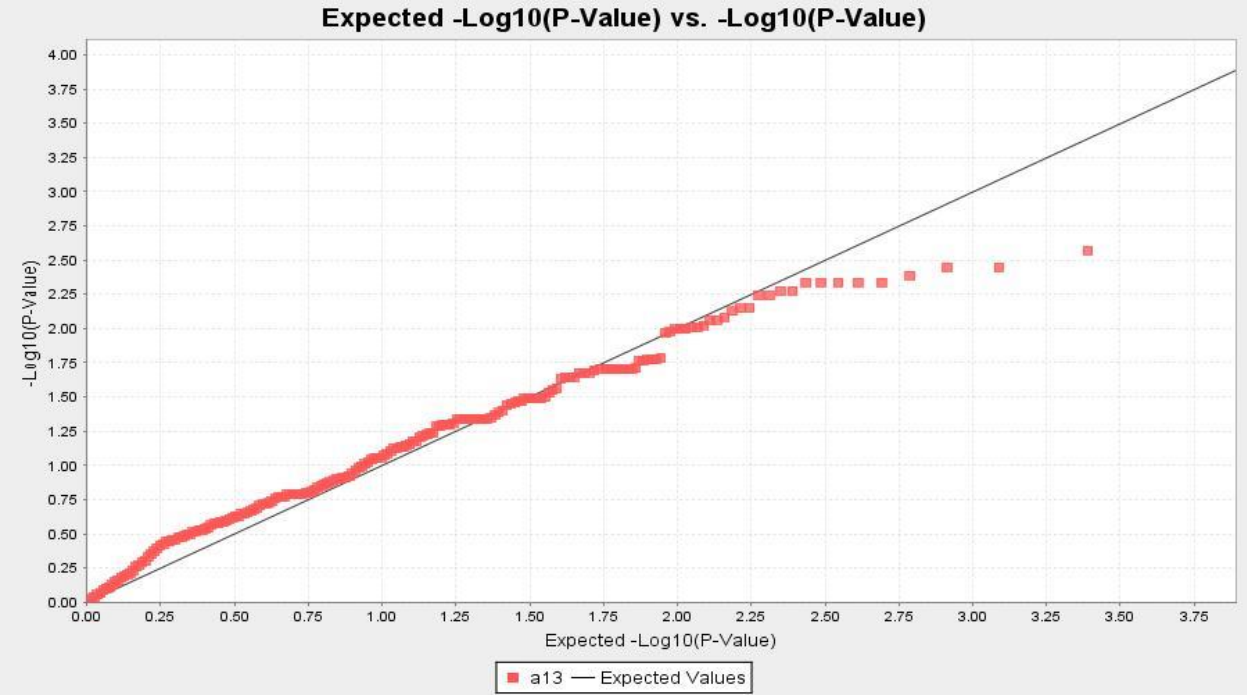


Figure 30. QQ plot for the trait of seed weight values averaged across 2013 of 61 black bean genotypes with 2800 SNP (>0.02 minor allele frequency) and mapping conducted with multiple linear model (MLM) using a kinship matrix and 3 principal components to account for population structure.

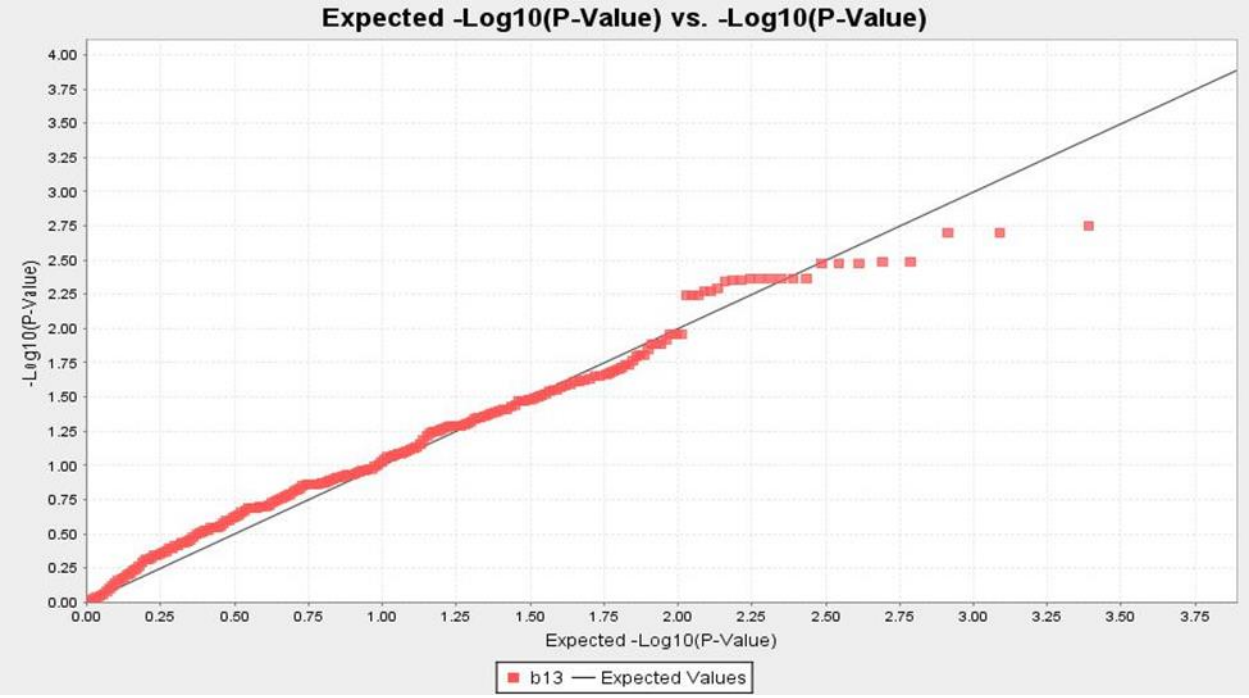


Figure 31. QQ plot for the trait of seed yield values averaged across 2014 of 61 black bean genotypes with 2800 SNP (>0.02 minor allele frequency) and mapping conducted with multiple linear model (MLM) using a kinship matrix.

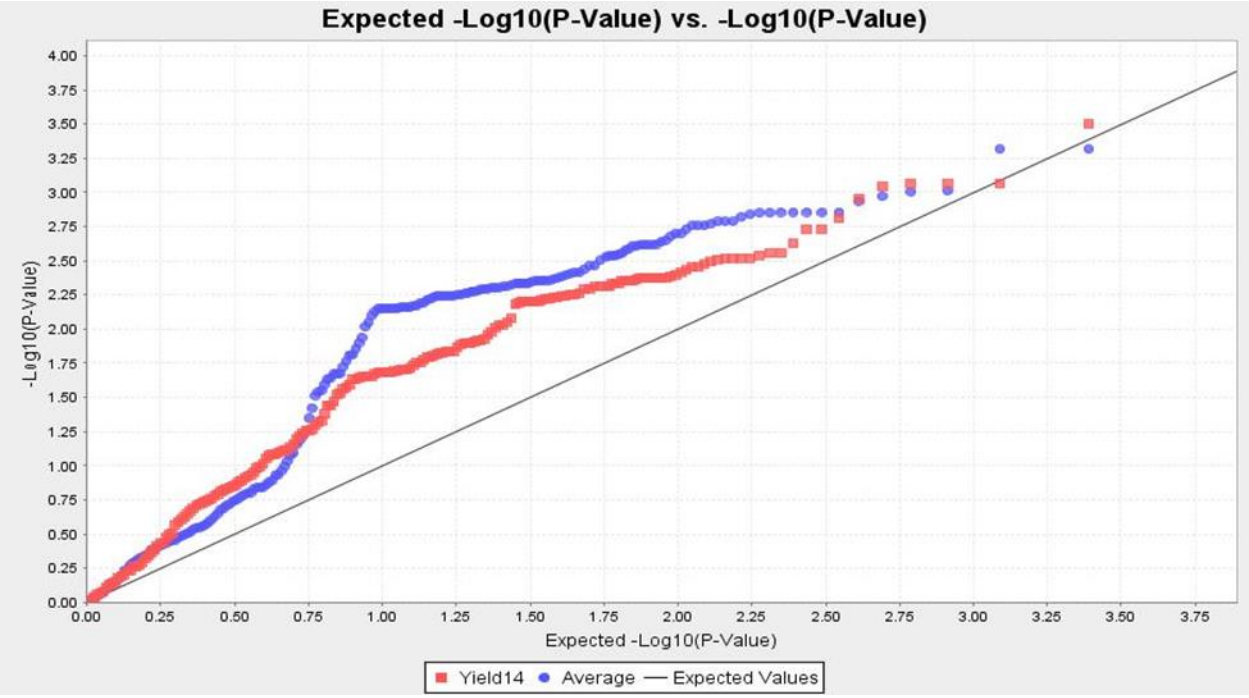


Figure 32. QQ plot for the trait of seed weight values averaged across 2014 of 61 black bean genotypes with 2800 SNP (>0.02 minor allele frequency) and mapping conducted with multiple linear model (MLM) using a kinship matrix and 7 principal components to account for population structure.

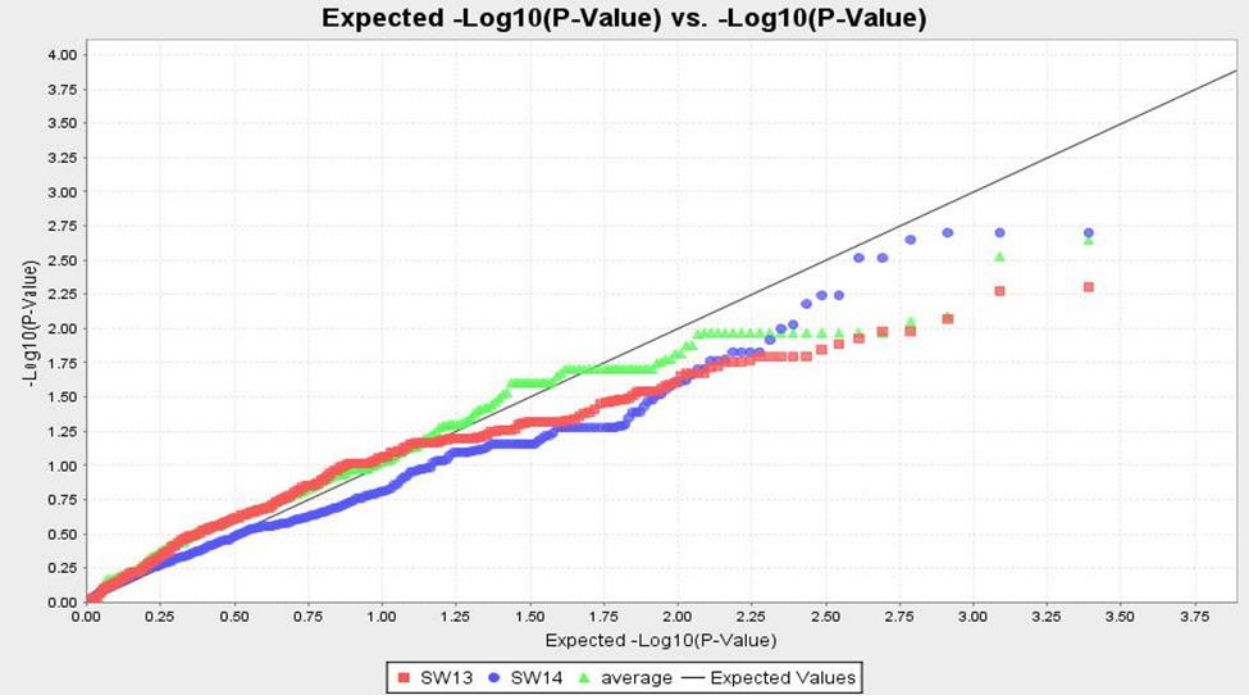


Figure 33. QQ plot for the trait of appearance ratings averaged across 2014 of 61 black bean genotypes with 2800 SNP (>0.02 minor allele frequency) and mapping conducted with multiple linear model (MLM) using a kinship matrix and 2 principal components to account for population structure.

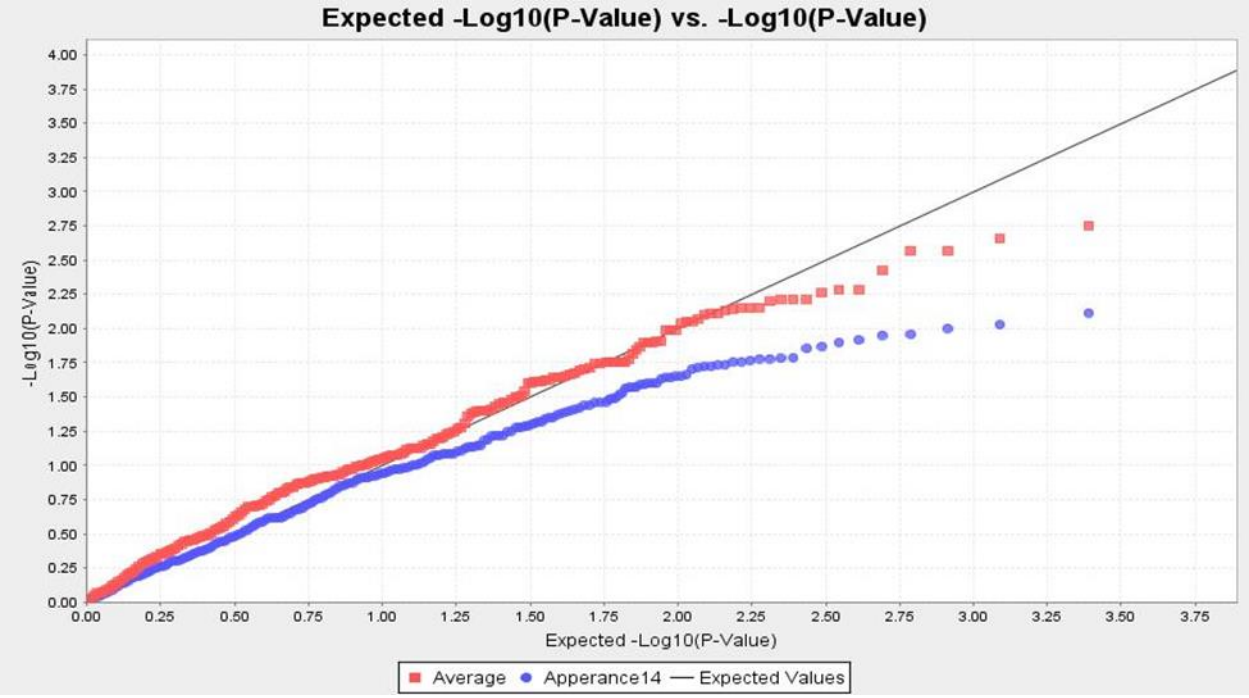


Figure 34. QQ plot for the trait of color ratings averaged across 2014 of 61 black bean genotypes with 2800 SNP (>0.02 minor allele frequency) and mapping conducted with multiple linear model (MLM) using a kinship matrix and 7 principal components to account for population structure.

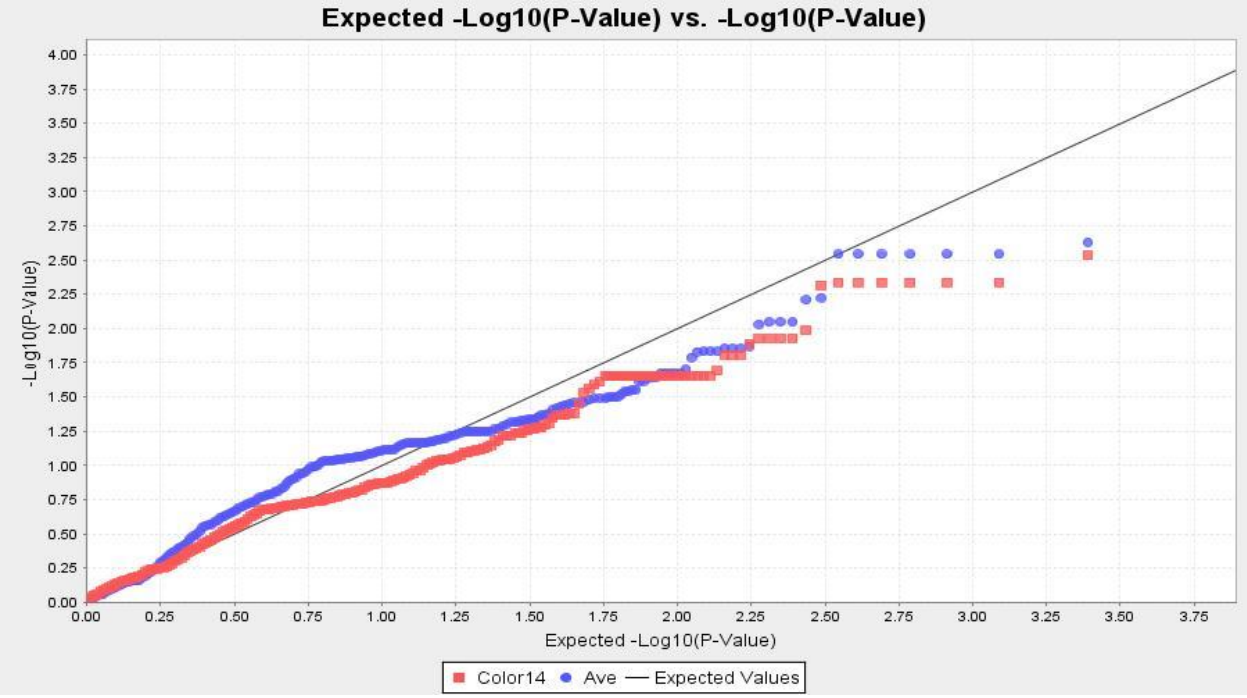


Figure 35. QQ plot for the trait of L* values averaged across 2014 of 61 black bean genotypes with 2800 SNP (>0.02 minor allele frequency) and mapping conducted with multiple linear model (MLM) using a kinship matrix and 7 principal components to account for population structure.

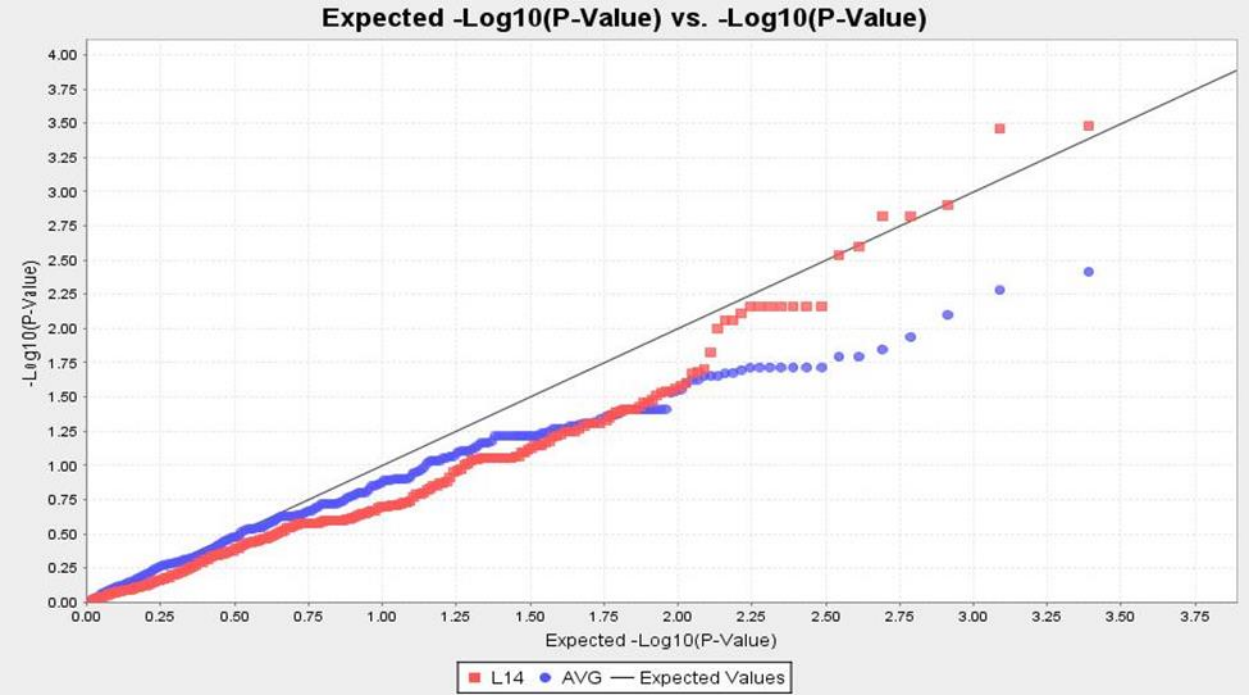


Figure 36. QQ plot for the trait of a* values averaged across 2014 of 61 black bean genotypes with 2800 SNP (>0.02 minor allele frequency) and mapping conducted with multiple linear model (MLM) using a kinship matrix and 5 principal components to account for population structure.

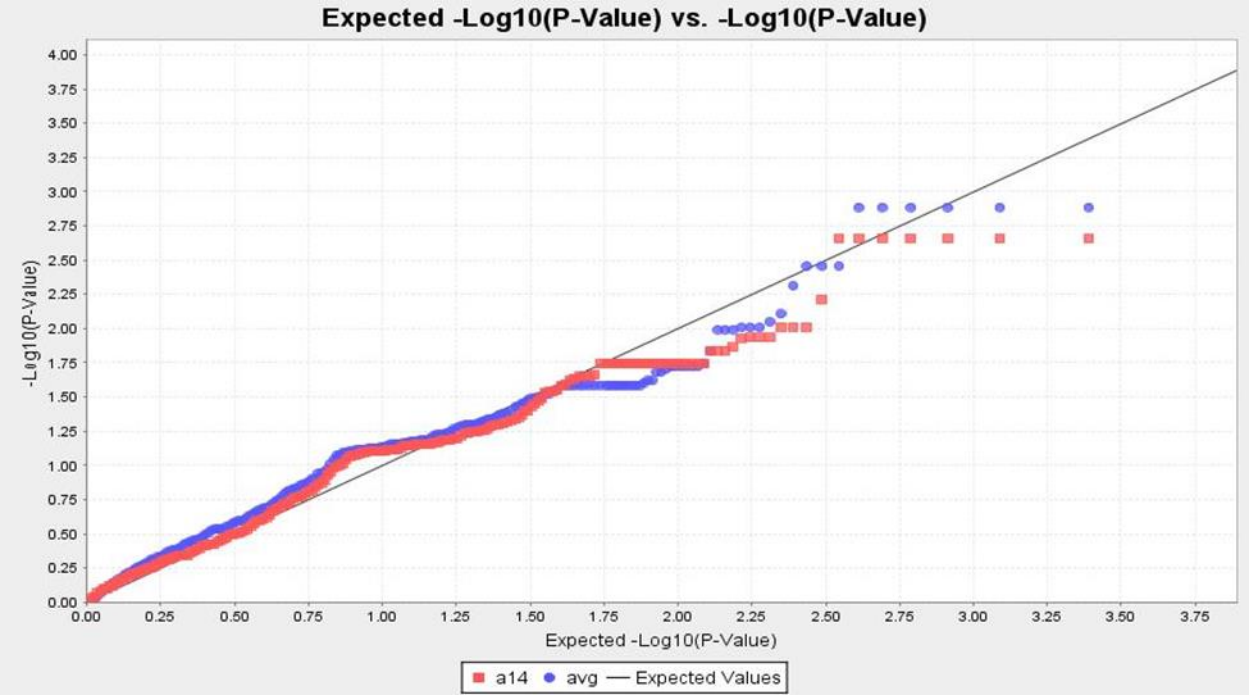
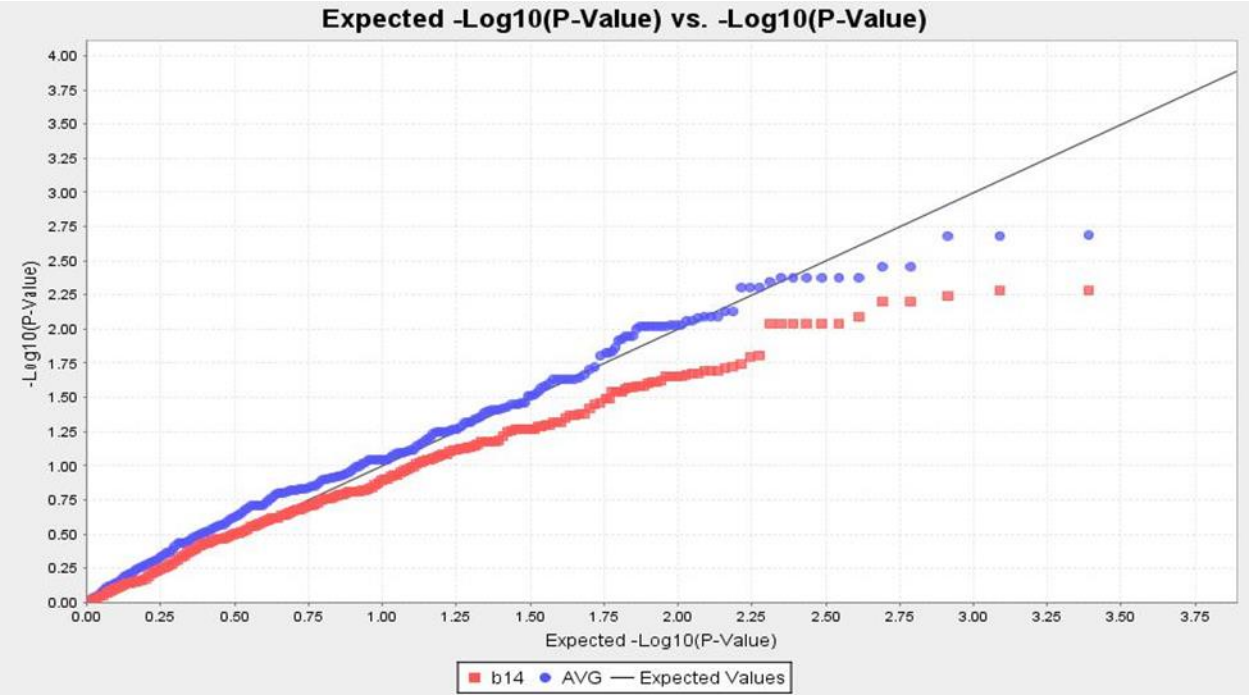


Figure 37. QQ plot for the trait of b* values averaged across 2014 of 61 black bean genotypes with 2800 SNP (>0.02 minor allele frequency) and mapping conducted with multiple linear model (MLM) using a kinship matrix.



BIBLIOGRAPHY

BIBLIOGRAPHY

Adams, J. B. (1973). Thermal degradation of anthocyanin with partiular reference on 3 glucosides of cyanidin. In acidified aqueoussolution at 100º C. *Journal of the Science of Food and Agriculture*, 24, 747-762.

Acevedo, A., Cruz, D., Hilbert, G., Rivière, C., Mengin, V., Ollat, N., Richard, T. (2012). Analytica Chimica Acta Anthocyanin identification and composition of wild Vitis spp . accessions by using LC – MS and LC – NMR. *Analytica Chimica Acta*, 732, 145–152.

Acosta-Gallegos, J., Kelly, J., & Gepts, P. (2007). Prebreeding in common bean and use of genetic diversity from wild germplasm. *Crop Science*, 47(Supplement_3), S44–S59.

Akibode, S. and Maredia, M. (2011). Global and regional trends in production, trade and consumption of food legume crops. [online] East Lansing: Michigan State University, p.42. Available at: http://impact.cgiar.org/sites/default/files/images/Legumetrendsv2.pdf [Accessed 23 Mar. 2016].

Akond, M., Laila, K., Janelle, B., Khwaja, H. (2011). Anthocyanin, Total Polyphenols and Antioxidant Activity of Common Bean. *Academic Journals Inc*, 5, 385–394.

Aparicio-Fernandez, X., Yousef, G. G., Loarca-Pina, G., De Mejia, E., & Lila, M. A. (2005). Characterization of Polyphenolics in the Seed Coat of Black Jamapa Bean (Phaseolus vulgaris L.). *Agric Food Chem*, 53, 4615−4622.

Balasubramanian, P., Slinkard, A., Tyler, R., & Vandenberg, A. (2000). A modified laboratory canning protocol for quality evaluation of dry bean (Phaseolus vulgaris L). *Sci Food Agric*, 738, 732–738.

Bauer, U.E., Briss, P. A., Goodman, R. A., Bowman, B.A. (2011). The Health of Americans 1 Prevention of chronic disease in the 21st century: elimination of the leading preventable causes of premature death and disability in the USA. *The Lancet*, 384(9937), 45–52.

Beebe A, S. E., Rao A, I. M., Jyostna Devi, M. B., & Polania, J. A. (2014). Common beans, biodiversity, and multiple stresses: challenges of drought resistance in tropical soils. *Crop & Pasture Science*, 65, 667–675.

Beebe, S., Skroch, P. W., Tohme, J., Duque, M. C., Pedraza, F., & Nienhuis, J. (2014). Structure of Genetic Diversity among Common Bean Landraces of Middle American Origin Based on Correspondence Analysis of RAPD. *Crop Sci*, 40, 264–273.

Beninger, C. W., Hosfield, G. L., Bassett, M. J., & Owens, S. (2000). Chemical and morphological expression of the B and Asp seedcoat genes in Phaseolus vulgaris. *Journal of the American Society for Horticultural Science*, 125(1), 52–58.

Bewley, J. D., Black, M., & Halmer, P. (2006). The Encyclopedia of Seeds: Science, Technology and Uses. *CABI*. Cromwell.

Bitocchi, E., Bellucci, E., Giardini, A., Rau, D., Rodriguez, M., Biagetti, E., Papa, R. (2012). Molecular analysis of the parallel domestication of the common bean (Phaseolus vulgaris L.) in Mesoamerica and the Andes. *Pnas*, 109(14), 788–796.

Boateng, J., Verghese, M., Walker, L. T., & Ogutu, S. (2008). Effect of processing on antioxidant contents in selected dry beans (Phaseolus spp. L.). *Food Science and Technology*, 41, 1541–1547.

Bowen-forbes, C. S., Zhang, Y., & Nair, M. G. (2010). Journal of Food Composition and Analysis Anthocyanin content , antioxidant , anti-inflammatory and anticancer properties of blackberry and raspberry fruits. *Food Composition and Analysis*, 23, 554–560.

Box GE, Cox DR (1964). An analysis of transformations. *J R Stat Soc Series B (Methodological)*, 26, 211–252.

Bradbury, PJ., Zhang, Z., Kroon DE., Casstevens, TM., Ramdoss, Y., Buck-ler, ES. (2007). TASSEL: software for association mapping of complex traits in diverse samples. *Bioinformatics*, 23, 2633–2635.

Broughton, W. J., Hern, G., Blair, M., Beebe, S., Gepts, P., & Vanderleyden, J. (2003). Beans (Phaseolus spp.) – model food legumes. *Plant and Soil*, 252, 55–128.

Bushey SM, Hosfield GL, Beninger CW. (2000). Water uptake and its relationship to pigment leaching in black beans (Phaseolus vulgaris L.). *Bean Improv Coop*, 43, 104–105.

Chandra, A., Nair, M. G., & Iezzoni, A. (1992). Evaluation and Characterization of the Anthocyanin Pigments in Tart Cherries (Prunus Cerasus L.). *Agrlc. Food Chem*, 40, 967–969.

Cichy, K., Fernandez, A., Kilian, A., Kelly, J., Galeano, C., Shaw, S., Troxtell, E. (2014). QTL analysis of canning quality and color retention in black beans (Phaseolus vulgaris L.). *Molecular Breeding*, 33(1), 139–154.

Cichy, K. A., G. V. Caldas, S. S. Snapp, and M. W. Blair. (2009). QTL Analysis of Seed Iron, Zinc, and Phosphorus Levels in an Andean Bean Population All rights reserved. *Crop Sci*, 49, 1742-1750.

Creamer, B. (2014). Major constraints and trends for common bean production and establishing priorities for future research. *Agronomía Colombiana*, 32(3), 423–431.

Connor, A. M., Luby, J. J., Tong, C. B. S., Finn, C. E., & Hancock, J. F. (2002). Genotypic and Environmental Variation in Antioxidant Activity , Total Phenolic Content , and

Anthocyanin Content among Blueberry Cultivars. *J Amer Soc Hort Sci*, 127(1), 89–97.

De Faria, L. C., Melo, P. G. S., Pereira, H. S., Wendland, A., Borges, S. F., Filho, I. A. P.. . Melo, L. C. (2014). Genetic progress during 22 years of black bean improvement. *Euphytica*, 199(3), 261–272.

De Lange, A., and M. Labuschagne. (2001). Multivariate assess- ment of canning quality, chemical characteristics and yield of small white canning beans (Phaseolus vulgaris L) in South Africa. *J. Sci. Food Agric*, 81, 30–35.

Díaz, A. M., Caldas, G. V., & Blair, M. W. (2010). Concentrations of condensed tannins and anthocyanins in common bean seed coats. *Food Research International*, 43(2), 595–601.

Dong, M., He, X., & Liu, R. H. (2007). Phytochemicals of black bean seed coats: Isolation, structure elucidation, and their antiproliferative and antioxidative activities. *Journal of Agricultural and Food Chemistry*, 55(15), 6044–6051.

Dzomba, P., Togarepi, E., & Mupa, M. (2013). Anthocyanin content and antioxidant activities of common bean species (Phaseolus vulgaris L.) grown in Mashonaland Central Zimbabwe. Afr. *J. Agric. Res*, 8(25), 3330–3333.

Ellis, R. H. (2007). The encyclopaedia of seeds: science, technology and uses. Wallingford, UK: CABI.

Fageria, N. K., & Santos, A. B. (2008). Yield Physiology of Dry Bean, *Journal of plant nutrition*, 31, 983–100.

Gebrelibanos, M., Tesfaye, D., Raghavendra, Y., & Sintayeyu, B. (2013). Nutritional and health implications of legumes. *International Journal of Pharmaceutical Sciences and Research IJPSR*, 4(4), 1269–1279.

Głowacka, A., Klikocka, H. & Onuch, J., (2015). Content of zinc and iron in common bean seeds (Phaseolus vulgaris L.) in different weed control methods. *Journal of Elementology*, 20(2), 293–303.

Goffnett, A., Sprague, C., Mendoza, F., Cichy, C. (2016). Preharvest Herbicide Treatments Affect Black Bean Desiccation, Yield, and Canned Bean Color. *Crop Science Society of America*, 56,1962–1969.

Gould, K., Davies, K. M., & Winefield, C. (2009). Anthocyanins: Biosynthesis, functions, and applications. New York, NY: Springer.

Homayouni, A., & Azizi, A. (2015). Date canning : a new approach for the long time preservation of date. *Journal of Food Science and Technology*, 52(April), 1872–1880.

Hutchins, A. M., Winham, D. M., & Thompson, S. V. (2012). Phaseolus beans: impact on

glycaemic response and chronic disease risk in human subjects. *British Journal of Nutrition*, 108(S1), S52–S65.

Jiang, L., Wang, J., Li, Y., Wang, Z., Liang, J., Wang, R., Zhang, M. (2014). Effects of ultrasound on the structure and physical properties of black bean protein isolates. *FRIN*, 62, 595–601.

Joseph, O., Phelomene, M., Helene, N., Valens, H., Patrick, O. M., Thavarajah, D., & Thavarajah, P. (2014). Phenolic Compound Profiles of Two Common Beans Consumed by Rwandans. *American Journal of Plant Sciences*, 5, 2943–2947.

Kelly, J. D., Varner, G. V., Cichy, K. A., & Wright, E. M. (2015a). Registration of "Zenith" Black Bean. *Journal of Plant Registrations*, 9(1), 15–20.

Khanal, R., Burt, A. J., Woodrow, L., Balasubramanian, P., & Navabi, A. (2015). Genotypic Association of Parameters Commonly Used to Predict Canning Quality of Dry Bean. *CROP SCIENCE*, 54, 2564–2573.

Kim, S., Plagnol, V., Hu, T. T., Toomajian, C., Clark, R. M., Ossowski, S., Nordborg, M. (2007). Recombination and linkage disequilibrium in Arabidopsis thaliana. *Nature Genetics*, 39(9), 1151–1155.

Kutoš, T., Golob, T., Kač, M., & Plestenjak, A. (2003). Dietary fibre content of dry and processed beans. *Food Chemistry*, 80(2), 231–235.

La Cruz, A. A. De, Hilbert, G., Mengin, V., Rivière, C., Ollat, N., Vitrac, C., Richard, T. (2013). Anthocyanin phytochemical profiles and anti-oxidant activities of vitis candicans and vitis doaniana. *Phytochemical Analysis*, 24(5), 446–452.

Lee, I. H., Hung, Y. H., & Chou, C. C. (2008). Solid-state fermentation with fungi to enhance the antioxidative activity, total phenolic and anthocyanin contents of black bean. *International Journal of Food Microbiology*, 121(2), 150–156.

Ma, F., Cholewa, E., Mohamed, T., Peterson, C. A., & Gijzen, M. (2004). Cracks in the palisade cuticle of soybean seed coats correlate with their permeability to water. *Annals of Botany*, 94(2), 213–228.

Mamidi, S., Rossi, M., Annam, D., Moghaddam, S., Lee, R., Papa, R., and McClean, P. (2011). Investigation of the domestication of common bean (Phaseolus vulgaris L.) using multilocus sequence data. *Journal compilation*, 38, 953–967.

Markakis, P., Livingstone, G. E., & Fillers, G. R. (1957). Quantitativeaspects of strawberry pigment degradation. *Food Research*, 22, 117–130.

Marles, M. A. S., Balasubramanian, P., & Bett, K. E. (2010). Differential Accumulation of Polyphenolics in Black Bean Genotypes Grown in Four Environments. *J. Agric. Food*

Chem, 58, 7001–7006.

Melotto, M., Monteiro-Vitorello, C. B., Bruschi, A. G., & Camargo, L. E. A. (2005). Comparative bioinformatic analysis of genes expressed in common bean (Phaseolus vulgaris L.) seed. *Genome Jun*, 48(3), 562–570.

Mendoza, F. A., Cichy, K., Lu, R., & Kelly, J. D. (2014). Evaluation of Canning Quality Traits in Black Beans (Phaseolus vulgaris L.) by Visible / Near-Infrared Spectroscopy. *Food Bioprocess Technol*, 7, 2666–2678.

Mendoza, F. A., Kelly, J. D., & Cichy, K. (2016). Automated prediction of sensory scores for color and appearance in canned black beans (Phaseolus vulgaris L.) using machine vision. *International Journal of Food Properties*, 2912, 1532–2386.

Merwe, D. Van Der, Osthoff, G., & Pretorius, A. J. (2006). Comparison of the canning quality of small white beans (Phaseolus vulgaris L.) canned in tomato sauce by a small-scale and an industrial method. *Journal of the Science of Food and Agriculture*, 1056(August 2005), 1046–1056.

Miklas, PN., Kelly, JD., Beebe, SE., Blair, MW. (2006a). Common bean breeding for resistance against biotic and abiotic stresses: from classical to MAS breeding. *Euphytica* 147, 105–131.

Miklas, PN., Hu, J., Grunwald, NJ., Larsen, KM. (2006b). Potential application of TRAP (targeted region amplified poly-483 morphism) markers for mapping and tagging disease resistance traits in common bean. *Crop Sci*, 46, 910–916.

Mojica, L., Meyer, A., Berhow, M. A., & de Mejía, E. G. (2015). Bean cultivars (Phaseolus vulgaris L.) have similar high antioxidant capacity, in vitro inhibition of α-amylase and α-glucosidase while diverse phenolic composition and concentration. *Food Research International*, 69, 38–48.

Moreno-jiménez, M. R., Cervantes-cardoza, V., Gallegos-infante, J. A., González-laredo, R. F., Estrella, I., García-gasca, T. D. J., Rocha-guzmán, N. E. (2015). Phenolic composition changes of processed common beans: their antioxidant and anti-inflammatory effects in intestinal cancer cells. *FRIN*, 76, 79–85.

Myles, S., Peiffer, J., Brown, P. J., Ersoz, E. S., Zhang, Z., Costich, D. E., & Buckler, E. S. (2009). Association mapping: critical considerations shift from genotyping to experimental design. *The Plant Cell*, 21(8), 2194–2202.

Nakitto, A. M., Muyonga, J. H., & Nakimbugwe, D. (2015). Effects of combined traditional processing methods on the nutritional quality of beans. *Food Science & Nutrition*, 131, 233–241.

Nyau, V., Prakash, S., Rodrigues, J., & Farrant, J. (2015). HPLC-PDA-ESI-MS Identification of

Polyphenolic Phytochemicals in Different Market Classes of Common Beans (Phaseolus vulgaris L.). *IJBCRR*, 8(4), 1–11.

Oseguera-toledo, M. E., Gonzalez, E., Mejia, D., Dia, V. P., & Amaya-llano, S. L. (2011). Common bean (Phaseolus vulgaris L.) hydrolysates inhibit inflammation in LPS-induced macrophages through suppression of NF- j B pathways. *Food Chemistry*, 127(3), 1175–1185.

Porch, T. G., Beaver, J. S., Debouck, D. G., Jackson, S. A., Kelly, J. D., Dempewolf, H., … Trust, D. (2013). Use of Wild Relatives and Closely Related Species to Adapt Common Bean to Climate Change. *Agronomy (Basel)*, 3, 433–461.

Rocha-Guzmán, N. E., Herzog, A., González-Laredo, R. F., Ibarra-Pérez, F. J., Zambrano-Galván, G., & Gallegos-Infante, J. A. (2007). Antioxidant and antimutagenic activity of phenolic compounds in three different colour groups of common bean cultivars (Phaseolus vulgaris L.). *Food Chemistry*, 103(2), 521–527.

Rockenbach, I., Jungfer, E., Ritter, C., Santiago-schübel, B., Thiele, B., Fett, R., & Galensa, R. (2012). Characterization of fl avan-3-ols in seeds of grape pomace by CE , HPLC-DAD-MS and LC-ESI-FTICR-MS. *FRIN*, 48(2), 848–855.

Rodrigues, S., & Fernandes, F. A. N. (2012). Contemporary Food Engineering: Advances in Fruit Processing Technologies(1). London, US: CRC Press.

Patras, A., Brunton, N. P., O'Donnell, C., & Tiwari, B. K. (2010). Effect of thermal processing on anthocyanin stability in foods; mechanisms and kinetics of degradation. *Trends in Food Science & Technology*, 21(1), 3–11.

Pritchard, JK., Stephens, M., Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics* 155, 945–959.

Porch, T. G., Beaver, J. S., Debouck, D. G., Jackson, S. A., Kelly, J. D., Dempewolf, H., … Trust, D. (2013). Use of Wild Relatives and Closely Related Species to Adapt Common Bean to Climate Change, *Agronomy*, 3(2), 433–461.

Sancho, R. A. S., Pavan, V., & Pastore, G. M. (2015). Effect of in vitro digestion on bioactive compounds and antioxidant activity of common bean seed coats. *FRIN*, 76, 74–78.

Schmutz, J., McClean, PE., Mamidi, S., Wu, GA., Cannon, SB., Grimwood, J., Jenkins, J., Shu, S., Song, Q., Chavarro, C., Torres-Torres, M., Gef- froy, V., Moghaddam, SM., Gao, D., Abernathy, B., Barry, K., Blair, M., Brick, MA., Chovatia, M., Gepts, P., Goodstein, DM., Gonzales, M., Hellsten, U., Hyten, DL., Jia, G., Kelly, JD., Kudrna, D., Lee, R., Richard, MMS., Miklas, PN., Osorno, JM., Rodrigues, J., Thareau, V., Urrea, CA., Wang, M., Yu, Y., Zhang, M., Wing, RA., Cregan, PB., Rokhsar, DS., Jackson, SA. (2014). A reference genome for com- mon bean and genome-wide analysis of dual domestications. *Nat Genet*, 46, 707–713.

Shi, C., Yu, K., Xie, W., & Deidre, P. N. M. (2012). Development of candidate gene markers associated to common bacterial blight resistance in common bean. *Theoretical and Applied Genetics*, 125(7), 1525–1537.

Singh, G. (2008). Seed Quality. Delhi, IND: Gene-Tech Books.

Sivolap, Y. M. (2013). Molecular markers and plant breeding. *Cytology and Genetics*, 47(3), 188–195.

Takeoka, G. R., Dao, L. T., Full, G. H., Wong, R. Y., Harden, L. A., Edwards, R. H., Berrios, J. (1997). Characterization of black bean (Phaseolus vulgarisL.) anthocyanins. *Journal of Agricultural and Food Chemistry*, 45(9), 3395–3400.

Thompson, M. D., Brick, M. A., McGinley, J. N., & Thompson, H. J. (2009). Chemical Composition and Mammary Cancer Inhibitory Activity of Dry Bean. *Crop Science*, 49, 179–186.

Tuberosa, R., Graner, A., & Frison, E. (2014). Genomics of plant genetic resources : Volume 2. crop productivity, food security and nutritional quality. Dordrecht, Netherlands: Springer.

Turner SD (2014) qqman: an R package for visualizing GWAS results using Q-Q and manhattan plots. bioRxiv (2014): 005165.

USDA Laboratory. (2014). Soybean Genomics and Improvement www.ars.usda.gov/main/site_main.htm?modecode=80-42-05-70 (accessed 5 April. 2016).

USDA-NASS (National Agricultural Statistics Service) (2015) 2014 State agriculture overview. http://nass.usda.gov/Quick_Stats/Ag_Overview/stateOverview.php?state=MICHIGAN. Accessed April 8, 2015.

Wang, X., Hansen, C., & Allen, K. (2013). Identification of Anthocyanins Isolated from Black Bean Canning Wastewater by Macroporous Resin Using Optimized Conditions. *Food and Nutrition Sciences*, 4, 174–181.

Wright, E. M., & Kelly, J. D. (2011). Mapping QTL for seed yield and canning quality following processing of black bean (Phaseolus vulgaris L.). *Euphytica*, 179(3), 471–484.

Wu, X., Beecher, G. R., Holden, J. M., Haytowitz, D. B., Gebhardt, S. E., & Prior, R. L. (2006). Concentrations of Anthocyanins in Common Foods in the United States and Estimation of Normal Consumption. *J.Agric.FoodChem*, 54, 4069−4075.

Xu, B., Huang, Y., & Watson, M. D. (2001). Cotton Color Distributions in the CIE L*a*b* System. *Textile Rex J*, 71(11), 1010–1015.

Zhang, C., Monk, J. M., Lu, J. T., Zarepoor, L., Wu, W., Liu, R., Power, K. a. (2014). Cooked navy and black bean diets improve biomarkers of colon health and reduce inflammation

during colitis. The British Journal of Nutrition, 111, 1549–1563.

Zhu, Z., Jiang, W., & Thompson, H. J. (2012). Edible dry bean consumption (Phaseolus vulgaris L.) modulates cardiovascular risk factors and diet-induced obesity in rats and mice. British Journal of Nutrition, 108, S66–S73.