GENETIC VARIABILITY AND MAPPING OF COOKING TIME AND SENSORY ATTRIBUTES IN ANDEAN DRY BEANS

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ABSTRACT

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Cooking time, flavor, and texture of dry beans (*Phaseolus vulgaris* L.) are valued by consumers but are not major considerations of dry bean breeding programs. The aim of this research is 1) to investigate mechanisms underlying fast cooking times of select genotypes, 2) to characterize the genetic control of cooking time, flavor, and texture of cooked beans in a diversity panel and a recombinant inbred line population, and 3) to evaluate how fast-cooking bean genotypes process into canned products. The genetic mechanism of fast cooking time was assessed via the physical and compositional seed characteristics in a set of 8 genotypes. Faster cooking beans had thinner cotyledon cell walls and seed coat layers and lower seed coat percentage, seed weight, and total and insoluble fiber. To identify genomic loci underlying cooking time, flavor, and texture, genome-wide association (GWA) and quantitative trait loci (QTL) mapping approaches were used with 430 lines of the Andean Diversity Panel and 242 yellow recombinant inbred lines. Sensory attributes included total flavor, beany, vegetative, earthy, starchy, sweet, and bitter intensity as well as seed coat perception and cotyledon texture. SNPs and QTL were identified for most of the attributes, with QTL for earthy intensity having the most phenotypic variation explained. In both populations, sweet and starchy intensity were positively correlated and associated via PCA, but other trait associations were minimal. A subset of lines from the RIL population were evaluated for canning quality following different retort processing durations. For fast-cooking lines, canning quality improved with reduced retort processing time, revealing a

potential cost-saving benefit to the canning industry. This information lays a foundation for targeting fast cooking times and specific sensory profiles in breeding programs.

To Frodo and Sammie, two brave rabbits who took this journey with me.

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INTRODUCTION

PROBLEM DEFINITION

Cooking time and sensory quality are important characteristics consumers consider when purchasing dry beans (*Phaseolus vulgaris* L.) (Castellanos et al., 1997; Scott and Maideni, 1998). Dry beans often require long cooking times, particularly when cooked without prior soaking. Many chemical and physical changes occur during the cooking process (Rockland and Jones, 1974; Cichy et al., 2015), but the mechanism of cooking time and the roles of the seed coat and cotyledon cell wall in cooking time are not well understood. Long cooking times deter consumers, particularly as convenience is increasingly valued concerning food and other aspects of modern life (Sloan, 2015). Taste is a major driver of food purchasing decisions among consumers (IFIC, 2019), and it is a common reason consumers choose not to eat beans (Leterme and Carmenza Muñoz, 2002; Eihusen and Albrecht, 2007; Winham et al., 2019). Food companies invest heavily in this aspect of product development (Banking, 2016), but limited research concerning dry bean flavor and associated volatiles is available (Vara-Ubol et al., 2004; Bott and Chambers, 2006; Oomah et al., 2007; Plans et al., 2014; Szczygiel et al., 2017).

Cooking time and sensory quality have largely been overlooked by breeders, who have focused instead on seed yield, processing quality, disease resistance, architecture, agronomic adaptation, stress tolerance, and grower friendliness, which encompasses traits that reduce labor and inputs required by growers (Kelly and Cichy, 2012). Cooking time and sensory attributes can be costly in time and resources to evaluate, but a lack of focus on these consumer-valued traits may be limiting consumption of dry beans below their potential. Wide genotypic variability exists for both cooking time and sensory attributes, even within market class (Rivera et al., 2013; Cichy

et al., 2015), which can be targeted to improve new varieties and appeal to more consumers and product developers. In addition, fast cooking time may appeal to the canning industry by reducing energy costs and improving efficiency of production through shorter retort processing times.

OBJECTIVES

This study aims to explore the mechanism of cooking time as it relates to the seed coat and cell wall, identify genomic loci relevant for cooking time and sensory attributes using quantitative genetics approaches, and determine the relevance of cooking time to the canning industry.

DISSERTATION OUTLINE

Chapter 1 assesses the genetic variability of cooking time across different soaking times as it relates to physical and compositional traits of the seed coat and cell wall. The study was performed using eight genotypes across four seed types. The relationships between cooking time and soaking time, seed size, seed coat/cotyledon percent, seed coat layer thickness, cell wall thickness, cotyledon cell wall isolate, and dietary fiber were determined.

Chapter 2 is a genome-wide association study aimed at understanding the genetic basis of cooking time and sensory attributes. Across three locations, 430 lines of the Andean Diversity Panel were evaluated for cooking time and sensory attributes intensities, including total flavor, beany, vegetative, earthy, starchy, sweet, bitter, seed coat perception, and cotyledon texture. Significant SNPs associated with these traits were identified and can be used for the development of molecular markers.

Chapter 3 is a quantitative trait loci mapping study aimed at understanding the genetic basis of cooking time and sensory attributes. Across two years in Michigan, a recombinant inbred line

population of 242 yellow bean lines was developed and evaluated for cooking time and sensory attributes, including total flavor, beany, vegetative, earthy, starchy, sweet, bitter, seed coat perception, and cotyledon texture. QTL were identified for these traits and can be used for the development of molecular markers.

Chapter 4 assesses canning quality as it relates to cooking time. Across five retort processing times, 20 yellow bean lines with varying cooking times were assessed for canning quality. The relationships between cooking time and intactness, washed-drained weight, texture, and color were determined.

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CHAPTER 1:

GENETIC VARIABILITY OF COOKING TIME IN DRY BEANS (*Phaseolus vulgaris* L.) RELATED TO SEED COAT THICKNESS AND THE COTYLEDON CELL WALL

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Genetic variability of cooking time in dry beans (*Phaseolus vulgaris* L.) related to seed coat thickness and the cotyledon cell wall

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ABSTRACT

Dry beans are an affordable, nutritious food that often require long cooking times. Seed age, storage conditions, growing environment, and genotype influence cooking times. Little is known about underlying factors responsible for genetic variation for cooking time. Using fast and slow cooking genotypes from four different seed types (brown, cranberry, red mottled, yellow), the objectives of this study were to (1) characterize genetic variability for cooking time across multiple soaking time points; (2) determine the roles of the seed coat and cotyledon cell wall thickness in genetic variability in cooking time; and (3) identify seed coat and cotyledon cell wall composition differences associated with genetic variability in cooking time. Genotypes were evaluated for cooking time on unsoaked beans and beans soaked for 3, 6, 12, 18, and 24 hr. Cooking times were sharply reduced after 3 hr of soaking and plateaued after 6 hr of soaking. Soaking time influenced the cooking times differently across genotypes. Greater seed coat percentage, cotyledon cell wall thickness, total and insoluble whole seed dietary fiber, and insoluble cotyledon cell wall isolate were genotypic factors associated with longer cooking times of soaked beans. Thicker seed coat macrosclereid- and osteosclereid-layers were genotypic factors associated with longer cooking times of unsoaked beans. These findings suggest that cotyledon

cell wall thickness and composition has a significant role in genotypic variability for cooking time of soaked beans and seed coat layer thickness relates to the genetic variability for cooking time of unsoaked beans.

INTRODUCTION

Dry beans are an affordable, nutritious food incorporated in many cuisines with versatile preparations, but beans frequently require long cooking times when prepared from dry seed. Cooking dry beans can require anywhere from ~15 minutes to several hours depending on factors such as genotype, seed type, seed age, storage and harvest conditions, and pretreatments (Rockland and Jones, 1974; Hernandez-Unzon and Ortega-Delgado, 1989; Coelho et al., 2007; Cichy et al., 2015). As consumers spend less time preparing meals, there is rising demand for convenience in food preparation (Furst et al., 1996; Jabs and Devine, 2006; Hamrick et al., 2012; Monsivais et al., 2014). For consumers who rely on fuel sources such as firewood and charcoal, long cooking times can be prohibitive due to the increased resources required to prepare beans. Since wide genetic variability exists for bean cooking times, plant breeding is one approach that can be used to develop fast-cooking beans to meet the needs of the growing global population.

Cooking dry beans by boiling is a hydrothermal process, and many physical and chemical changes occur during cooking. These physical and chemical changes include the uptake of water, denaturation of proteins, starch swelling and gelatinization, and partial solubilization of polysaccharides in the cell wall leading to separation of adjacent whole cells (Rockland and Jones, 1974). Soaking can greatly reduce cooking time and has been associated with changes in pectin content, starch gelatinization, and protein solubility (Bellido, Arntfield, Cenkowski, & Scanlon, 2006; Chigwedere, Njoroge, Van Loey, & Hendrickx, 2019; Martínez-Manrique et al., 2011). Seed

age and storage conditions also impact cooking time through changes in phytic acid content, enzyme activity, seed coat permeability, pectin solubility, cell wall content and thickness, oxidation of phenolic compounds, and membrane deterioration (Jackson and Varriano-Marston, 1981; Moscoso et al., 1984; Hincks and Stanley, 1987; Stanley, 1992; Garcia et al., 1994, 1998; Yousif and Deeth, 2003; Waldron et al., 2003; Galiotou-Panayotou et al., 2008; Shiga et al., 2009; Daher and Braybrook, 2015). Cooking time varies across genotypes. Within a screening of 206 bean genotypes across multiple seed types, cooking times ranged from 15 to 90 min as determined by the Mattson cooker method (Cichy et al., 2015). There was variability among seed types, such that white and yellow beans cook faster on average than red mottled beans. There was also variation within seed types such that one cranberry bean cooked in 15 minutes whereas another cooked in 90 min. The cooking time trait has been found to be highly to moderately heritable (Cichy et al., 2019; Katuuramu et al., 2020).

The genetic variability for cooking time could be caused by multiple physical or chemical factors expressed at various stages of the cooking process. The first point of contact during cooking process is the seed coat, which serves as a physical barrier to water uptake (Jackson and Varriano-Marston, 1981). While the micropyle, hilum, strophiole, and raphe have been implicated as the primary means of water entry into the bean (Snyder, 1936; Powrie et al., 1960; Deshpande and Cheryan, 1986a; Agbo et al., 1987; Gargiulo et al., 2020), some studies have identified a relationship between water uptake and total seed coat thickness as well as the thickness of individual seed coat layers, including the macrosclereid, osteosclereid, and parenchyma, finding that thin seed coats increase water uptake rates (Deshpande and Cheryan, 1986a; Agbo et al., 1987). Studies have also shown that seed coat color influences water uptake, with darker beans taking up water more slowly (Marbach and Mayer, 1974, 1975; Tully et al., 1981; Valle et al.,

1992). Therefore, to better understand genetic factors that contribute to cooking time, it is useful to compare fast- and slow-cooking genotypes both within and across seed types.

When it comes to the prolonged cooking times exhibited by aged, improperly stored beans (i.e. hard-to-cook), the cotyledon has been shown to be more important than the seed coat in dictating cooking times for soaked beans (Chigwedere et al., 2018). The cotyledon cell walls are especially important, as they influence cooking time due to the physiological role they play during the soaking and cooking process. The cotyledon cell wall is made up of cellulose, hemicellulose, pectin, neutral sugars, proteins, glycoproteins, lignin, and phenolic compounds and presents a barrier surrounding a matrix of protein and starch (Ginzburg, 1961; Letham, 1962; Varriano-Marston and Jackson, 1981; Shiga and Lajolo, 2006; Yi et al., 2016). As beans cook, the cell wall partially solubilizes and the pectin-rich middle lamella breaks down, which allows separation of adjacent cells and adequate softening of the bean (Rockland and Jones, 1974; Shomer et al., 1990). Cell wall content, integrity, and thickness as well as dietary fiber content as a whole have been linked increased cooking time resulting from seed age and storage conditions (Moscoso et al., 1984; Hincks and Stanley, 1987; Yousif and Deeth, 2003; Shiga et al., 2009; Yi et al., 2016; Siqueira et al., 2018). As seeds age, they exhibit less soluble fiber and increased cell wall content and take longer to cook (Moscoso et al., 1984; Yousif and Deeth, 2003; Shiga et al., 2009). The hard-to-cook phenomenon associated with improperly stored beans has been linked to increased cotyledon cell wall thickness due to lignin deposition, increased prevalence of cotyledon cell wall ruptures, and increased covalent bonding of pectin and other cell wall polysaccharides as demonstrated by Chigwedere, Nkonkola, et al. (2019), which could potentially be hindering cell separation (Hincks and Stanley, 1987; Yi et al., 2016; Siqueira et al., 2018). Exploring differences in the seed coat layer thickness and cotyledon cell wall thickness and composition of fast- and

slow-cooking genotypes within different seed types will be useful to reveal heritable traits associated with genetic variability of cooking time.

Evaluation of cooking time is frequently performed on soaked beans. However, consumers often prepare dry beans without soaking (unsoaked) or with variable soaking times. While previous studies have identified a relationship between cooking times of unsoaked and soaked beans, the correlation is only moderate (R = 0.67) (Mendoza et al., 2018). In addition, cooking time of unsoaked beans has been found to be less heritable as compared to cooking time of soaked beans (Cichy et al., 2019). Understanding the stability of the fast cooking trait across unsoaked and soaked treatments can inform phenotyping methods so that germplasm can be accurately assessed for cooking time. In addition, observing responses to different soaked treatments could help reveal genetic factors associated with the fast cooking trait.

The overall goal of this study was to characterize genetic variability of cooking time as it relates to seed coat layers and cotyledon cell wall traits within fast- and slow-cooking genotypes across four seed types: brown, cranberry, red mottled, and yellow. The genotypes were identified and categorized as fast or slow cooking in a large germplasm screening evaluating cooking time following a 12 hr soak (Cichy et al., 2015). The three objectives of this study are (1) to determine cooking times and water uptake of the 8 genotypes across 6 soaking times (0, 3, 6, 12, 18, and 24 hr), (2) to determine physical differences in seed coat macrosclereid- and osteosclereid layers and cotyledon cell wall thickness as well as seed weight and percentage of cotyledon, seed coat, and embryo and how these factors relate to genetic variability for cooking time, and (3) to determine compositional differences in total, soluble, and insoluble whole seed dietary fiber content and cotyledon cell wall isolate and how these factors relate to genetic variability for cooking time (Figure S1.1).

MATERIALS AND METHODS

Germplasm

The *Phaseolus vulgaris* germplasm relevant to this study consists of 8 genotypes with a faster and slower cooking genotype represented from four seed types: brown, cranberry, red mottled, and yellow. Seed type refers to seed appearance in terms of color, pattern, and shape, with like seeds considered to have the same seed type. The genotypes include brown beans W616488, ADP0037 (Brown-1) and Incomparable, ADP0027 (Brown-2), cranberry beans G23086, ADP0367 (Cranberry-1) and Katarina Kibala, ADP0515 (Cranberry-2), red mottled beans JB-178, ADP0436 (RedMottled-1) and PR0737-1, ADP0434 (RedMottled-2), and yellow beans Ervilha (Yellow-1) and PI527538 (Yellow-2) (Figure 1.1).

The selected germplasm were identified after screening 206 lines of the Andean Diversity Panel for cooking time (Cichy et al., 2015). The genotypes designated "-1" are considered faster cooking compared to those marked "-2" within a seed type pair when cooked after soaking for 12 hr. W616488 (Brown-1), Incomparable (Brown-2), and PI527538 (Yellow-2) are part of the US Phaseolus germplasm collection. G23086 (Cranberry-1) is part of the International Center of Tropical Agriculture germplasm collection. Katarina Kibala (Cranberry-2) and Ervilha (Yellow-1) were originally collected in an Angolan marketplace in 2010. JB-178 (RedMottled-1) was developed and released by the Centro de Investigación Agrícolas del Suroeste, Ministry of Agriculture of the Dominican Republic in cooperation with the University of Puerto Rico and the University of Nebraska (Arnaud-Santana et al., 2000). PR0737-1 (RedMottled-2) was developed and released cooperatively by the University of Puerto Rico Agricultural Experiment Station, the USDA–ARS, the Instituto Dominicano de Investigaciones Agropecuarias y Forestales, and the

Ministry of Agriculture, Natural Resources and Rural Development of the Republic of Haiti (Prophete et al., 2014).

All genotypes used in this study were planted in 2017 at the Montcalm Research Farm in MI, which has Eutric Glossoboralfs (coarse-loamy, mixed) and Alfic Fragiorthods (coarse-loamy, mixed, frigid) soil types. The genotypes were grown in duplicate in a randomized complete block design of two row plots 4.75 m long with 0.5 m spacing between rows. Standard agronomic practices were followed as described in the MSU SVREC 2017 Farm Research Report (Kelly et al., 2017). Plants were hand-pulled at maturity and threshed with a Hege 140 plot harvester. Following harvest, beans were cleaned by hand to remove field debris, off types, and damaged beans. All beans were stored at room temperature for 3 months following harvest. Seed weights in g/100 seeds were determined for each genotype with 6 technical replications per field replicate.

Water uptake and Cooking Time Evaluation

Cooking times were determined for each field replicate of each genotype using automated Mattson cookers (Wang and Daun, 2005). Thirty beans per sample at 10-14% moisture were soaked in 250 ml distilled water for 0, 3, 6, 12, 18, or 24 hr. The beans were then patted dry of excess water and weighed to determine water uptake. For each sample, twenty-five beans were loaded onto 25 well Mattson cookers (Michigan State University Machine Shop, East Lansing, MI) with weighted (65 g) 2 mm diameter pins positioned in the center of each bean. Loaded Mattson cookers were placed into 4 L stainless steel beakers with 1.8 L of boiling distilled water on Cuisinart CB-30 Countertop Single Burners. A low boil was maintained until 80% of the beans were pierced completely. The 80% cooking time was recorded, and samples were cooled to room temperature and weighed to determine water uptake during cooking. Cooked samples were frozen

at -80 °C and freeze dried using a in a VirTis Genesis 12EL freeze dryer (Figure S1.1- Objective 1).

Scanning Electron Microscopy

For each genotype, unsoaked and soaked beans (12 hr in distilled water) were imaged using a JEOL JSM-6610LV scanning electron microscope at the Center for Advanced Microscopy at Michigan State University with an accelerating voltage of 12kV, spot size of 30, and working distance of 11 mm. For unsoaked beans, four beans per field replicate were cut into cross sections using double-edged razor blades, with four halves per field replicate mounted on an aluminum stub using high vacuum carbon tabs. For soaked beans, four beans per field replicate were cut to obtain approximately 4 mm thick slices including the hilum. These slices were fixed at 4 °C for 1 hr in 4% glutaraldehyde buffered with 0.1 M sodium phosphate (pH 7.4). After fixation, the slices were soaked for 30 min in 0.1 M sodium phosphate buffer (pH 7.4) and dehydrated in an ethanol series (25%, 50%, 75%, 95%) for 40 min at each gradation followed by three 30 min soaks in 100% ethanol. The slices were then critical-point dried in a Leica Microsystems EM CPD300 critical point dryer using carbon dioxide as the transitional fluid. The dried slices were mounted on aluminum stubs using high vacuum carbon tabs. Following mounting, each sample was platinum coated to a thickness of 8 nm while rotating. Four micrographs per cross-section were collected: two of seed coat cells and two of cotyledon cells (Figure S1.2). Seed coat cells were imaged at 750X, and cotyledon cells were imaged at 1200X for unsoaked samples and 600X for soaked samples. Example images of seed coat and cotyledon cells are depicted in Figures 1.2 and 1.3. Measurements of macrosclereid thickness (5/micrograph), osteosclereid thickness (5/micrograph), and cotyledon cell wall thickness (10/micrograph) were collected using ImageJ version 1.51j8 (Schneider et al., 2012). Macrosclereid cells form the outer layer of the seed coat,

and osteosclereid cells are located between the macrosclereid layer and the parenchyma layer of the seed coat. For macrosclereid and osteosclereid layers, measurements were collected in regions with well-defined edges separating the seed coat components. Cotyledon cell wall thickness was recorded only for cell walls perpendicular to the plane of view with well-defined edges to ensure accurate measurements were collected (Figure S1.1- Objective 2).

Dietary Fiber Quantification

For each field replicate of each genotype, raw beans were ground in a Kinematica PX-MFC 90 D laboratory hammer mill and passed through a 0.5 mm sieve. Milled samples were submitted to Great Lakes Scientific (Stevensville, USA) for dietary fiber analysis. Soluble, and insoluble dietary fiber content of whole seeds by dry weight were determined using the enzymatic-gravimetric methods AOAC 993.19 and AOAC 991.42 (AOAC, 1995b, 1995a). The sum of soluble and insoluble fiber is total fiber (Figure S1.1- Objective 3).

Seed Component Percentage and Cotyledon Cell Wall Isolation

For each genotype, 25 raw beans were wrapped in moist paper towels for 2 hr prior to separating seed coats and embryos from the cotyledons with forceps. The cotyledons, seed coats, and embryos were then frozen at -80 °C, freeze-dried in a VirTis Genesis 12EL freeze dryer, and weighed, and for each component, the percentage of total weight was calculated. Cotyledons were ground in a Kinematica PX-MFC 90 D laboratory hammer mill and passed through a 0.5 mm sieve (Figure S1.1- Objective 2). Water soluble and insoluble cell wall components were isolated in triplicate from the cotyledon cells from one field replicate of each genotype using the method described in Shiga and Lajolo, 2006 (Shiga and Lajolo, 2006). In brief, milled samples were defatted and digested via α-amylase, protease, and amyloglucosidase. After centrifuging, supernatants were dialyzed for 48 hr, freeze dried, and weighed to determine soluble cell wall

content, and the residues were washed, treated with 0.5 M sodium phosphate buffer, and sonicated in dimethyl sulfoxide with thorough rinsing between steps. The final residues were rinsed, freeze dried, and weighed to determine insoluble cell wall content. The sum of soluble and insoluble cell wall isolate is total cell wall isolate. Isolates were normalized to g per 1g cotyledon (Figure S1.1-Objective 3).

Statistical Analysis

All analyses of variance (ANOVA) in this study were conducted using the MIXED procedure in SAS version 9.4 of the SAS System for Windows. For Objective 1, genotype, soaking time, and genotype by soaking time were included as fixed effects with field replicate as a random effect. For Objective 2, genotype was included as a fixed effect with field replicate as a random effect for seed coat percentage, cotyledon percentage, and embryo percentage. Genotype, soaking time, and genotype by soaking time were included as fixed effects and field replicate, technical replicate(field replicate), micrograph(technical replicate), and measurement(micrograph) as random effects for cotyledon cell wall, macrosclereid, and osteosclereid thickness ANOVAs. For Objective 3, genotype was included as a fixed effect and field replicate as a random effect for total, insoluble, and soluble dietary fiber of whole seeds. For total, insoluble, and soluble cell wall isolate, genotype was included as a fixed effect and technical replicate as a random effect. In each case, mean separation was determined using pdiff within the mixed procedure and a Tukey multiple comparison adjustment. Spearman correlation coefficients (rs) were determined in R using the Cor function. Spearman correlations were used rather than Pearson correlations due to the monotonic, non-linear relationship between soaking time and cooking time, the two fundamental variables studied in this work. Principal component analysis (PCA) was performed with averages of all traits using the Prcomp function in R.

RESULTS

Cooking Time and Water Uptake

Objective 1 was to determine cooking times and water uptake of the 8 genotypes across 6 soaking times (0, 3, 6, 12, 18, and 24 hr) (Figure S1.1). Cooking time and water uptake were influenced by genotype and soaking time (Table S1.1). Individual genotypes were affected differently by soaking time, resulting in differences cooking time curves, rates of water uptake, and significant genotype by soaking interactions. This was observed in some genotypes cooking relatively more quickly than others when not soaked prior to cooking, but those same genotypes taking relatively longer to cook than others when soaked. Within each seed type, the same genotype was not necessarily the fastest cooking across the different soaking treatments. The seed type pairs were originally selected after screening for cooking times of 12 hr soaked beans. The genotypes selected as faster cooking lines (indicated "-1") based on this screening generally cooked faster following all soaked treatments longer than 6 hr. However, for the unsoaked treatment, the genotypes selected as slower cooking lines (indicated "-2") cooked faster than their counterpart in every seed type except brown.

Cooking times ranged 16.7 - 108.1 min across all soaking times (Figures 1.1, S1.2). For the unsoaked treatment, Cranberry-2 had the fastest cooking time (82.3 min) and RedMottled-1 had the slowest (108.1 min). For the 3 hr soaking time, Yellow-1 had the fastest cooking time (24.9 min) and Brown-2 had the slowest (49.7 min). Cooking times began to stabilize for all genotypes beginning at 6 hr of soaking when the beans were fully hydrated. For all soaking times 6 hr and longer, Cranberry-1 generally was the fastest cooking genotype (16.7 – 18.0 min) and RedMottled-2 was the slowest (32.8 – 36.0 min).

The water uptake ranged 47.8 - 110.2 % across all soaking times (Figures 1.1, S1.2). For the 3 hr soaking time, Brown-1 and Brown-2 had the lowest water uptake (47.8% & 53.0%) and Cranberry-1 and Cranberry-2 had the highest (80.6% & 75.4%). For soaking times of 6 hr and longer, the water uptake varied less across genotypes as they approached full hydration. Yellow-2 exhibited the lowest (89.2 - 95.7%) and Yellow-1 the highest (100.4 – 110.2%) water uptake for all soaking times 6 hr and longer.

Seed and Physical Traits

Objective 2 to determine physical differences in seed coat macrosclereid- and osteosclereid layers and cotyledon cell wall thickness as well as seed weight and percentage of cotyledon, seed coat, and embryo and how these factors relate to genetic variability for cooking time (Figure S1.1). Genotype significantly affected seed weight and percentage of seed coat, cotyledon, and embryo (Table S1.1).

Seed weights ranged 38.9 – 64.4 g/100 seeds (Table 1.1). Percentage of seed coat, cotyledon, and embryo percentage exhibited narrow ranges across genotypes (0.4 – 3.1%). Within each seed type, the genotypes that cooked faster when soaked had higher seed weight, lower seed coat percentage, and higher cotyledon percentage than their slower-cooking counterparts.

Seed coat macrosclereid-layer, seed coat osteosclereid-layer, and cotyledon cell wall thicknesses varied by genotype and soaking time and there was a significant genotype by soaking time interaction for these traits (Table S1.1). The macrosclereid layer is the outer layer of the seed coat, and the osteosclereid layer is located between the macrosclereid layer and the parenchyma layer of the seed coat (Figure 1.2).

Seed coat macrosclereid-layer thickness for unsoaked beans ranged 39.6 – 48.6 µm and for the soaked beans ranged 23.4 – 39.6 µm (Figure 1.2; Table S1.2). Soaking decreased

macrosclereid-layer thickness 14.7 – 49.9%. The brown genotypes had the thickest macrosclereid layers when unsoaked, and these layers remained thicker than most other genotypes after soaking. Within seed type pairs, no trends were identified between unsoaked macrosclereid-layer thickness and cooking time of unsoaked or soaked beans. However, genotypes that cook faster when unsoaked had macrosclereid layers that thinned to a greater extent following soaking than those of their slower-cooking counterparts within all seed type pairs. This conveys that macrosclereid-layer thickness is relevant for cooking time of unsoaked beans as they hydrate during the cooking process. For soaked beans, macrosclereid-layer thickness does not appear to relate to cooking time.

Seed coat osteosclereid-layer thickness of unsoaked beans ranged 10.4 – 15.5 µm and for soaked beans ranged 7.4 – 13.7 µm (Figure 1.2; Table S1.2). Soaking decreased osteosclereid-layer thickness 2.1 – 37.2%. For all seed types except yellow, thicker unsoaked osteosclereid layers were observed in genotypes that cooked slower when unsoaked. For all seed types except cranberry, osteosclereid layers were thinner after soaking in the genotypes that cook faster when unsoaked as compared to their slower-cooking counterparts. These trends convey that osteosclereid-layer thickness is relevant for cooking time of unsoaked beans both before and after they are partially or fully hydrated during the cooking process.

Cotyledon cell wall thickness for unsoaked beans ranged 0.96 – 1.41 µm and for soaked beans ranged 0.63 – 1.12 µm (Figure 1.3; Table S1.2). Soaking decreased cotyledon cell wall thickness 17.5 – 37.0%. Within all seed type pairs, the genotypes that cooked faster when soaked had thinner cotyledon cell walls in both unsoaked and soaked treatments as compared to their slow-cooking counterparts.

Seed Coat and Cotyledon Cell Wall Compositional Traits

Objective 3 was to determine compositional differences in total, soluble, and whole seed dietary fiber content and insoluble cotyledon cell wall isolate and how these factors relate to genetic variability for cooking time (Figure S1.1). Total and insoluble whole seed fiber and insoluble cotyledon cell wall isolate varied by genotype (Table S1.1).

Total whole seed dietary fiber ranged 13.6 – 21.8 g/100 g milled beans; soluble whole seed fiber ranged 3.1 – 6.9 g/100 g milled beans; and insoluble whole seed fiber ranged 8.3 – 16.8 g/100 g milled beans (Figure 1.4; Table S1.3). Total and insoluble whole seed dietary fiber were lower in the genotypes that cook faster when soaked within all seed types except cranberry, which showed no significant differences for insoluble whole seed dietary fiber.

Total cotyledon cell wall isolate ranged 106.4 – 134.7 mg/g cotyledon; soluble cotyledon cell wall isolate ranged 27.6 – 44.1 mg/g cotyledon; and insoluble cotyledon cell wall isolate ranged 67.0 – 95.0 mg/g cotyledon (Figure 1.4; Table S1.3). There were no significant differences between genotypes for total or soluble cotyledon cell wall isolate, but there were significant genotypic differences for insoluble cotyledon cell wall isolate. Within all seed types except brown, insoluble cotyledon cell wall isolate was lower in the genotypes that cook faster when soaked compared to their slow-cooking counterparts, although only RedMottled-1 and RedMottled-2 had insoluble cotyledon cell wall isolate values that were significantly different from each other.

Principal Component Analysis and Correlations

Principal component analysis (PCA) was performed to relate genetic variability for cooking time with seed coat and cotyledon cell wall physical and compositional traits to support the overall goal of this study. The first two principal components (PCs) explained about 65% of the variation (Figure 1.5). The first principal component (PC) separated the genotypes

approximately by cooking time of soaked beans and seed coat/cotyledon percentage and represented almost half of the variation (44.4%). The second PC represented over a sixth of the variation (20.0%) and separated the genotypes loosely by cooking time of unsoaked beans. The remaining PCs accounted for 14.8, 11.4, 5.3, 3.6, 0.4, and 0% of the variance respectively (data not shown).

For each seed type pair, the genotype that cooks faster when soaked separated toward the bottom right of the biplot with the slower cooking genotypes separating toward the top left (Figure 1.5). The loading for cooking time after a 3 hr soak is positioned between those for cooking time of unsoaked beans and 12 hr soaked beans, representing the transition point from cooking time patterns of unsoaked beans and of soaked beans. The separation of the unsoaked, 3 hr soaked, and 12 hr soaked time points is also an indication of distinct physical and compositional factors related to genetic variability for cooking time depending upon whether beans were soaked and for how long. Significant correlations were identified among soaking time, cooking time, and water uptake such that longer soaking times decreased cooking times ($r_s = -0.37$, p-value < 0.0001) and increased water uptake ($r_s = 0.81$, p-value < 0.0001). (Figure S1.3). Cooking time decreased with increased water uptake ($r_s = -0.42$, p-value < 0.0001).

The PCA biplot indicates that genotypes with fast cooking times when unsoaked had increased levels of soluble cell wall isolate and soluble whole seed dietary fiber and thinner macrosclereid- and osteosclereid-layers (Figure 1.5). However, soluble whole seed dietary fiber and soluble cotyledon cell wall isolates were highly variable with insignificant ANOVAs so there is insufficient evidence to suggest a relationship between these traits and cooking time for this study (Figure 1.4; Tables S1.1 & S1.3). Osteosclereid-layer thickness of unsoaked beans was the only physical characteristic that significantly correlated with cooking time of unsoaked beans, such

that cooking time of unsoaked beans increased with osteosclereid-layer thickness (rs = 0.93, p-value = 0.0081) (Figure S1.4; Table 1.2). However, longer cooking times were associated with thicker macrosclereid (rs = 0.73, p-value = 0.0002) and osteosclereid layers in general (rs = 0.80, p-value = 0.0019) (Figure S1.5), and unsoaked beans exhibited the longest cooking times (Figures 1.1, S1.2). Soaking decreased the thickness of both macrosclereid (rs = -0.84, p-value < 0.0001) and osteosclereid layers (rs = -0.68, p-value = 0.0069) (Figure S1.5).

The PCA biplot also indicates that genotypes with fast cooking times when soaked had higher seed weight, higher cotyledon percentage, lower seed coat percentage, lower cotyledon cell wall thickness, lower total and insoluble whole seed dietary fiber, and lower insoluble cell wall isolate (Figure 1.5). Significant correlations support the relationships between these traits and soaked cooking time. Cooking time of 12 hr soaked beans decreased as seed weight increased (rs = -0.81, p-value = 0.0047); seed coat percentage decreased (rs = 0.95, p-value = 0.0021), and cotyledon percentage increased (rs = -0.95, p-value = 0.0019) (Figure S1.6). The same trend was observed for the 6, 18, and 24 hr soaking times (Table 1.2). Bean genotypes with greater seed weight had a lower seed coat percentage (rs = -0.83, p-value = 0.0174) and higher cotyledon percentage (rs = 0.93, p-value = 0.0084), and seed coat percentage was strongly and negatively correlated with cotyledon percentage (rs = -1.00, p-value < 0.0001) (Figure S1.6). Cooking time increased with cotyledon cell wall thickness (rs = 0.78, p-value = 0.0033), and soaking decreased cotyledon cell wall thickness (rs = -0.73, p-value = 0.0038) (Figure S1.5). Unsoaked and 12 hr soaked cotyledon cell wall thickness significantly correlated with cooking time of 6 hr soaked beans, but not with cooking times of other soak treatments despite high rs (Figure S1.4; Table 1.2). Total and insoluble whole seed dietary fiber correlated positively with cooking time of 6 hr soaked beans, but not with cooking times from other soaked treatments (Figure S1.7; Table 1.2). Insoluble

cotyledon cell wall isolate was correlated with longer cooking times after a 12 hr soak (rs = 0.60, p-value = 0.0160) (Figure S1.7; Table 1.2). Similar correlations were identified for the 6, 18, and 24 hr soaking times (Table 1.2).

DISCUSSION

For all genotypes, cooking time decreased as soaking time approached six or more hours. This negative association between cooking time and soaking time was expected, as it has been identified in prior studies (Bellido et al., 2006; Chigwedere et al., 2019a). Soaking has been shown to activate cell wall enzymes that change the polysaccharide arrangement in the cell wall, which increases the rate of pectic polysaccharide thermosolubility and therefore decreases cooking time (Martínez-Manrique et al., 2011). In addition, beans that are fully or almost fully hydrated prior to cooking require limited water uptake during cooking. Beans soaked for 6 or more hours in this study took up an additional 20-40% of their dry weight in water during cooking, while unsoaked beans took up about 120% of their dry weight during cooking (data not shown).

The genetic variability of how soaking influenced cooking time was interesting because genotypes that cooked faster when soaked (for 6 or more hours) were not the same ones that cooked faster unsoaked as observed in the cranberry, red mottled, and yellow seed types. This suggests that different physical or compositional factors determine cooking times of unsoaked and soaked beans. The extent to which soaking reduces cooking time has been shown to vary by genotype (Martínez-Manrique et al., 2011), which could explain why some genotypes are fast-cooking when soaked but not unsoaked. In addition, water uptake rates vary among genotypes, and some genotypes experience a lag time for water uptake, which would be irrelevant for soaked beans but could be affecting cooking time of unsoaked beans (Ross et al., 2010). Combining the factors that

decrease cooking times of soaked and unsoaked beans into a single cultivar could be a worthwhile quality attribute to appeal to a broad base of consumers. Figure 1.6 summarizes the traits associated with fast cooking time of unsoaked and soaked beans as determined in this study.

For unsoaked beans, fast cooking time is associated with thin macrosclereid and osteosclereid layers in the seed coat (Figure 1.6). Darker beans tend to have thicker macrosclereid layers, as was observed in the brown genotypes, in part due to polyphenol storage (Agbo et al., 1987; Smýkal et al., 2014). Previous studies showed that the thickness of seed coat layers affects water uptake, particularly in the early stages of hydration (Sefa-Dedeh and Stanley, 1979; Deshpande and Cheryan, 1986a; Agbo et al., 1987). Water uptake rate has been shown to impact cooking time (Deshpande and Cheryan, 1986b) and could explain the differences in cooking time observed for the unsoaked and 3 hr soaked treatments, which require a large amount of water to be taken up during cooking as compared to longer soaked treatments. Raw seed macrosclereid- and osteosclereid layer thickness were not correlated with water uptake for any soaking time, although both layers thinned in all genotypes following a 12 hr soak as the beans expanded to accommodate water uptake. Earlier time points may be needed to capture the impact of unsoaked seed coat layer thickness on water uptake in these genotypes. However, negative correlations were observed for 12 hr soaked macrosclereid- and osteosclereid-layer thickness and water uptake after a 3 hr soak. This finding could indicate a relationship between the extent to which the seed coat thins when hydrating and the rate of water uptake, which helps to explain why genotypes with thinner hydrated macrosclereid and osteosclereid layers had faster cooking times when cooked unsoaked. This is in line with the finding that thinner seed coats are associated with faster water uptake (Sefa-Dedeh and Stanley, 1979; Deshpande and Cheryan, 1986a; Agbo et al., 1987).

For soaked beans, fast cooking time is associated with high seed weight, low seed coat percentage/high cotyledon percentage, thin cotyledon cell walls, low total and insoluble fiber, and low insoluble cotyledon cell wall isolate (Figure 1.6). Larger beans have lower seed coat percentage and higher cotyledon percentage, which were found to associate with fast cooking time of soaked beans. The relationship between seed weight and cooking time of soaked beans has been previously identified (Cichy et al., 2015). Hydrated seeds coats allow free movement of water into the bean, and larger beans have more surface area, allowing for an increased rate of water movement into the bean during cooking (Deshpande and Cheryan, 1986a). Seed weight, seed coat percentage, and cotyledon percentage were not associated with cooking time of unsoaked beans. The surface area of an unsoaked bean may be less relevant than the permeability of the seed coat for water uptake during cooking, explaining a lack of correlation between cooking time of unsoaked beans and seed weight. Seed coat impermeability has been previously associated with hardshell, a textural defect with both genetic and environmental causes that results in seed hardness (Bourne, 1967; Jackson and Varriano-Marston, 1981; Stanley, 1992). In dry beans and cowpea, seed hardness was found to decrease with water uptake and increase with cooking time (Sefa-Dedeh et al., 1978, 1979; Castellanos et al., 1995; Marques Corrêa et al., 2010).

The relationship between cotyledon cell wall thickness and cooking time of soaked beans is consistent with a prior study that found thicker cotyledon cell walls were associated with poor cell separation and longer cooking times in the context of the hard-to-cook phenomenon (Yousif and Deeth, 2003). Lignification and associations between hemicellulose and nitrogenous compounds have been associated with cell wall thickening during storage (Hincks and Stanley, 1987; Yousif and Deeth, 2003). Differences in cotyledon cell wall thickness and capacity for cell wall thickening during storage could be associated with genetic variability for cooking time.

Thicker cells walls translate to increased fiber, which is largely comprised of cell wall polysaccharides (Shiga et al., 2009), and cotyledon cell wall isolate. Total and insoluble whole seed dietary fiber and insoluble cotyledon cell wall isolate were all positively correlated with cooking time of soaked beans in this study. Softening of the cotyledon during cooking is mainly attributed to cell wall polysaccharide solubilization and pectin solubilization in the middle lamella (Chigwedere et al., 2018). An increase in insoluble cotyledon cell wall isolate and thereby insoluble whole seed dietary fiber could delay or prolong this solubilization, causing the observed increase in cooking time of soaked beans. In addition, crude fiber content has been found to increase resistance to water uptake (Saio, 1976; Deshpande and Cheryan, 1986a). A fiber-associated resistance to water uptake into cotyledon cells during cooking could be contributing to increased cooking time of soaked beans as an expression of the genetic variability for cooking time. A relationship has been previously identified between increased fiber content and the hard-to-cook phenomenon, which prevents cotyledons from taking up water and expanding (Gloyer, 1921; Agbo et al., 1987; de Godínez, 1990; Rodriguez and Mendoza, 1990; Gonzalez and Paredes-Lapez, 1993).

CONCLUSIONS

This study evaluated cooking time, soaking time, physical traits, and cell wall and seed coat compositional traits across four seed types of dry beans. The relationship between soaking time and cooking time was explored across these seed types to reveal that genetic factors related to the fast-cooking trait are not consistent across unsoaked and soaked treatments. Physical and compositional traits of the seed coat and cotyledon cell wall were identified that relate to cooking time for unsoaked or soaked beans via spearman correlation, PCA, and general trends. These

relationships help to reveal factors associated with fast cooking time in both unsoaked and soaked beans. Cooking time of soaked beans appears to be related to seed weight, cotyledon/seed coat percentage, cotyledon cell wall thickness, insoluble cell wall isolate, and total and insoluble whole seed dietary fiber. These traits affect cell separation, water uptake, and water transport during cooking. The thicknesses of seed coat layers appear to be related to cooking time of unsoaked beans. These traits also affect water uptake and transport, but at an earlier stage in the hydration process.

Understanding the factors associated with genetic variability for cooking time in unsoaked and soaked beans is useful to direct progress in breeding fast-cooking beans as well as to recognize the potential consequences of faster-cooking germplasm, including trade-offs like reduced fiber or seed coat integrity.

ACKNOWLEDGEMENTS

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APPENDICES

APPENDIX A:

CHAPTER 1 TABLES AND FIGURES

Table 1.1 Means for all genotypes of seed weight; cotyledon, seed coat, and embryo percentage.

	Brown		Cranberry		Red Mottled		Yellow	
Trait	1	2	1	2	1	2	1	2
Seed Weight (g/100 seeds)								
	58.9^{b}	49.4^{cd}	64.4^{a}	44.4^{e}	48.9^{d}	38.9^{f}	56.3^{b}	52.1^{c}
Seed Coat Percentage (%)								
	7.1^{cde}	8.5^{ab}	6.7^{de}	7.6^{bcd}	8.0^{abc}	9.1^{a}	6.0^{e}	7.1^{cde}
Cotyledon Percentage (%)								
	92.0^{abc}	90.3^{de}	92.5^{ab}	91.1^{cd}	91.0^{cd}	89.8^{e}	92.9^{a}	91.8^{bc}
Embryo Percentage (%)								
	1.0^{bcd}	1.2^{ab}	0.8^{d}	1.2^{a}	0.9^{cd}	1.1^{abc}	1.1^{abc}	1.1^{abc}

Mean separation within seed type pairs is indicated by the letter superscript.

Table 1.2 Spearman correlations of all traits with cooking times from 0, 3, 6, 12, 18, and 24 hr soaked samples.

	Cooking Time					
Soaking Time	0	3	6	12	18	24
Seed Weight	-0.24	-0.31	-0.64*	-0.81**	-0.83**	-0.83**
Seed Coat Percentage	0.36	0.67	0.93**	0.95**	0.93**	0.93**
Cotyledon Percentage	-0.36	-0.67	-0.93**	-0.95**	-0.93**	-0.93**
Embryo Percentage	-0.11	0.14	0.18	0.38	0.26	0.26
Macrosclereid Layer (U)‡	-0.07	0.60	0.38	0.14	0.05	0.05
Macrosclereid Layer (S)	0.55	0.57	0.07	0.02	0.00	0.00
Osteosclereid Layer (U)	0.93**	0.48	0.74	0.74	0.76	0.76
Osteosclereid Layer (S)	0.67	0.45	0.12	0.24	0.19	0.19
Cotyledon Cell Wall (U)	0.12	0.43	0.74*	0.76	0.69	0.69
Cotyledon Cell Wall (S)	0.29	0.52	0.76*	0.83	0.74	0.74
Total Whole Seed Dietary Fiber	-0.05	0.52	0.71*	0.69	0.55	0.55
Soluble Whole Seed Dietary Fiber	-0.36	-0.14	-0.50	-0.33	-0.45	-0.45
Insoluble Whole Seed Dietary Fiber	0.43	0.55	0.88**	0.79	0.71	0.71
Total Cotyledon Cell Wall Isolate	-0.17	0.48	0.69	0.74	0.67	0.67
Soluble Cotyledon Cell Wall Isolate	-0.40	-0.33	-0.55	-0.45	-0.52	-0.52
Insoluble Cotyledon Cell Wall Isolate	0.07	0.55	0.62*	0.6*	0.67**	0.67*

† *P*-values are indicated by asterisks, where *, **, and *** represent <0.05, <0.01, and <0.001 respectively.
‡ U and S indicate unsoaked and soaked (12 hr), respectively

Figure 1.1 Scatterplots of cooking time and water uptake vs soaking time and images of the genotypes used in this study. Circles indicate cooking time and squares indicate water uptake.

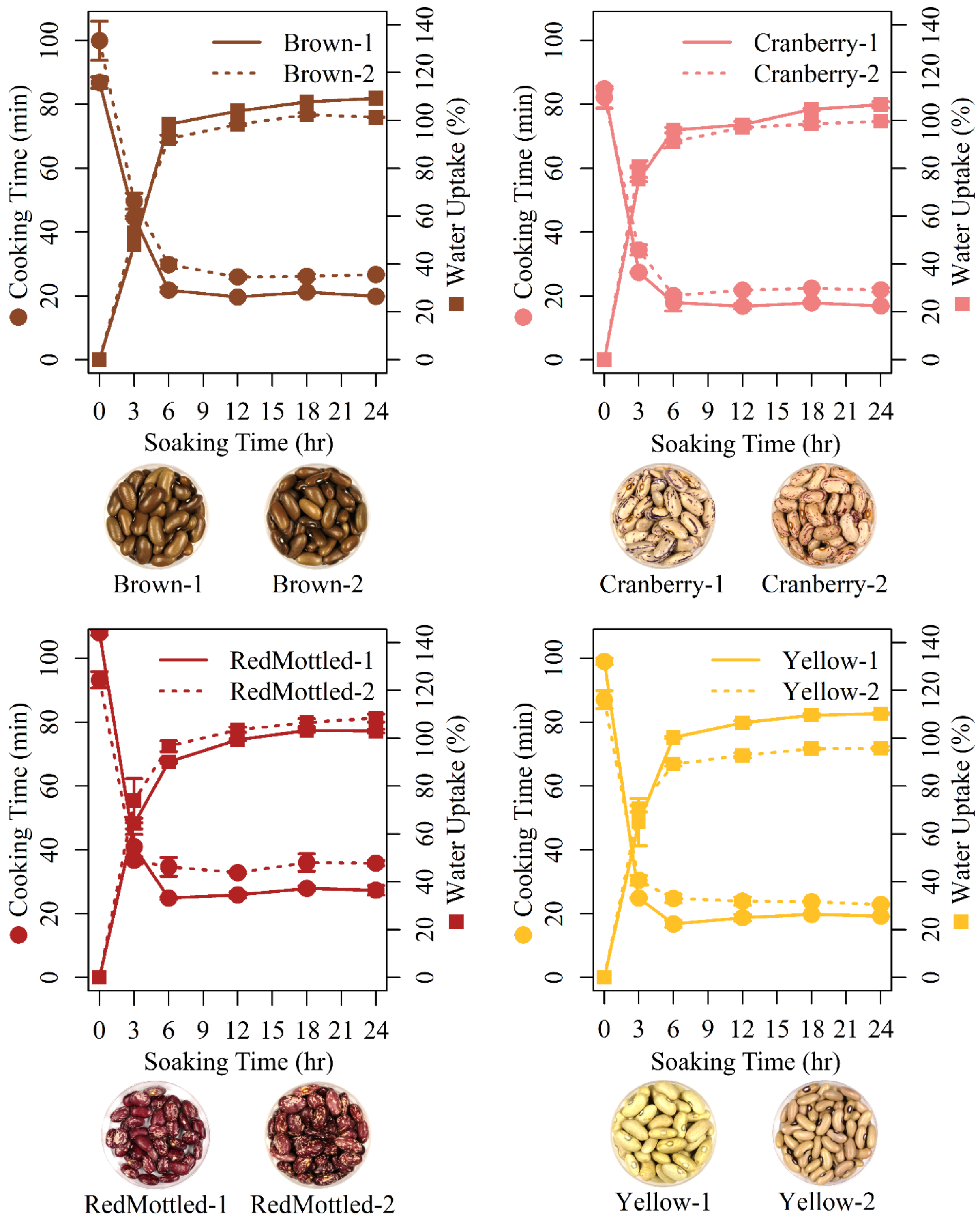


Figure 1.2 Bar plots of seed coat layer thickness for unsoaked and soaked (12 hr) beans with seed type and genotype indicated. Example SEM images (RedMottled-1) of the seed coat layers are presented with the measured layers indicated. MS = Macrosclereid layer; OS = osteosclereid layer.

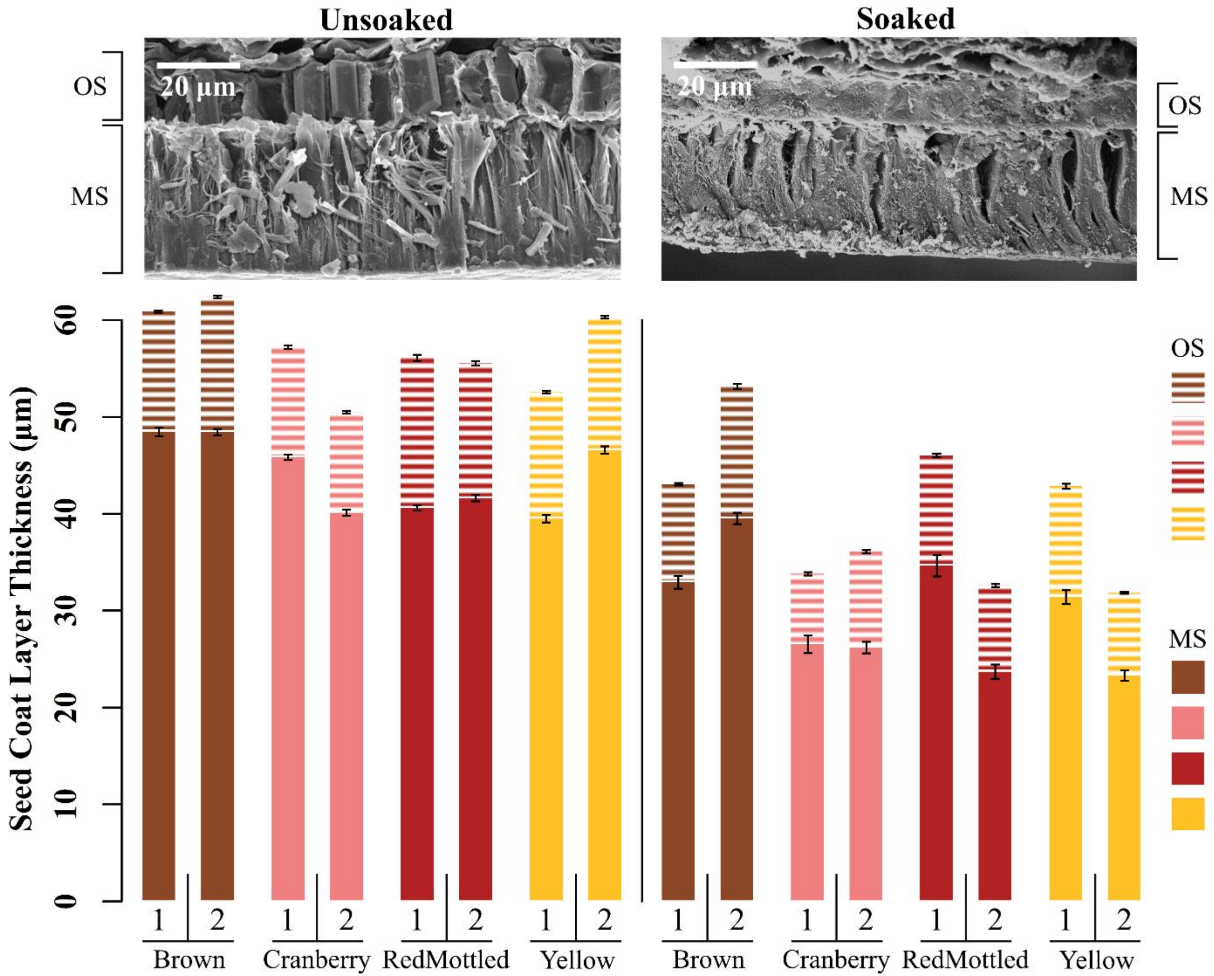


Figure 1.3 Bar plots of cotyledon cell wall thickness for unsoaked and soaked (12 hr) beans with seed type and genotype indicated. Example SEM images (RedMottled-1) of cotyledon cells are presented with locations of measurements indicated by white arrows.

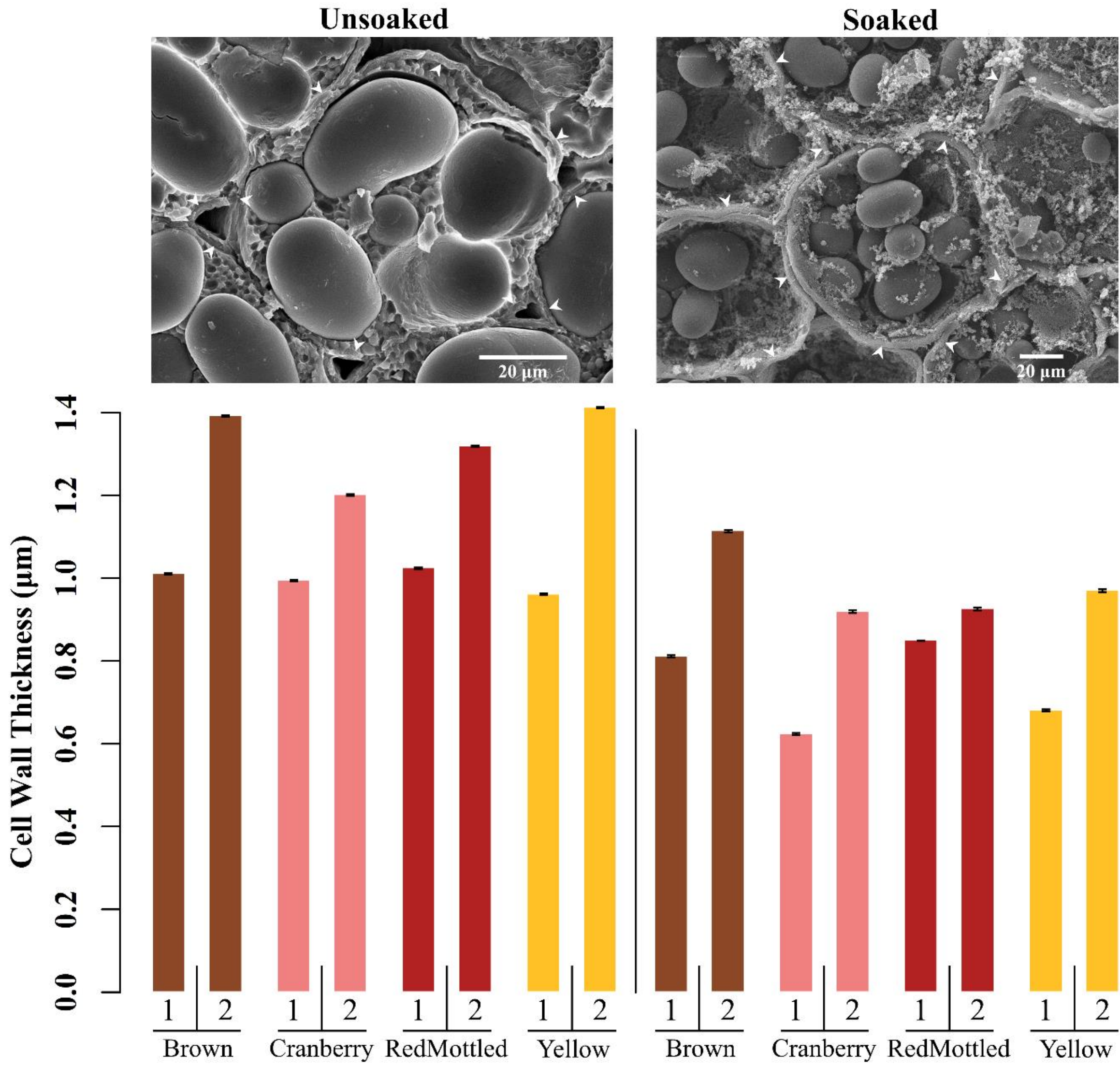


Figure 1.4 Bar plots of soluble and insoluble whole seed dietary fiber and cotyledon cell wall isolate of raw beans with seed type and genotype indicated.

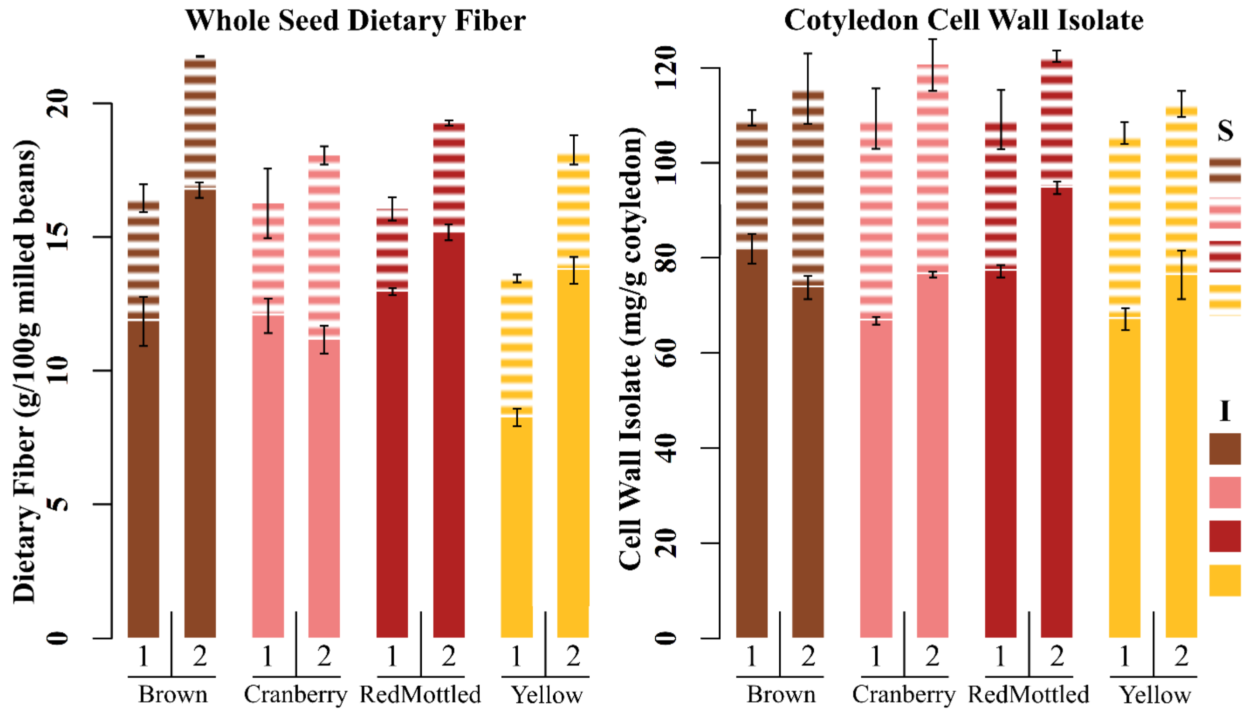


Figure 1.5 Principal component analysis biplot with each genotype indicated and loadings for cooking times across 0, 3, and 12 hr soaking times (CT0, CT3, and CT12); seed weight (SeedWt); seed coat (SeedCoat), cotyledon (Cotyledon), and embryo (Embryo) percentage; unsoaked and soaked (12 hr) macrosclereid-layer (MST0 and MST12), osteosclereid-layer thickness (OST0 and OST12), and cotyledon cell wall (CWT0 and CWT12); raw total (TCWI), soluble (SCWI), and insoluble (ISCWI) cotyledon cell wall isolate; and raw total (TFiber), soluble (SFiber), and insoluble (IFiber) whole seed dietary fiber.

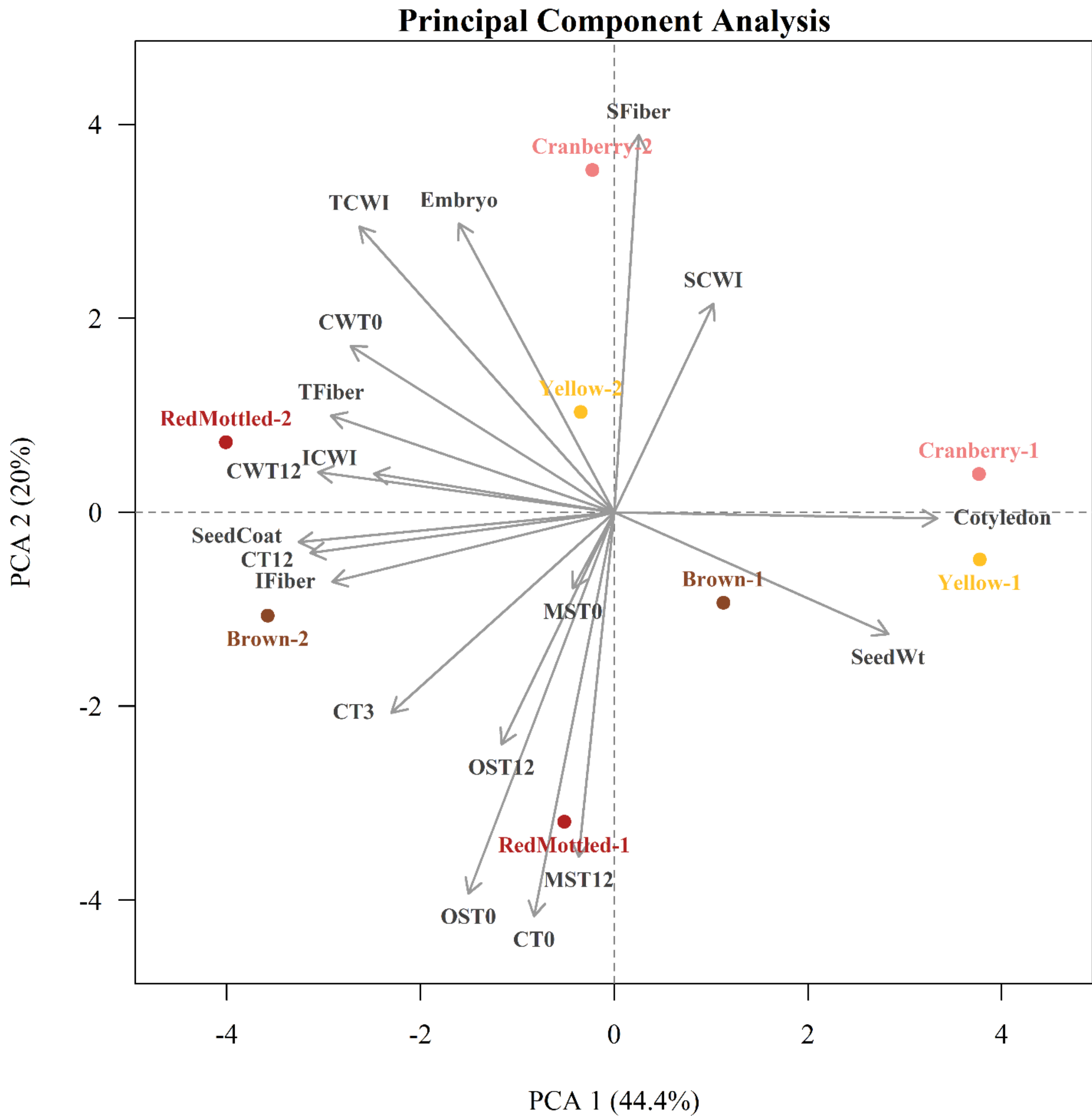
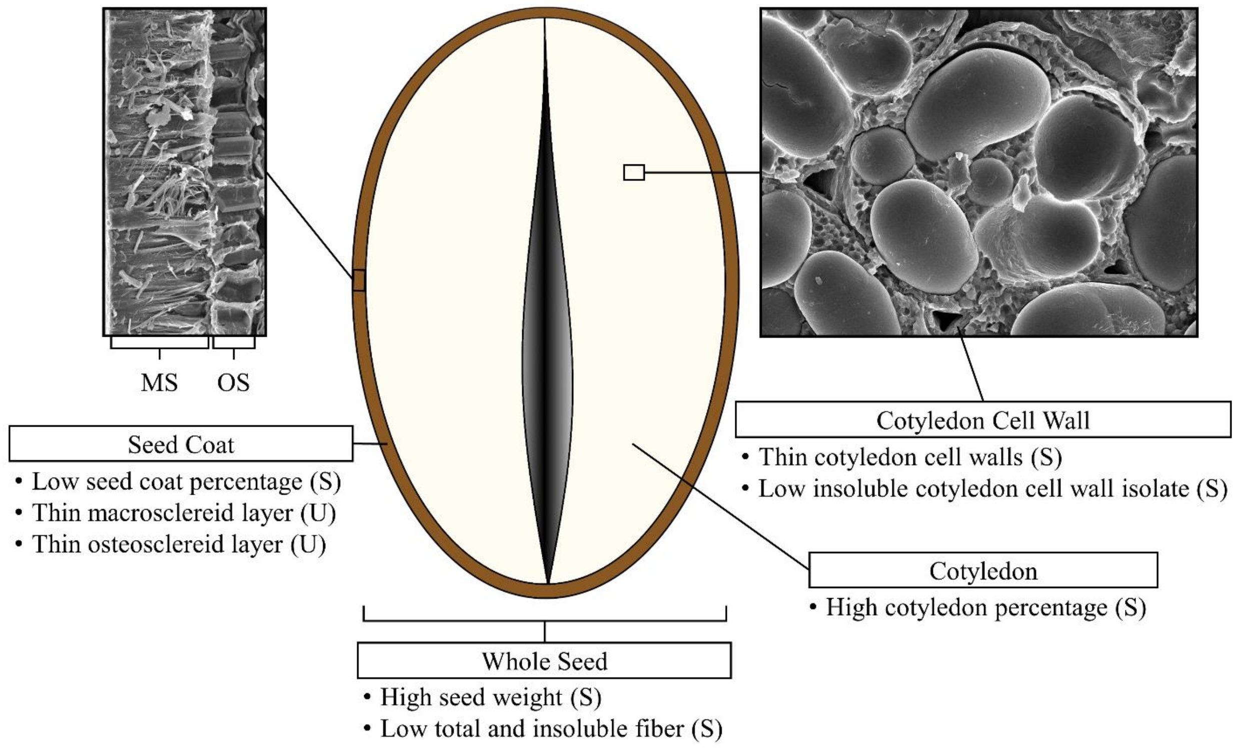


Figure 1.6 Diagram of a dry bean cross section indicating the traits associated with fast cooking time of unsoaked (U) or soaked (S) beans. MS = macrosclereid layer; OS = Osteosclereid layer.



APPENDIX B:

CHAPTER 1 SUPPLEMENTAL TABLES AND FIGURES

Table S1.1 ANOVA results[†] indicating the significance of the fixed effects genotype, soaking time, and genotype by soaking time for all traits.

Trait	Genotype	Soak	Genotype by Soak
Water Uptake	<0.0001	<0.0001	<0.0001
Cooking Time	<0.0001	<0.0001	<0.0001
Seed Weight	<0.0001	.	.
Seed Coat Percentage	0.0001	.	.
Cotyledon Percentage	<0.0001	.	.
Embryo Percentage	0.0008	.	.
Macrosclereid-layer Thickness	<0.0001	<0.0001	<0.0001
Osteosclereid-layer Thickness	<0.0001	<0.0001	<0.0001
Cotyledon Cell Wall Thickness	<0.0001	<0.0001	<0.0001
Total Whole Seed Dietary Fiber	0.0003	.	.
Soluble Whole Seed Dietary Fiber	NS	.	.
Insoluble Whole Seed Dietary Fiber	0.0006	.	.
Total Cotyledon Cell Wall Isolate	NS	.	.
Soluble Cotyledon Cell Wall Isolate	NS	.	.
Insoluble Cotyledon Cell Wall Isolate	0.0001	.	.

† *P*-values, where NS indicates *p*-values that are not significant at $\alpha = 0.05$

Table S1.2 Means for all genotypes of unsoaked and soaked (12 hr) macrosclereid-layer, osteosclereid-layer, and cotyledon cell wall thickness.

		Brown		Cranberry		Red Mottled		Yellow	
Trait	Soak	1	2	1	2	1	2	1	2
Macrosclereid-layer Thickness (µm)									
	0	48.6^{a}	48.5^{a}	46.0^{b}	40.2^{d}	40.7^{cd}	41.7^{c}	39.6^{d}	46.7^{b}
	12	33.0^{b}	39.6^{a}	26.6^{c}	26.3^{c}	34.7^{b}	23.8^{c}	31.5^{b}	23.4^{c}
Osteosclereid-layer Thickness (µm)									
	0	12.4^{cd}	14.0^{b}	11.4^{de}	10.4^{e}	15.5^{a}	13.9^{b}	13.1^{bc}	13.7^{b}
	12	10.2^{c}	13.7^{a}	7.4^{f}	9.9^{cd}	11.4^{b}	9.0^{de}	11.5^{b}	8.6^{e}
Cotyledon Cell Wall Thickness (µm)									
	0	1.01^{ef}	1.39^{b}	1.00^{f}	1.20^{d}	1.03^{e}	1.32^{c}	0.96^{g}	1.41^{a}
	12	0.81^{e}	1.12^{a}	0.63^{g}	0.92^{c}	0.85^{d}	0.93^{c}	0.68^{f}	0.97^{b}

Mean separation within seed type pairs is indicated by the letter superscript.

Table S1.3 Means for all genotypes (raw) of whole seed total, soluble, and insoluble fiber and total, soluble, and insoluble cotyledon cell wall isolate.

	Brown		Cranberry		Red Mottled		Yellow	
Trait	1	2	1	2	1	2	1	2
Total Whole Seed Dietary Fiber (g/100 g milled beans)								
	16.5^{bcd}	21.8^{a}	16.3^{bcd}	18.0^{bc}	16.1^{cd}	19.3^{ab}	13.6^{d}	18.4^{bc}
Soluble Whole Seed Dietary Fiber (g/100 g milled beans)								
	4.6^{a}	5.0^{a}	4.2^{a}	6.9^{a}	3.1^{a}	4.1^{a}	5.2^{a}	4.5^{a}
Insoluble Whole Seed Dietary Fiber (g/100 g milled beans)								
	11.9^{bcd}	16.8^{a}	12.1^{bc}	11.2^{cd}	13.0^{bc}	15.2^{ab}	8.3^{d}	13.8^{abc}
Total Cotyledon Cell Wall Isolate (mg/g cotyledon)								
	109.7^{a}	115.8^{a}	109.4^{a}	120.8^{a}	122.7^{a}	134.7^{a}	106.4^{a}	112.6^{a}
Soluble Cotyledon Cell Wall Isolate (mg/g cotyledon)								
	27.7^{a}	41.8^{a}	42.4^{a}	44.1^{a}	32.0^{a}	27.6^{a}	39.1^{a}	36.0^{a}
Insoluble Cotyledon Cell Wall Isolate (mg/g cotyledon)								
	82.0^{ab}	74.0^{bc}	67.0^{c}	76.7^{bc}	77.4^{bc}	95.0^{a}	67.3^{c}	76.6^{bc}

Mean separation (by row) is indicated by the letter superscript.

Figure S1.1 Workflow depicting the steps for each objective.

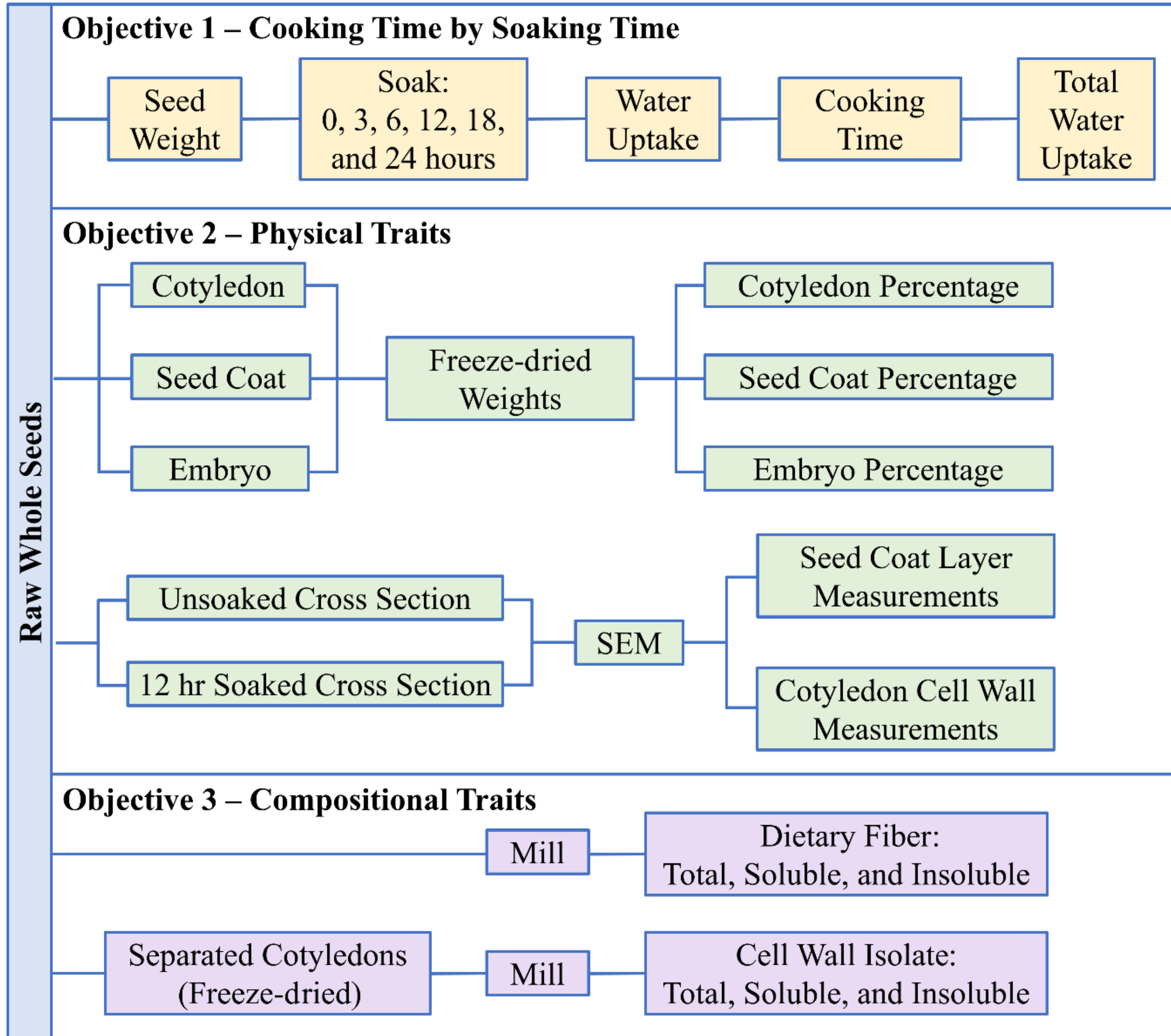


Figure S1.2 Means of cooking time and water uptake for all genotypes across 0, 3, 6, 12, 18, and 24 hr soaking times. Within seed type pairs, the faster cooking time for each soaking time is indicated in red. Mean separation of each trait (by row) is indicated by the letter superscript.

Seed Type	Soak (hr)	Cooking Time (min)		Water Uptake (%)	
Brown		1	2	1	2
	0	86.9^{b}	100.0^{a}	.	.
	3	44.6^{a}	49.7^{a}	47.8^{a}	53.0^{a}
	6	21.8^{b}	29.8^{a}	98.4^{a}	92.3^{b}
	12	**19.7^{b}**	**26.0^{a}**	**103.9^{a}**	**98.3^{b}**
	18	21.2^{b}	26.1^{a}	107.7^{a}	102.4^{b}
	24	19.9^{b}	26.7^{a}	109.2^{a}	101.3^{b}
Cranberry		1	2	1	2
	0	84.9^{a}	82.3^{a}	.	.
	3	27.4^{b}	34.4^{a}	80.6^{a}	75.4^{a}
	6	18.0^{a}	20.1^{a}	91.2^{b}	95.9^{a}
	12	**16.7^{b}**	**21.8^{a}**	**97.0^{a}**	**98.2^{a}**
	18	17.9^{b}	22.4^{a}	98.6^{b}	104.6^{a}
	24	16.9^{b}	21.9^{a}	99.6^{b}	106.6^{a}
Red Mottled		1	2	1	2
	0	108.1^{a}	93.3^{b}	.	.
	3	41.0^{a}	36.7^{a}	64.3^{a}	73.9^{a}
	6	24.9^{b}	34.7^{a}	90.1^{b}	96.7^{a}
	12	**25.9^{b}**	**32.8^{a}**	**99.3^{b}**	**103.5^{a}**
	18	27.9^{b}	36.0^{a}	103.2^{b}	106.5^{a}
	24	27.3^{b}	35.9^{a}	103.0^{b}	108.4^{a}
Yellow		1	2	1	2
	0	99.1^{a}	87.1^{b}	.	.
	3	24.9^{a}	30.5^{a}	64.9^{a}	70.6^{a}
	6	16.8^{b}	24.8^{a}	100.4^{a}	89.2^{b}
	12	**18.8^{b}**	**23.9^{a}**	**106.4^{a}**	**92.8^{b}**
	18	19.8^{b}	23.7^{a}	109.6^{a}	95.6^{b}
	24	19.3^{b}	22.9^{a}	110.2^{a}	95.7^{b}

Figure S1.3 Pairwise comparison matrix of soaking time (Soak), cooking time (CT), and water uptake (WU) across 0, 3, 6, 12 18, and 24 hr soaking times. Spearman correlation coefficients are indicated in the lower left, and scatterplots for each pairwise comparison with LOWESS regression lines are shown in the upper right. *P*-values are indicated by asterisks, where *, **, and *** represent <0.05, <0.01, and <0.001 respectively.

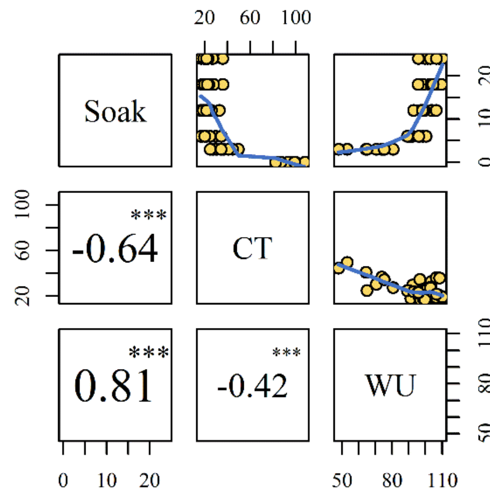


Figure S1.4 Pairwise comparison matrix of cooking times of unsoaked (CT0) and 12 hr soaked (CT12) beans, unsoaked (MST0) and 12 hr soaked (MST12) macrosclereid-layer thickness, unsoaked (OST0) and 12 hr soaked (OST12) osteoclereid-layer thickness, and unsoaked (CWT0) and 12 hr soaked (CWT12) cotyledon cell wall thickness. Spearman correlation coefficients are indicated in the lower left, and scatterplots for each pairwise comparison with LOWESS regression lines are shown in the upper right. *P*-values are indicated by asterisks, where *, **, and *** represent <0.05, <0.01, and <0.001 respectively.

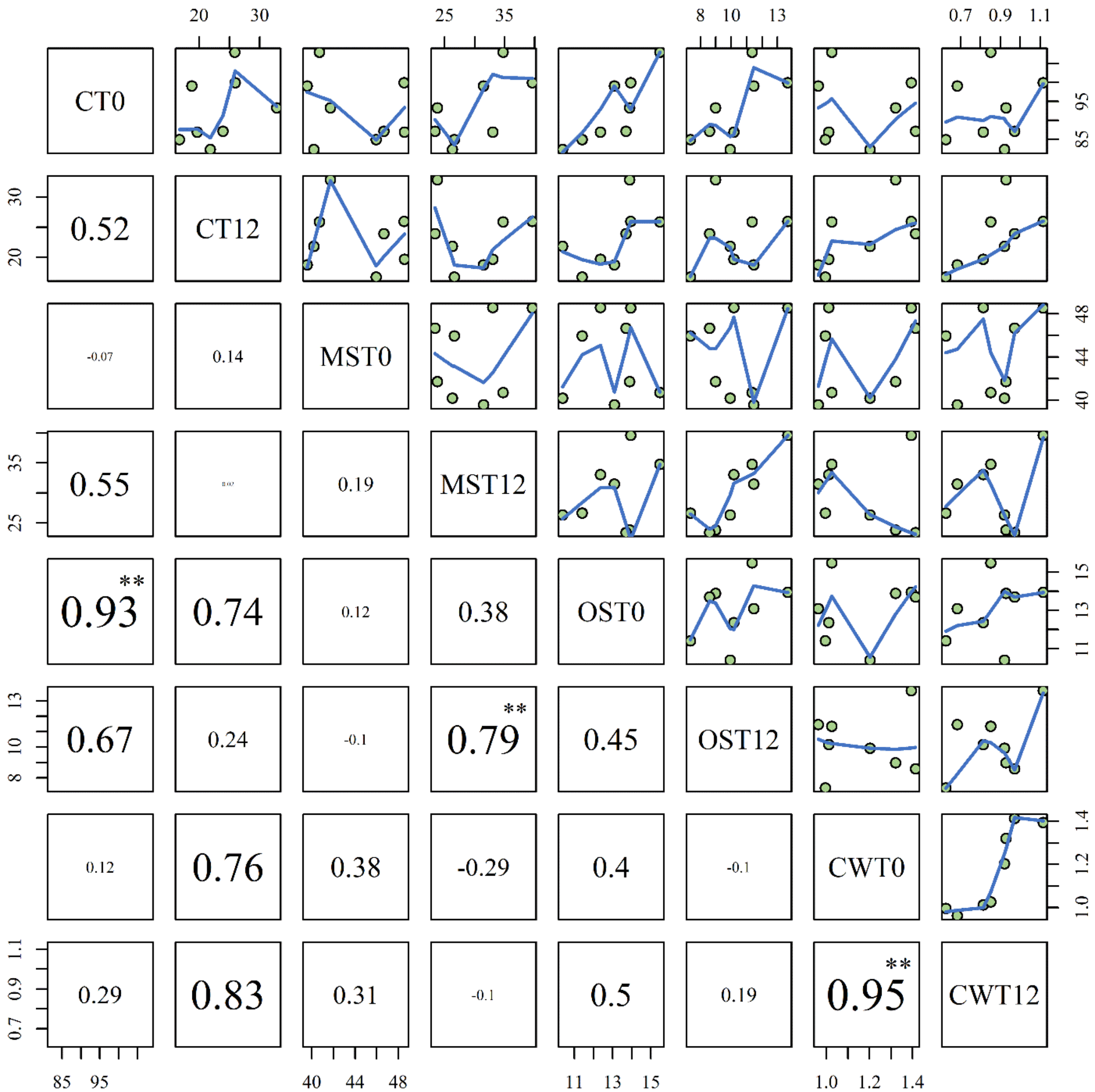


Figure S1.5 Pairwise comparison matrix of soaking time (Soak), cooking time (CT), macrosclereid-layer thickness (MST), osteoclereid-layer thickness (OST), and cotyledon cell wall thickness (CWT). Spearman correlation coefficients are indicated in the lower left, and scatterplots for each pairwise comparison with LOWESS regression lines are shown in the upper right. *P*-values are indicated by asterisks, where *, **, and *** represent <0.05, <0.01, and <0.001 respectively.

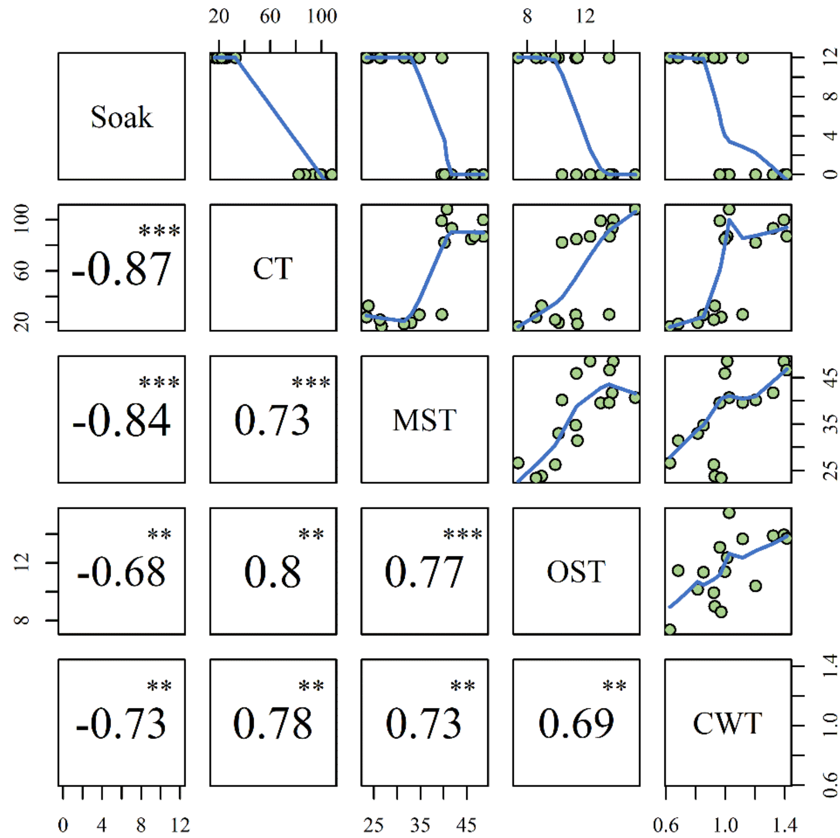


Figure S1.6 Pairwise comparison matrix of cooking time of unsoaked beans (CT0) and cooking time of 12 hr soaked beans (CT12), seed weight (SeedWt), and seed coat (SeedCoat), cotyledon (Cotyledon), and embryo (Embryo) percentage. Spearman correlation coefficients are indicated in the lower left, and scatterplots for each pairwise comparison with LOWESS regression lines are shown in the upper right. *P*-values are indicated by asterisks, where *, **, and *** represent <0.05, <0.01, and <0.001 respectively.

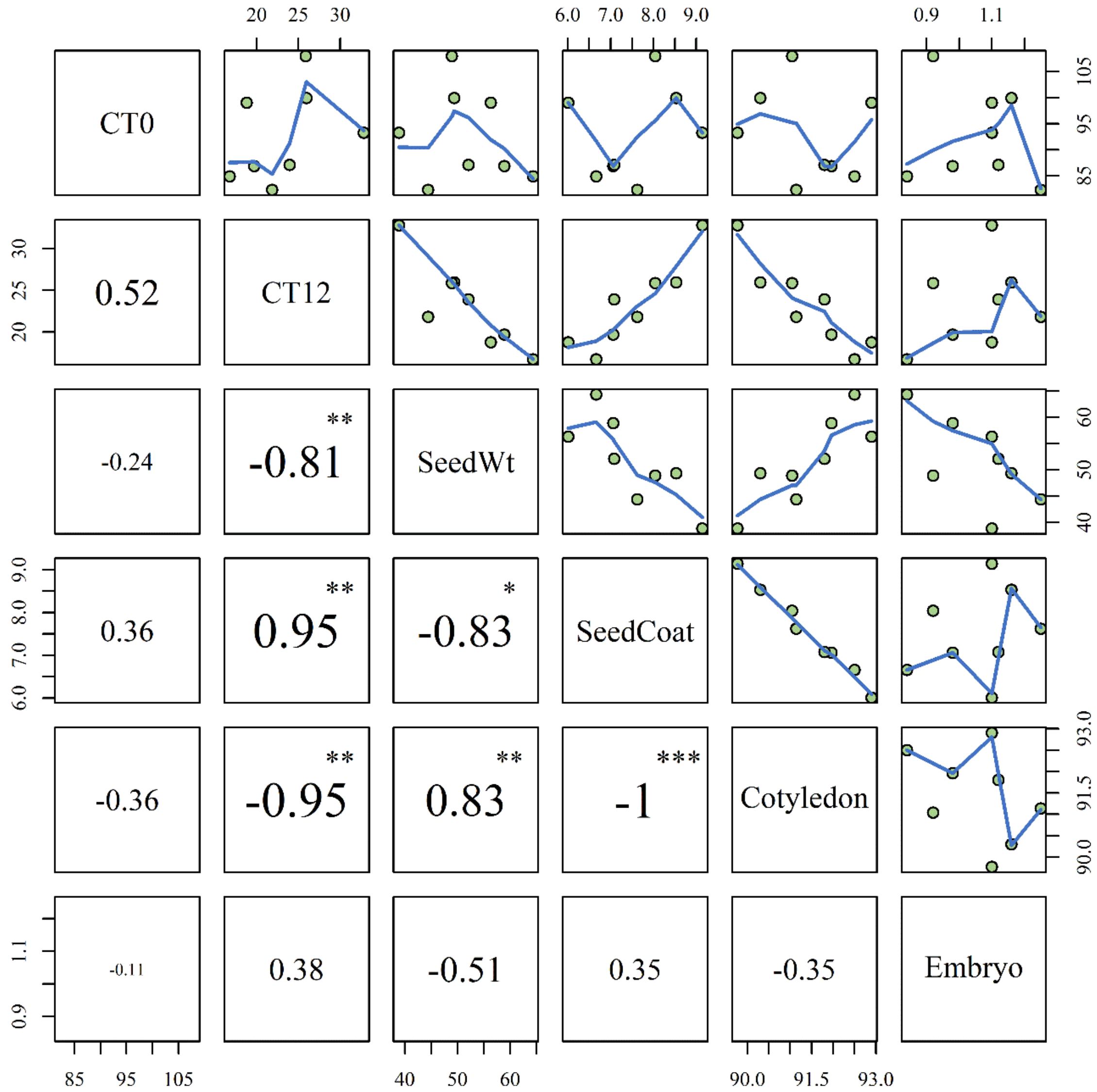
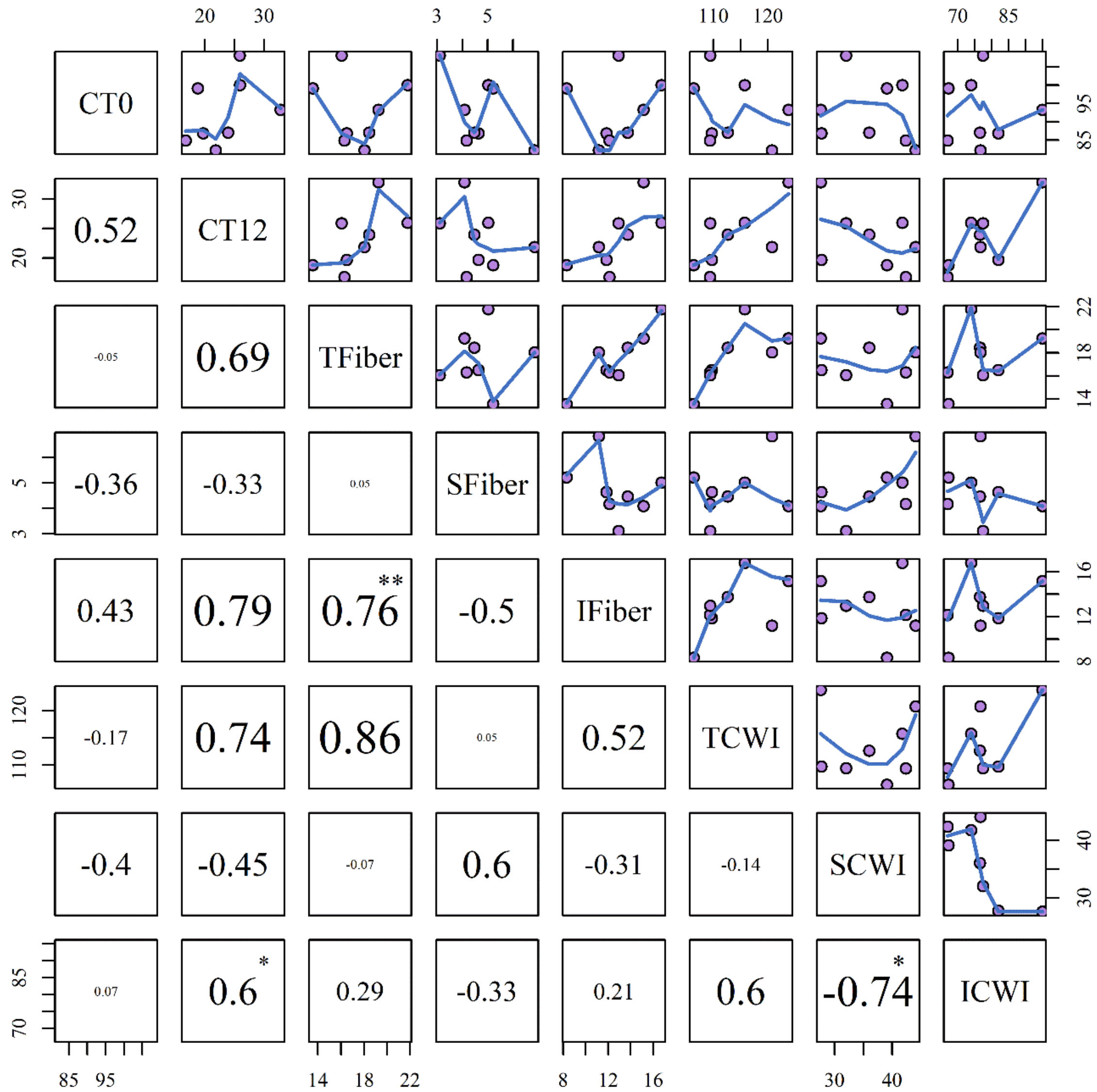


Figure S1.7 Pairwise comparison matrix of cooking times of unsoaked (CT0) and 12 hr soaked (CT12) beans; total (TFiber), soluble (SFiber), and insoluble (IFiber) whole seed dietary fiber; and total (TCWI), soluble (SCWI), and insoluble (ICWI) cotyledon cell wall isolate. Spearman correlation coefficients are indicated in the lower left, and scatterplots for each pairwise comparison with LOWESS regression lines are shown in the upper right. *P*-values are indicated by asterisks, where *, **, and *** represent <0.05, <0.01, and <0.001 respectively.



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CHAPTER 2:

GENETIC VARIABILITY AND GENOME-WIDE ASSOCIATION ANALYSIS OF FLAVOR AND TEXTURE IN COOKED BEANS (*Phaseolus vulgaris* L.)

[Submitted for publication in Theoretical and Applied Genetics]

Genetic variability and genome-wide association analysis of flavor and texture in cooked beans (*Phaseolus vulgaris* L.)

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ABSTRACT

Dry beans are a nutritious food recognized as a staple globally, but consumption is low in the US. Improving dry bean flavor and texture through breeding has the potential to improve consumer acceptance and suitability for new end-use products. Little is known about the genetic variability and inheritance of bean sensory characteristics. A total of 430 genotypes of the Andean Diversity Panel of 20 seed types were grown in three locations, and cooked seeds were evaluated by a trained sensory panel for flavor and texture attribute intensities, including total flavor, beany, vegetative, earthy, starchy, sweet, bitter, seed coat perception, and cotyledon texture. Extensive variation in sensory attributes was found across and within seed types. A set of genotypes was identified that exhibit extreme attribute intensities generally stable across all three environments. Seed coat perception and total flavor intensity had the highest broad-sense heritability (0.39 and 0.38 respectively), while earthy and vegetative intensities exhibited the lowest (0.14 and 0.15

respectively). Starchy and sweet flavors were positively correlated and highest in white bean genotypes according to PCA. SNPs associated with total flavor intensity (6 SNPs across three chromosomes), beany (5 SNPs across 4 chromosomes), earthy (3 SNPs across two chromosomes), starchy (1 SNP), bitter (1 SNP), seed coat perception (3 SNPs across 2 chromosomes), and cotyledon texture (2 SNPs across 2 chromosomes) were detected. These findings lay a foundation for incorporating flavor and texture in breeding programs for the development of new varieties that entice growers, consumers, and product developers alike.

INTRODUCTION

Dry beans (*Phaseolus vulgaris* L.) are a nutritious food that serve as a staple in many majority-world countries (Akibode and Maredia, 2011). Despite their global pervasiveness, they have limited consumption in the US, with only 2.2 kg per capita consumed in 2019 (Parr and Lucier, 2020). In the US, primary breeding goals for dry beans include yield, processing quality, disease resistance, architecture, agronomic adaptation, stress tolerance, and grower friendliness, which encompasses traits that reduce labor and inputs required by growers (Kelly and Cichy, 2012). Quality characteristics such as flavor and texture, however, have largely been overlooked in breeding programs. Quality is most commonly addressed through processing and the addition of sauces and flavors, especially to canned beans and bean products, often at the expense of nutritional value (Borchgrevink, 2013; Roland et al., 2017; Gilham et al., 2018). Taste is a primary factor driving consumer purchasing decisions of food, which motivates food companies to invest heavily in this aspect of product development (William Blair, 2016; IFIC, 2019). Consumers are also very interested in clean labels and food products with few additives (Asioli et al., 2017). Therefore improving dry bean flavor and texture through breeding has the potential to increase

consumer acceptance and utilization of beans and inclusion of beans as ingredients in products while appealing to consumers' interest in flavor without many additives.

Along with cooking time and price, flavor and texture are important characteristics that consumers consider when purchasing dry beans, influencing their decisions regarding market class and product type (Castellanos et al., 1997; Scott and Maideni, 1998; Leterme and Carmenza Muñoz, 2002; Eihusen and Albrecht, 2007; Winham et al., 2019). However, for many consumers, beans are not palatable, and the beany flavor they impart when used as ingredients is often perceived as undesirable (Nachay, 2017; Dougkas et al., 2019). Flavor and texture are not typically evaluated prior to variety release in the U.S., and this lack of focus on sensory quality may be limiting consumption of dry beans below their potential.

A breeding approach to address flavor and texture in beans has not been explored in part due to the complexity and cost associated with sensory evaluations. Protocols have been developed for the preparation and evaluation of cooked bean samples as well as the training and maintenance of sensory panels (Koehler et al., 1987; Sanz-Calvo and Atienza-del-Rey, 1999; Romero del Castillo et al., 2008; Romero del Castillo et al., 2012), but these protocols are designed for few samples with plentiful seed and are not feasible to implement in breeding programs. The application of these sensory methods have identified genetic variability for texture and flavor acceptability (Koehler et al., 1987) and attribute intensities, including seed-coat perception, roughness, mealiness, and beany flavor (Rivera et al., 2013). This indicates that sensory quality can be addressed by harnessing the genetic variability present through breeding, provided appropriate phenotyping methods are available. There is a need for further evaluation of genetic variability for sensory attributes within P. vulgaris to understand the full range of attribute

intensities available and to assess the genetic control of these attributes. These are important steps to develop a breeding program that incorporates flavor and texture.

For this study, a modified quantitative descriptive analysis approach was developed and applied to the screening of 1,940 samples for cooked bean flavor and texture. This approach was used to address three objectives: (1) to evaluate nine sensory attributes in 430 genotypes of a dry bean diversity panel grown in three locations, (2) to examine the relationships among sensory attributes, seed types, and cooking time, and (3) to identify genetic markers associated with sensory attributes across multiple locations.

MATERIALS AND METHODS

Germplasm

Subsets of the Andean Diversity Panel were grown and evaluated across three locations for this study. The genetic composition and germplasm origin of the ADP is described by Cichy et al. (2015) and included in Table S2.1. Only Andean genotypes were included in statistical and GWAS analyses. The Southern Agricultural Research Institute provided seeds from 373 Andean genotypes grown in Hawassa, Ethiopia in Fall 2015, and the University of Zambia provided seeds from 251 Andean genotypes grown in Kabwe, Zambia and 356 Andean genotypes grown in Lusaka, Zambia in Spring 2018. Combined, a total of 430 genotypes were represented covering 20 seed types. Raw seed weights were recorded for each field rep as grams per 100 seeds.

In Hawassa, the ADP was grown during the main cropping season (July to October) in 2015 at the Hawassa Research Station, which has soil classified as Eutric Fluvisol with a pH of 7.0. The ADP genotypes were planted using an augmented design with genotypes arranged in 21 blocks, which each contained 13 test entries and 5 standard checks randomly allocated. Each

genotype was planted in two-row plots with 0.4 m and 0.1m inter-row and intra-row spacing, respectively. Each block was spaced 1 m apart. Fertilizer in the forms of urea (46% N, 0% P2O5, 0% K2O) and DAP (8% N, 46% P2O5, 0% K2O) were applied at a rate of 100 kg/ha.

In Kabwe, the ADP was grown at the Zambia Agricultural Research Institute Farm, which has soil classified as Ultisol and had a pH of 5.0. In Lusaka, the ADP was grown in the field during the rainy season in 2017 at the University of Zambia Research Farm, which has soil classified as fine loamy Isohyperthermic Paleustalf with a pH of 5.5. During the 2017 rainy season a total of 850 mm of rain was received at the experimental site at the University Farm. In both Zambia locations, the ADP genotypes were planted using a randomized complete block designs with two replications. In each replication a genotype was planted in a single-row plot that was 4 M long with 0.60 M inter-row spacing. A compound fertilizer (10P: 20P: 10K) was applied to the experimental site at a rate of 100 Kg Ha-1 just before planting.

Genotypes exhibiting extreme attribute intensities along with Red Hawk (dark red kidney) and Etna (cranberry) were grown at the Montcalm Research Farm in MI in 2018. The soil type is Eutric Glossoboralfs (coarse-loamy, mixed) and Alfic Fragiorthods (coarse-loamy, mixed, frigid). Two row plots 4.75 m long with 0.5 m spacing between rows were arranged in a randomized complete block design with two replications per genotype. Standard agronomic practices were followed as described in the MSU SVREC 2018 Farm Research Report (Kelly et al., 2018).

Cooking Time Evaluation

For each location, two replicates of 30 seed per genotype were equilibrated to 10-14% moisture in a 4 °C humidity chamber prior to evaluating for cooking time. For the seed from both locations in Zambia, each replicate corresponded to a field replicate. For the seed from Hawassa, Ethiopia, the single field replicate for each genotype was split to create two replicates. Each 30

seed sample was soaked for 12 hours in distilled water and weighed prior to cooking time evaluation using an automated Mattson cooker method (Wang and Daun, 2005). Genotypes were cooked in a random order to minimize seed aging effects. Mattson cookers were loaded with soaked seeds and placed in boiling distilled water to cook. The Mattson cookers (Michigan State University Machine Shop, East Lansing, MI) use twenty-five 65g stainless steel rods with 2mm diameter pins to pierce beans as they finish cooking in each well. As the pins drop, a custom software reports the cooking time associated with each pin. The cooking times were recorded, with the 80% cooking time regarded as the time required to fully cook each sample. Cooked samples were weighed and total water uptake following cooking was calculated.

Sensory Evaluation

The ADP subsets from each location were evaluated in duplicate by four panelists each using a Quantitative Descriptive Analysis (QDA) approach (Stone et al., 1974) in which each panelist independently evaluated samples using a non-consensus approach to limit group bias. QDA has been found to yield reproducible measurements with small differences for boiled dry beans, although it is typically applied to small numbers of samples due to the substantial time and personnel commitment it requires (McTigue et al., 1989). For the purposes of this study, the QDA approach was modified to make it feasible to screen hundreds of samples with replication using a small number of panelists, which is necessary for implementation in public breeding programs with limited resources. For each location, seeds were prepared for sensory evaluation in the same order as for cooking time evaluation. Four panelists were present at each sensory evaluation session, scheduled according to their availability. Sensory evaluation sessions were held daily until each genotype had been evaluated twice for each location. For the Ethiopia location, twenty genotypes were evaluated at each session. For the Zambia locations, twelve genotypes including

cranberry (Etna) and dark red kidney (Red Hawk) bean controls grown at the Montcalm Research Center were evaluated at each session. Each sample was evaluated using 5-point attribute intensity scales (low → high intensity) for total, beany, vegetative, earthy, starchy, bitter, and sweet flavor intensities as well as seed coat perception and cotyledon texture. The scale for seed coat perception ranged from imperceptible (1) to tough and lingering (5). For cotyledon texture, the scale ranged from mushy (1) to very gritty/firm (5) (Table S2.2). This sensory evaluation protocol was approved by the Institutional Review Board of Michigan State University (IRB# x16-763e Category: Exempt 6).

Panel Training and Assessment

Panelists were recruited from the USDA-ARS (East Lansing, MI) and Michigan State University Dry Bean Breeding programs due to their familiarity with dry beans and their availability for long term sensory evaluation projects.

An initial training session was conducted with eight panelists using a consensus approach to determine which attributes to evaluate and how to evaluate them. A diverse set of dry bean genotypes was selected from the USDA and MSU dry bean programs with the intention of exposing panelists to a wide range of attribute intensities. This initial set included black, cranberry, dark red kidney, great northern, Jacob's cattle, navy, pink, pinto, small red, and yellow beans. Following screening of the ADP grown in Hawassa, Ethiopia, a training set of genotypes exhibiting extreme attribute intensities was developed (Table 2.1, Figure S2.1). This set was used to train eleven panelists to rate the selected attributes prior to evaluating the ADP grown in the Zambia locations. For the sensory evaluation of the ADP from both Zambia locations, Red Hawk and Etna were used as controls. Red Hawk (Kelly et al., 1998), a dark red kidney bean, is a variety

released by the Michigan State University dry bean breeding program. Etna (PI 546490), a cranberry bean, is a private variety developed by Seminis of Monsanto Vegetable Seeds.

Panelists were trained over multiple sessions using a non-consensus approach to improve their familiarity with the selected scales and their sensory evaluation skills. Panelist performance was assessed via ANOVA with FGenotype (p-value < 0.05) indicating ability to discriminate and Frep (p-value > 0.05) indicating consistency (Meilgaard et al., 1999; Armelim et al., 2006). Sensory evaluation of each location commenced after successful training of each panelist. Following screening of the ADP from each location, panel performance was assessed as during training.

Sample Preparation for Sensory Evaluation

A standardized method for preparing boiled dry beans for sensory evaluation was previously developed (Romero del Castillo et al., 2012), but could not be applied in this study due to limited seed per genotype. Instead, the preparation method used by Mkanda et al. (2007) was modified to suit smaller seed volumes and a larger number of samples, as well as maintain consistent soaking time with the cooking evaluation method. In preparation for each sensory evaluation session, large tea bags filled with 12 hour soaked seeds were boiled in distilled water for the cooking time determined by the Mattson cooker method, timed so they all finished cooking together. No salt was added. The cooked samples were poured into preheated (105 °C) ceramic ramekins, covered with aluminum foil, and placed in a chafing dish to maintain temperature. Samples were given a random letter code to mask their identity. Panelists were asked to refrain from wearing strong scents or eating during the hour before each session. Samples were served out of the ceramic ramekins with a plastic spoon onto paper plates. Lemon water was made available as a palette cleanser, and panelists were asked to drink water between samples.

Statistics

PROC MIXED in SAS version 9.4 of the SAS System for Windows (SAS Institute Inc. Cary, NC, USA) was used to conduct ANOVAs for each recorded trait. For raw seed weight, soak water uptake, cooking time, and total water uptake traits, the fixed effects were genotype, location, and genotype by location with replicate as a random effect. For the sensory attribute intensity traits, the fixed effects were genotype, location, and genotype by location with rep, panelist(location), and session(location) as random effects. Least squares estimates (LSEs) for sensory traits were calculated via the LSMeans statement in PROC MIXED for visualization of trait distributions with outliers excluded. To evaluate differences among seed types, ANOVAs were also performed with the seed type, location, and seed type by location as fixed effects and rep, panelist(location), and session(location) as random effects.

To analyze all locations combined while minimizing environmental effects, best linear unbiased predictors (BLUPs) were generated for each trait using the lme4 package (Bates et al., 2015) in R (R Core Team, 2017) with genotype, location, genotype by location, and rep nested in location as random effects. For sensory traits, panelist nested in location and session nested in location were also included as random effects. For analysis within individual locations, BLUPs were calculated for sensory traits with genotype, rep, panelist, and session included as random effects.

Broad sense heritability (H2) was calculated on a family mean basis for each trait using the equation var(G)/(var(G)+(var (G*L)/no. loc)+(var(error)/no. loc * rep), where var is variance, G is genotype, and G*L is genotype by location, and no. loc is number of locations. Variance components were calculated using PROC VARCOMP in SAS version 9.4 with method = restricted maximum likelihood method (reml) (Holland et al., 2003). Pearson correlation coefficients among

traits were determined with BLUPs from all locations combined using the Cor function in R. Principle component analysis among traits was conducted with BLUPs from all locations combined using the prcomp function in R.

Genotyping

The ADP has been genotyped previously via genotyping-by-sequencing (GBS), and associated data including hapmaps are available at the Feed the Future – Development and Characterization of the Common Bean Diversity Panel (ADP) website (http://arsftfbean.uprm.edu/bean/) (Katuuramu et al., 2018). In brief, two GBS libraries were constructed at 364-plex and 137-plex as described by Elshire et al. (2011) with modifications described by Hart and Griffiths (2015). The raw sequencing data are available in association with BioProject accession number PRJNA290028 in the NCBI BioProject database (https://www.ncbi.nlm.nih.gov/bioproject/).

For this study, the raw sequence data were cleaned of adapters and trimmed for quality score ≥ 30 and minimum length ≥ 30 via Cutadapt (Martin, 2011) and evaluated via FastQC (Andrews, 2010). Cleaned reads were demultiplexed using the Next Generation Sequencing Eclipse Plugin (NGSEP) pipeline with NGSEP version 3.0.2 (Duitama et al., 2014; Perea et al., 2016), aligned to the Phaseolus vulgaris v2.1 genome (DOE-JGI and USDA-NIFA, http://phytozome.jgi.doe.gov/) using Bowtie 2 (Langmead and Salzberg, 2012), and sorted using Picard (http://www.bioinformatics.babraham.ac.uk/projects/fastqc). Variant calling and annotation were performed via NGSEP. Raw SNPs were filtered to eliminate those with more than 90% missing data, and remaining missing data were imputed using FILLIN in Tassel 5.2.31 (Bradbury et al., 2007a; Swarts et al., 2014).

Genome Wide Association

Genome-wide association analyses were performed with Bayesian-information and Linkage-disequilibrium Iteratively Nested Keyway (BLINK) (Huang et al., 2018) in R. BLINK has increased statistical power as compared to other methods and better controls for false negatives and false positives (Liu et al., 2016; Huang et al., 2018). Instead of using kinship, BLINK uses iterations to select a set of markers associated with a trait of interest, which are fitted as covariates. The first 3 principle components were determined using prcomp in R and included in each analysis to control for population structure. Single nucleotide polymorphisms (SNPs) with MAF < 0.05 or with more than two alleles were excluded from analysis. BLUPs were used in genome-wide association analyses for all locations combined and for sensory traits for individual locations, and means were used for analyses of all other traits for individual locations. BLINK does not report R2 for identified SNPs.

To support the BLINK findings, additional genome-wide association analyses were performed using a mixed linear model (MLM) approach in TASSEL v 5.2.31 (Bradbury et al., 2007b). Kinship was calculated using normalized IBS (Yang et al., 2011), and the first 3 PCs were included to control for population structure. SNPs with MAF < 0.05 or with more than two alleles were excluded from analysis.

Manhattan plots and QQ plots were generated using the CMPlot R package (https://github.com/YinLiLin/R-CMplot), and significance levels were established using the False Discovery Rate (Benjamini and Hochberg, 1995) for the BLINK analyses and using a Bonferroni correction based on the effective number of markers tested determined via SimpleM for the MLM analyses (Gao et al., 2008). When reporting significant SNPs from each GWAS analysis, the SNP with the lowest p-value was chosen to represent each locus of interest.

RESULTS

Sensory Extremes

Twelve genotypes were identified which exhibited extreme sensory attributes (Table 2.1, Figure S2.1). These genotypes include Zawadi (ADP0106), a purple speckled variety from Tanzania with low total flavor intensity; Bellagio (ADP0681), a cranberry variety from the United States (Kelly et al., 2010) with high total flavor intensity; USDK-4 (ADP0654), a dark red kidney germplasm line from the United States (Miklas et al., 2004) with high beany intensity; SELIAN94 (ADP0530), a red speckled variety from Tanzania with high vegetative intensity; Kijivu, W616460 (ADP0057), a dark red kidney landrace from Tanzania with high earthy intensity; Perry Marrow, G4499 (ADP0206), a white variety from the United States with high starchy intensity; Baetao-Manteiga 41, G1678 (ADP0190), a purple speckled landrace from Brazil with high sweet intensity; Carioca, Kibala (ADP0517), a carioca landrace from Angola with high bitter intensity; Kabuku, W616464 (ADP0005), a small red landrace from Tanzania with low seed coat perception; Blanco Belén, INIAP422 (ADP0450), a white variety from Ecuador (Minchala et al., 2003) with high seed coat perception; PR1146-123 (ADP0791), a yellow germplasm line and sibling of the germplasm release PR1146-138 (Beaver et al., 2016) from Puerto Rico with smooth cotyledon texture; and Kijivu, W616491 (ADP0044), a purple speckled landrace from Tanzania with grainy cotyledon texture.

These genotypes were selected for training panelists because they exhibited the range of attributes likely present in the entire sample set. While the attribute intensities of these genotypes varied somewhat across the three locations, they collectively represented a large portion of the attribute intensity ranges that were observed, reflected by their averages across locations (Table 2.2, S2.4). Significant genotype effects for each sensory attribute and insignificant rep effects

indicated that the panelists were trained sufficiently to detect differences among genotypes and were consistent across reps despite significant panelist and session effects (Table 2.2, S2.3).

Sensory Evaluation

The pin drop Mattson cooker was used to determine cooking times of the beans used in the sensory evaluation. The trained panel rated doneness of each cooked bean sample based on mouthfeel and concluded that the cooking times determined via the Mattson cooker equated to fully cooked samples (data not shown).

Least squares estimates for all sensory attribute intensities exhibited approximately normal distributions (Figure 2.1). Genotype significantly affected all sensory attributes (p-value < 0.05) (Table 2.2). Location significantly affected total flavor intensity and cotyledon texture (p-value < 0.05), but was not significant for other sensory attributes. Genotype by location significantly affected total flavor intensity, vegetative intensity, sweet intensity, seed coat perception, and cotyledon texture (p-value < 0.05).

Across all three locations, least squares estimates ranged 1.6 – 4.5 for total flavor intensity, 1.5 – 5.0 for beany intensity, 1.1 – 4.0 for vegetative intensity, 1.2 – 3.4 for earthy intensity, 2.1 – 4.4 for starchy intensity, 0.8 – 3.5 for sweet intensity, 0.5 – 3.5 for bitter intensity, 1.6 – 4.4 for seed coat perception, and 1.1 – 4.2 for cotyledon texture. While panelists were able to differentiate among genotypes using 5-point scales, sensory attribute ranges did not exceed 3.2 in any single location, suggesting panelists did not make full use of the scales.

Twenty seed types were represented in the ADP, and seed type significantly affected all sensory attribute intensities (p-value < 0.0001) (Table S2.5). However, large ranges of attribute intensities are observed for each seed type (Figure 2.2), indicating variability of flavor and texture within a seed type. Brown genotypes (N = 10) tended to vary the least across sensory attributes

followed by light red kidney (N = 41), with cranberry (N = 63) and red mottled/red speckled (N = 80) varying the most. Earthy intensity followed by bitter intensity had the least variability across all seed types, and seed coat perception and cotyledon texture had the most.

Broad-sense heritability for sensory attribute intensities was low, ranging from 0.14 to 0.39 (Table 2.2). Seed coat perception and total flavor intensity exhibited the highest broad-sense heritability (0.39 and 0.38), while earthy intensity and vegetative intensity exhibited the lowest (0.14 and 0.15).

Cooking Time Evaluation

Genotype, location, and genotype by location significantly affected raw seed weight, soak water uptake, cooking time, and total water uptake (Table 2.3). The means and ranges of raw seed weight, soak water uptake, cooking time, and total water uptake varied across locations (Figure 2.3). Across all 3 locations, raw seed weight ranged from 20.7 – 72.2 g per 100 seeds; soak water uptake ranged from 29.5 – 140.4%; cooking time ranged from 16.7 – 85.8 min; and total water uptake ranged from 100.4 – 169.7% (Table 2.3). Raw seed weight, soak water uptake, cooking time, and total water uptake exhibited approximately normal distributions (Figure 2.3). Broad-sense heritability was moderate to high for raw seed weight (0.90), soak water uptake (0.85), cooking time (0.73), and total water uptake (0.65).

Correlations and PCA

Significant correlations among sensory attribute intensities and cooking time were observed (Figure 2.4). Total flavor intensity correlated with all other sensory attributes such that earthy ($R = 0.44$, p-value < 0.0001), beany ($R = 0.39$, p-value < 0.0001), sweet ($R = 0.38$, p-value < 0.0001), vegetative ($R = 0.33$, p-value < 0.0001), bitter ($R = 0.27$, p-value < 0.0001), and starchy ($R = 0.17$, p-value $= 0.0004$) intensity all increased with total flavor intensity. The correlations

between total flavor intensity and seed coat perception ($R = 0.17$, p-value $= 0.0003$) and cotyledon texture ($R = 0.14$, p-value $= 0.0050$) were weak, but indicate that more flavor is associated with tougher, lingering seed coats and grittier, firmer cotyledons in fully cooked seeds. Total flavor intensity was negatively correlated with cooking time ($R = -0.16$, p-value $= 0.0009$), suggesting that genotypes with shorter cooking times have more total flavor, potentially due to less time for leaching during the cooking process.

Individual sensory attributes also correlated with one another, suggesting that some attributes tend to be observed together. Genotypes with high beany intensity tended to be somewhat earthy ($R = 0.27$, p-value < 0.0001) and bitter ($R = 0.25$, p-value < 0.0001) and less starchy ($R = -0.13$, p-value $= 0.0073$). Genotypes with high vegetative intensity also tended to be somewhat earthy ($R = 0.21$, p-value < 0.0001) and bitter ($R = 0.27$, p-value < 0.0001). Genotypes with high earthy intensity were bitter ($R = 0.36$, p-value < 0.0001) as well as beany and vegetative as already noted. Genotypes with high starchy intensity were notably sweet ($R = 0.48$, p-value < 0.0001), less bitter ($R = -0.26$, p-value < 0.0001), and less beany as already mentioned. Genotypes with high sweet intensity were also observed as being less bitter ($R = -0.18$, p-value $= 0.0002$). Genotypes with high bitter intensity were somewhat beany, vegetative, and earthy and less starchy or sweet as previously noted. Genotypes with tougher seed coats were beany ($R = 0.22$, p-value < 0.0001) and bitter ($R = 0.10$, p-value $= 0.0386$) and less starchy ($R = -0.17$, p-value $= 0.0003$) or sweet ($R = -0.10$, p-value $= 0.0343$). Genotypes with grittier/firmer cotyledon texture were vegetative ($R = 0.15$, p-value $= 0.0024$), earthy ($R = 0.24$, p-value < 0.0001), and bitter ($R = 0.12$, p-value $= 0.0147$) and less beany ($R = -0.12$, p-value $= 0.0167$). Many of these correlations are relatively weak, suggesting that these tendencies are not always observed and that these attributes can be packaged together in multiple ways.

Cooking time also correlated with individual sensory attributes. Faster-cooking genotypes were starchy (R = -0.36, p-value < 0.0001) and sweet (R = -0.34, p-value < 0.0001) and had smoother cotyledon texture (R = -0.12, p-value = 0.0123), while slower cooking genotypes were beany (R = 0.23, p-value < 0.0001) and bitter (R = 0.12, p-value = 0.0167) and had tougher seed coats (R = 0.2, p-value < 0.0001). These correlations were relatively weak, indicating that fast cooking time can be packaged with target sensory profiles.

For the PCA, the first two principal components (PCs) explained about 45% of the variation (Figure 2.5). The first PC separated the genotypes approximately by total flavor, vegetative, earthy, beany, and bitter intensity as well as cotyledon texture and somewhat seed coat perception and represented almost a quarter of the variation (22.8%). The second PC represented a similar amount of the variation (20.9%) and separated the genotypes by starchy and sweet intensity and cooking time. The remaining PCs accounted for 13.1, 8.9, 8.4, 6.7, 6.2, 5.5, 4.4, and 3.1% of the variance respectively (data not shown).

The PCA highlights a positive relationship among total flavor, vegetative, earthy, beany, and bitter intensity as well as seed coat perception and cotyledon texture. Total flavor, vegetative, and earthy intensity and cotyledon texture are positioned closer together as are beany and bitter intensity and seed coat perception, indicating stronger relationships within each group. A positive relationship was also observed between starchy and sweet and sweet intensity, which appear to be negatively associated with cooking time.

Each genotype within the PCA is colored by seed type, which reveals substantial variation within seed type. All seed types are spaced somewhat evenly across the biplot with the exception of the white seed type. White genotypes tend to cluster near starchy and sweet and away from cooking time and seed coat perception, indicates that white genotypes tend to be starchy and sweet

with shorter cooking times. Dark red kidney, light red kidney, and red mottled genotypes are distributed somewhat closer toward loadings for total flavor intensity, vegetative intensity, earthy intensity, and cotyledon texture, and purple speckled genotypes are distributed somewhat away, but the clustering is very loose.

Genome-Wide Association Mapping

Across the 430 Andean genotypes evaluated in this study, 31,273 SNPs remained after imputing and filtering. For each location, a similar number of SNPs were used in GWAS: 29,926 SNPs from Hawassa, Ethiopia (N = 373), 29,545 SNPs from Kabwe, Zambia (N = 251), and 31,484 SNPs from Lusaka, Zambia (N = 356).

Across all locations combined, significant SNPs were identified using BLINK and MLM for several sensory attributes, including total flavor intensity, beany intensity, earthy intensity, starchy intensity, bitter intensity, seed coat perception, and cotyledon texture (Figure 2.6, S2.2). Significant SNPs detected for sensory traits were not consistent across the BLINK and MLM analyses methods, except for cotyledon texture (Table 2.4). MLM identified fewer significant SNPs overall, as expected due to its lower power and poor control of false negatives as compared to BLINK (Liu et al., 2016; Huang et al., 2018). For each sensory attribute with significant marker associations, an increase in the number of alleles conferring positive effects corresponded to an increase in mean attribute intensity (Figure 2.7).

For total flavor intensity, 6 significant SNPs were identified on Pv01, Pv02, Pv05, and Pv09 (Table 2.4). MLM identified S01_5952237 on Pv01, which had no significant SNPs detected by BLINK. S02_34288083, S02_38579748, and S09_235919 were most significant. Genotypes with 5 significant SNPs conferring positive effects had a mean total flavor intensity rating 1.2 higher than those with no positive significant SNPs (Figure 2.7). There were no genotypes with all

6 positive significant SNPs. For beany intensity, 5 significant SNPs were identified on Pv02, Pv06, Pv07, and Pv10 (Table 2.4). S02_47727086, S06_5174714, and S10_42475118 were most significant. Genotypes with all 5 significant SNPs conferring positive effects had a mean beany intensity rating 0.8 higher than those with no positive significant SNPs (Figure 2.7). For earthy intensity, 3 significant SNPs were identified on Pv04 and Pv11, with S04_528286 being most significant (Table 2.4). Genotypes with all 3 significant SNPs conferring positive effects had a mean earthy intensity rating about equal to those with no positive significant SNPs when presented as means of least squares estimates (Figure 2.7) and slightly increased (0.1) when presented as means of BLUPs (data not shown). Starchy intensity had 1 significant marker on Pv01 (S01_42652564), which was detected by MLM and not BLINK (Table 2.4). Genotypes with the significant marker conferring a positive effect had a mean starchy intensity rating 0.1 higher than those without the positive significant marker (Figure 2.7). Bitter intensity also had 1 significant marker on Pv01 (S01_51119029), which was detected by MLM and not BLINK (Table 2.4). Genotypes with the significant marker conferring a positive effect had a mean bitter intensity rating 0.2 higher than those without the positive significant marker (Figure 2.7). For seed coat perception, 3 significant SNPs were detected on Pv02 and Pv08 (Table 2.4). All three were highly significant. Genotypes with all 3 significant SNPs conferring positive effects had a mean seed coat perception rating 0.7 higher than those with no positive significant SNPs (Figure 2.7). For cotyledon texture, 2 significant SNPs were detected on Pv03 and Pv08, which were detected by both BLINK and MLM (Table 2.4). Both SNPs were highly significant. Genotypes with both significant SNPs conferring positive effects had a mean cotyledon texture rating 0.4 higher than those with no positive significant SNPs (Figure 2.7).

For each individual location, significant SNPs were also identified using BLINK for total flavor intensity, beany intensity, earthy intensity, and seed coat perception (Table S2.6). MLM was not performed for individual locations. The identified SNPs somewhat reflect the findings for all locations combined, but largely point to different SNPs relevant for specific locations. For total flavor intensity, a total of 15 significant SNPs were identified on Pv02, Pv03, Pv04, Pv05, and Pv11 in the samples from Hawassa Ethiopia; Pv03, Pv08, Pv09, Pv10, and Pv11 in the samples from Kabwe, Zambia; and Pv05, Pv06, and Pv10 in the samples from Lusaka, Zambia (Table S2.6, Figure S2.3). For beany intensity, a total of 6 significant SNPs were identified on Pv10 and Pv11 in the samples from Kabwe, Zambia and Pv02, Pv06, Pv10, and Pv11 in the samples from Lusaka, Zambia (Table S2.6, Figure S2.4). For earthy intensity, a total of 3 significant SNPs were identified on Pv04 in the samples from Kabwe, Zambia and Pv02 and Pv11 in the samples from Lusaka, Zambia (Table S2.6, Figure S2.5). For seed coat perception, a total of 5 significant SNPs were identified on Pv02 and Pv05 in the samples from Hawassa, Ethiopia; Pv05 in the samples from Kabwe, Zambia; and Pv02 and Pv07 in the samples from Lusaka, Zambia (Table S2.6, Figure S2.6).

Across all locations combined, significant SNPs were identified using BLINK and MLM for raw seed weight, soak water uptake, cooking time, and total water uptake (Figure S2.7, S2.8). Both methods identified different SNPs, with some overlap for raw seed weight and soak water uptake (Table S2.7). MLM identified fewer significant SNPs overall, as was the case for the sensory attributes.

For raw seed weight, 15 significant SNPs were identified on Pv01, Pv02, Pv03, Pv04, Pv05, Pv06, Pv08, Pv09, and Pv11 (Table S2.7). MLM identified S03_41895570, which was also detected by BLINK, and S05_1138961, which was not. Genotypes with 13 significant SNPs

conferring positive effects had a mean raw seed weight 31 grams per 100 seeds higher than those with only 3 positive significant SNPs (Figure S2.9). There were no genotypes with fewer than 3 or more than 13 positive significant SNPs. For soak water uptake, 17 significant SNPs were identified on Pv02, Pv03, Pv04, Pv05, Pv07, Pv08, Pv10, and Pv11 (Table S2.7). MLM identified 6 of those SNPs, of which 1 was also detected by BLINK. Genotypes with 15 significant SNPs conferring positive effects had a mean soak water uptake 64% higher than those with only 4 positive significant SNPs (Figure S2.9). There were no genotypes with fewer than 4 or more than 15 positive significant SNPs. For cooking time, 11 significant SNPs were identified on Pv03, Pv04, Pv06, Pv07, Pv08, and Pv11 (Table S2.7). MLM identified S04_3957256 and S08_62659170, which were not detected by BLINK. Genotypes with 9 significant SNPs conferring negative effects had a mean cooking time 23 min faster than those with 3 or fewer negative significant SNPs (Figure S2.9). There were no genotypes with 0, 2, or more than 9 negative significant SNPs, and there was a single genotype with only 1 negative significant marker. For total water uptake, 5 significant SNPs were identified on Pv03, Pv04, Pv09, and Pv11 (Table S2.7). No SNPs were identified by MLM for total water uptake. S04_30764016 was associated with both soak water uptake and total water uptake. Genotypes with all 5 significant SNPs conferring positive effects had a mean total water uptake 10% higher than those with 1 or fewer positive significant SNPs (Figure S2.9). There was only 1 genotype with no positive significant SNPs.

DISCUSSION

The modified QDA approach used in this study successfully detected differences among genotypes for the purposes of identifying extremes, evaluating the relationships among sensory attributes and seed type, and performing genome-wide association analyses to reveal SNPs

associated with sensory attributes. Although significant panelist effects were identified (Table S2.3), these effects are not concerning because QDA does not rely on consensus among panelists. However, limited use of the scales by the panelists prevents detection of small differences between samples. This can be remedied by increasing the size of the scales or using line scales that allow for continuous rather than discrete ratings. As for panelists, differences among sessions are expected and can be accounted for in the ANOVAs and by using BLUPs where appropriate. Genotypes exhibiting extreme attribute intensities were identified (Table 2.1) and successfully used for training panelists for sensory evaluation (Tables 2.2, S2.3). These genotypes could serve as a training set for future sensory research or for training sensory panels for germplasm evaluation in breeding programs.

Location of production and crop management practices have previously been identified as factors affecting sensory quality (Mkanda et al., 2007; Ferreira et al., 2012), which complicates efforts to understand and breed for sensory quality in beans. The location and genotype by location effects were significant for many of the sensory attributes in this study (Table 2.2), supporting these findings. Differences among locations were also apparent in density plots for some flavor and texture attributes (Figure 2.1). Despites small fluctuations in sensory profile across locations, the genotypes exhibiting extreme sensory attribute intensities remained extreme for their attribute of interest in each location (Table S2.4). This suggests that differences across location affect magnitude of sensory attribute intensities, but do not substantially alter sensory attribute intensities relative to each other.

Many significant correlations were identified among flavor, texture, and cooking time, although correlation coefficients were generally weak, suggesting that traits can combine in multiple ways (Figure 2.4). Sweet and starchy intensity were the two most strongly correlated

attributes, and the loadings for these attributes were positioned near each other in the PCA, away from other attributes (Figure 2.5). White seeds were generally sweet and starchy, but otherwise, few trends were identified in regard to seed type, which indicates that seed type does not define the sensory profile of a genotype (Figure 2.2, 2.5). This supports a previous study that found similarities in morphology and genetic background do not indicate similarity of sensory attributes among genotypes (Rivera et al., 2013). The genetic variability existing within seed type could be harnessed to achieve a target sensory profile and ensure greater consistency and uniformity of flavor and texture. In addition, fast cooking time could be targeted without substantially influencing sensory profile, which would address another major factor influencing consumer purchasing decisions (Leterme and Carmenza Muñoz, 2002; Eihusen and Albrecht, 2007; Winham et al., 2019).

Many SNPs significantly associated with flavor and texture were identified using BLINK and MLM, and they appear to confer minor effects, highlighting the complexity of the genetics underlying these traits. (Table 2.4, Figure 2.6, S2.2). Significant SNPs varied for each individual location (Table S2.6, Figure S2.3-S2.6), emphasizing the importance of location in expression of genetic variability for sensory attributes. The significant SNPs identified have not been previously associated with sensory attributes as this is the first study of its kind in beans. No significant SNPs were associated with vegetative or sweet intensity, but alternative approaches such as QTL mapping or genomic prediction with a population of related individuals may provide increased power to detect relevant loci for these traits. Other studies in fruits have successfully used volatiles and instrumental measures in GWAS as proxies for flavor and texture, allowing for easier phenotyping and in some cases higher heritability than traits evaluated via descriptive panels (Zhang et al., 2015; Amyotte et al., 2017; Bauchet et al., 2017; Zhao et al., 2019). However,

volatiles and instrumental measures do not always successfully predict flavor and texture as it is perceived by a descriptive panel (Amyotte et al., 2017), and for dry beans, little is known about how volatiles or other measures relate to flavor and texture. The screening of the ADP performed in this study provides a resource for future population development to further understanding of the genetic control of sensory attributes and how volatiles and instrumental measurements relate to sensory attributes.

One of the unique flavor characteristics found in dry beans and other legumes consumed as seeds is the "beany" flavor, which has proven a challenge to define and is often described as an "off" flavor in products using beans as ingredients (Kinsella, 1979; Bott and Chambers, 2006; Hooper et al., 2019). One study defined the flavor as undesirable, with multiple contributing volatiles (Vara-Ubol et al. 2004). In soybean, significant SNPs have been associated with volatiles contributing to beany flavor, and some of these SNPs are present in regions syntenic with dry bean chromosomes where SNPs associated with beany flavor were identified in this study (Schmutz et al., 2014; Xia et al., 2019b; Xia et al., 2019a; Wang et al., 2020). In particular, the end of Pv02 where S02_47727086 and S02_49605939 are located is syntenic with soybean chromosomes 5 and 8 (Schmutz et al., 2014). Using Minimap2 (Li, 2018) and the soybean reference genome (Williams 82) from SoyBase (Grant et al., 2009), the 50 kb regions around S02_47727086 and S02_49605939 align near rs39728576 and rs4039554, respectively, markers on soybean chromosome 5 and 8 associated with hexanal content in soybean (Wang et al., 2020).

Off-flavors in soy products are generated by lipoxygenases, primarily Lipoxygenase-2, or the oxidative rancidity of unsaturated fatty acids (Wolf et al., 1971; Kim et al., 2004). Markers linked to Lipoxygenase-2 are available and in use for breeding efforts targeting the reduction of beany flavor in soybean (Lenis et al., 2010; Talukdar and Shivakumar, 2016). Several

lipoxygenase genes are located within a megabase of S07_28996873 and S10_42475118 (http://phytozome.jgi.doe.gov/). In addition, a single lipoxygenase is located within three megabases of S06_5174714. While some lipoxygenases are present on Pv02, they are not close to S02_47727086 or S02_49605939.

It is not yet understood whether beany flavor in a boiled beans translates to off-flavor in products made using beans as ingredients. In addition, consumer preference as it relates to sensory attribute intensities has not been explored for boiled beans beyond a general preference for beans that are sweet and soft when fully cooked (Mkanda et al., 2007). Further research relating consumer preference to attribute intensities in boiled beans as well as products using beans as ingredients could allow breeders to identify target sensory profiles for different seed types or varieties intended for use as ingredients.

In regard to raw seed weight, soak water uptake, cooking time, and total water uptake, many significant SNPs were identified in association with these traits as well via BLINK and MLM (Table S2.7, Figure S2.7, S2.8). Most of the SNPs identified were novel, but some were proximal to QTL and markers identified in previous studies. Of particular interest, S11_10805992, which was significantly associated with cooking time (Table S2.7), is near a QTL identified for cooking time by Berry et al. (2020). S02_47837868, S03_50652595, S03_51140861, S04_30764016, S07_3919560, S10_37637761, which were significantly associated with soak water uptake (Table S2.7), appear to be supported by hydration coefficient and water absorption QTL previously identified (Pérez-Vega et al., 2010; Cichy et al., 2014; Kelly and Bornowski, 2018; Sandhu et al., 2018).

While broad-sense heritability for each sensory attribute was generally low (Table 2.2), heritability could be improved in the context of a breeding program by screening only promising

lines with greater replication. This could allow for better understanding of panelists and session effects and a balanced statistical design while maintaining a manageable time and personnel commitment. If fewer samples are evaluated each session, sensory fatigue could be reduced, allowing for better detection of small differences between samples. Potential alternative methods for screening sensory attributes could also be explored, including screening volatile profiles via GC-MS and collecting NIR spectra. NIR spectra of both raw seeds and cooked and dried seeds have been analyzed for their ability to predict beany flavor, mealiness, seed-coat roughness, and seed-coat brightness, although correlations between NIR spectra and these attributes were poor for raw beans (Plans et al., 2014). Using alternative methods for screening sensory attributes could increase the throughput of sensory profile characterization, but more research is needed to identify predictive measurements.

CONCLUSION

This study lays a foundation for incorporating sensory quality traits into dry bean breeding programs. The broad range of sensory attribute intensities observed across and within seed types indicates a lack of uniformity within seed type, but also a wealth of genetic variability for sensory quality. This presents an opportunity for specific sensory profiles to be defined for each seed type. The limited correlation among sensory attributes indicates that they can combine in multiple ways, suggesting it is feasible to target specific sensory profiles according to consumer preference. Using the modified QDA approach to screen materials and the significant genetic SNPs identified for flavor and texture attributes, breeders could continue to improve agronomic traits without sacrificing desirable sensory quality. The set of genotypes exhibiting extreme sensory attribute intensities identified during this study can be used for panel training as well as future work

exploring sensory attributes and consumer preference. In addition, further understanding of sensory profiles suitable for bean products would allow varieties to be developed for use as ingredients, increasing the chance of success for bean products on the market. Improving flavor and texture in dry beans can ensure they are appreciated as a delicious and tasteful component of a healthful diet in all the versatile ways consumers choose to eat them.

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APPENDICES

APPENDIX A:

CHAPTER 2 TABLES AND FIGURES

Table 2.1 Genotypes exhibiting extreme sensory attribute intensities identified from screening accessions of the Andean Diversity Panel grown in Hawassa, Ethiopia.

Genotype	ADP ID	Seed Type	Region of origin	Sensory Attribute
Zawadi	ADP0106	Purple speckled	Tanzania	Low total flavor intensity
Bellagio	ADP0681	Cranberry	United States	High total flavor intensity
USDK-4	ADP0654	Dark red kidney	United States	High beany intensity
SELIAN94	ADP0530	Red speckled	Tanzania	High vegetative intensity
Kijivu (W616460)	ADP0057	Dark red kidney	Tanzania	High earthy intensity
Perry Marrow (G4499)	ADP0206	White	United States	High starchy intensity
Baetao-Manteiga 41 (G1678)	ADP0190	Purple speckled	Brazil	High sweet intensity
Carioca,Kibala	ADP0517	Carioca	Angola	High bitter intensity
Kabuku (W616463)	ADP0005	Small red	Tanzania	Low seed coat perception
INIAP422	ADP0450	White	Ecuador	High seed coat perception
PR1146-123	ADP0791	Yellow	Puerto Rico	Smooth cotyledon texture
Kijivu (W616491)	ADP0044	Purple speckled	Tanzania	Grainy cotyledon texture

Table 2.2 Least squares estimates, range, and coefficient of variation of sensory attribute intensities of the Andean Diversity Panel grown in three locations with ANOVA *p*-values[a] for genotype, location (Loc), and genotype by location indicated.

Trait	Location	LSE	Range	CV (%)[b]	Genotype	Loc	Genotype x Loc	H2[c]
Total Flavor Intensity								
	Hawassa, ET	2.8	1.6 - 3.7	14.4	<.0001	<.0001	<.0001	0.38
	Kabwe, ZM	3.4	2.2 - 4.4	12.6				
	Lusaka, ZM	3.4	2.0 - 4.5	13.3				
Beany Intensity								
	Hawassa, ET	2.8	1.7 - 3.8	13.3	<.0001	NS	NS	0.30
	Kabwe, ZM	2.9	1.5 - 4.1	14.9				
	Lusaka, ZM	3.4	1.8 - 5.0	16.1				
Vegetative Intensity								
	Hawassa, ET	2.0	1.1 - 3.4	17.8	<.0001	NS	0.0013	0.15
	Kabwe, ZM	2.4	1.3 - 3.7	16.0				
	Lusaka, ZM	2.6	1.6 - 4.0	16.4				
Earthy Intensity								
	Hawassa, ET	2.0	1.2 - 3.0	15.7	<.0001	NS	NS	0.14
	Kabwe, ZM	2.1	1.2 - 3.2	17.0				
	Lusaka, ZM	2.1	1.2 - 3.4	18.6				
Starchy Intensity								
	Hawassa, ET	3.2	2.2 - 4.4	10.4	<.0001	NS	NS	0.21
	Kabwe, ZM	3.2	2.1 - 4.0	11.7				
	Lusaka, ZM	3.2	2.2 - 4.1	12.2				
Sweet Intensity								
	Hawassa, ET	1.7	1.0 - 3.5	21.2	<.0001	NS	<.0001	0.26
	Kabwe, ZM	1.9	0.9 - 3.2	21.2				
	Lusaka, ZM	1.8	0.8 - 3.1	21.2				
Bitter Intensity								
	Hawassa, ET	1.6	0.8 - 3.5	22.0	<.0001	NS	NS	0.22
	Kabwe, ZM	1.5	0.8 - 3.0	22.0				
	Lusaka, ZM	1.4	0.5 - 2.8	24.6				
Seed Coat Perception								
	Hawassa, ET	3.0	1.6 - 4.4	13.3	<.0001	NS	<0.0001	0.39
	Kabwe, ZM	3.1	2.2 - 4.1	13.1				
	Lusaka, ZM	3.0	1.6 - 4.1	13.8				
Cotyledon Texture								
	Hawassa, ET	2.7	1.4 - 4.0	16.1	<.0001	0.0025	<.0001	0.31
	Kabwe, ZM	2.3	1.4 - 4.2	15.2				
	Lusaka, ZM	2.2	1.1 - 3.4	14.2				

Table 2.2 (cont'd)

[a] NS indicates non-significant *p*-values at $\alpha = 0.05$
[b] Coefficient of variation
[c] Broad sense heritability

Table 2.3 Mean, range, and coefficient of variation of raw seed weight, soak water uptake, cooking time, and total water uptake of the Andean Diversity Panel grown in three locations with ANOVA *p*-values for genotype, location (Loc), and genotype by location indicated.

Trait	Location	Mean	Range	CV (%)[a]	Genotype	Loc	Genotype x Loc	H2[b]
Raw Seed Weight (g per 100 seed)								
	Hawassa, ET	37.2	20.7 – 54.0	16.4	<.0001	<.0001	<.0001	0.90
	Kabwe, ZM	44.8	25.9 – 62.0	15.6				
	Lusaka, ZM	45.1	24.3 - 72.2	17.0				
Soak Water Uptake (%)								
	Hawassa, ET	112.1	51.9 - 140.4	8.9	<.0001	<.0001	<.0001	0.85
	Kabwe, ZM	100.3	54.0 - 118.6	9.3				
	Lusaka, ZM	101.0	29.5 - 128.1	8.7				
Cooking Time (min)								
	Hawassa, ET	31.5	16.7 - 68.9	22.8	<.0001	<.0001	<.0001	0.73
	Kabwe, ZM	31.5	17.8 - 75.5	23.8				
	Lusaka, ZM	33.8	21.0 - 85.8	24.9				
Total Water Uptake (%)								
	Hawassa, ET	139.5	100.4 - 165.2	5.7	<.0001	<.0001	<.0001	0.65
	Kabwe, ZM	134.8	110.7 - 156.2	5.1				
	Lusaka, ZM	135.0	105.0 - 169.7	5.6				

[a] Coefficient of variation
[b] Broad sense heritability

Table 2.4 GWAS significant markers associated with sensory attribute intensities with marker, chromosome (Chr), position, *P*-value, minor allele frequency (MAF), major and minor alleles (Maj/Min), significance (Sig), and method indicated.

Trait	Marker	Chr	Position[a]	*P*-value	MAF	Maj/Min[b]	Sig[c]	Method
Total Flavor Intensity								
	S01_5952237	1	5952237	1.87E-05	0.06	**G**/T	*	MLM
	S02_34288083	2	34288083	1.94E-07	0.27	**A**/G	***	BLINK
	S02_38579748	2	38579748	2.31E-07	0.07	T/**A**	***	BLINK
	S05_36225444	5	36225444	1.91E-06	0.15	C/**T**	**	BLINK
	S05_39325999	5	39325999	1.23E-05	0.28	**C**/T	*	BLINK
	S09_235919	9	235919	6.53E-07	0.10	C/**T**	***	BLINK
Beany Intensity								
	S02_47727086	2	47727086	3.67E-08	0.22	G/**C**	***	BLINK
	S02_49605939	2	49605939	2.48E-06	0.06	**C**/T	**	BLINK
	S06_5174714	6	5174714	6.15E-07	0.14	G/**T**	***	BLINK
	S07_28996873	7	28996873	6.66E-06	0.37	**G**/T	**	BLINK
	S10_42475118	10	42475118	5.51E-09	0.15	**T**/C	***	BLINK
Earthy Intensity								
	S04_528286	4	528286	8.63E-08	0.07	C/**T**	***	BLINK
	S04_4661131	4	4661131	1.98E-06	0.19	G/**A**	**	BLINK
	S11_47172346	11	47172346	1.23E-06	0.30	A/**T**	**	BLINK
Starchy Intensity								
	S01_42652564	1	42652564	5.42E-06	0.30	G/**A**	**	MLM
Bitter Intensity								
	S01_51119029	1	51119029	1.47E-05	0.20	C/**T**	*	MLM
Seed Coat Perception								
	S02_34629777	2	34629777	2.43E-07	0.10	A/**C**	***	BLINK
	S02_48936819	2	48936819	9.06E-11	0.26	C/**T**	***	BLINK
	S08_60104671	8	60104671	4.90E-07	0.23	**C**/G	***	BLINK
Cotyledon Texture								
	S03_31659572	3	31659572	9.43E-11	0.18	**G**/T	***	BLINK, MLM
	S08_2356200	8	2356200	3.32E-07	0.08	A/**G**	***	BLINK, MLM

[a] Position is based on the P. vulgaris v2.1 reference genome (DOE-JGI and USDA-NIFA, http://phytozome.jgi.doe.gov/)

[b] Alleles in bold confer a positive effect on the indicated trait

[c] Significance is indicated by asterisks, such that *, **, *** indicate significance at α = 0.1, α = 0.05, α = 0.01 using the false discovery rate for the BLINK method and a Bonferroni correction based on the effective number of markers determined using the SimpleM algorithm for the MLM method

Figure 2.1 Density plots of least squares estimates of sensory attribute intensities for the Andean Diversity Panel for all locations combined (C); Hawassa, ET (H); Kabwe, Zambia (K); and Lusaka, Zambia (L).

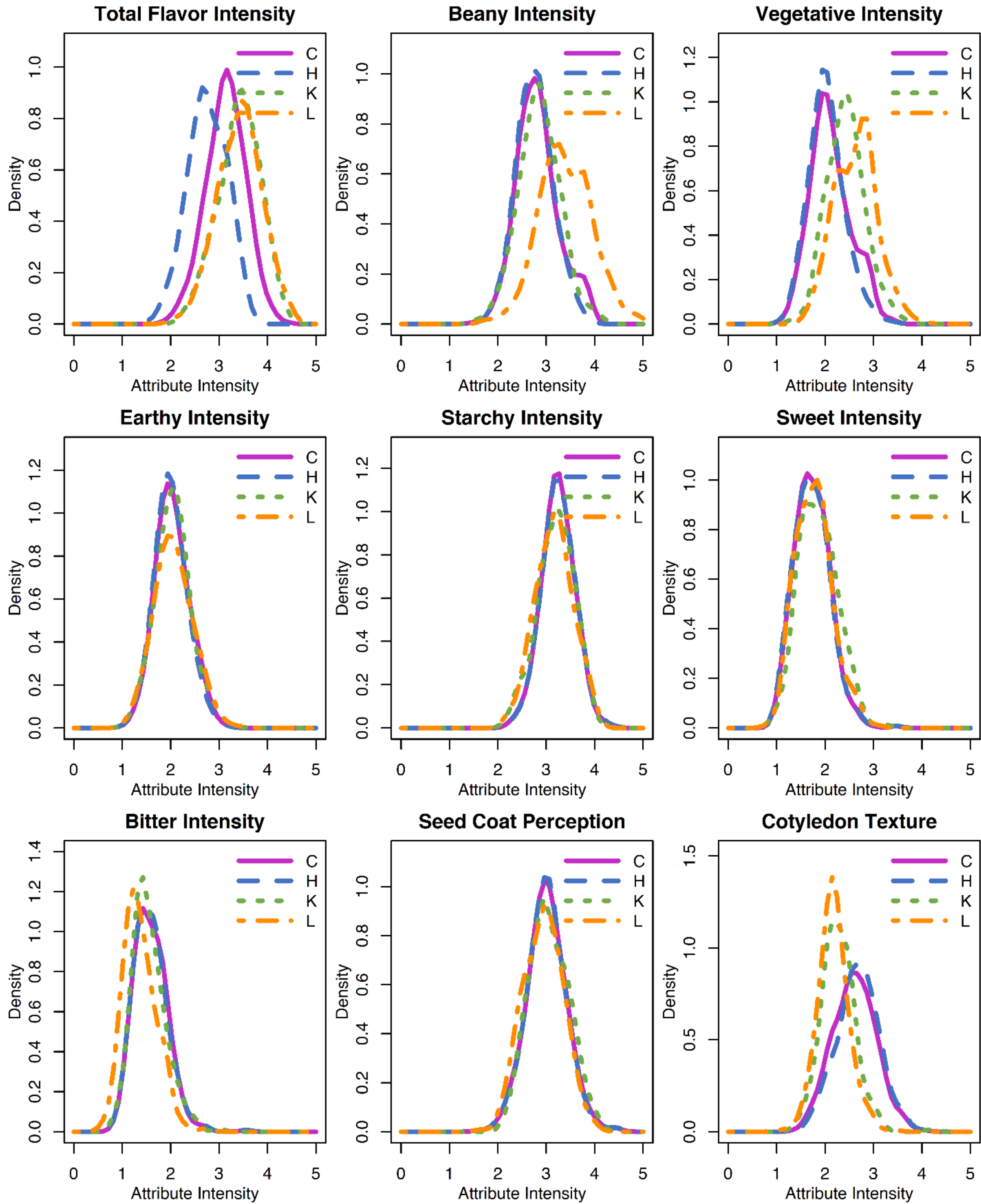


Figure 2.2 Boxplots of sensory attribute intensities separated by seed type. All boxplots are presented as least squares estimates averaged across all locations for seed types with N > 10, where "Other" includes the remaining seed types with N < 10.

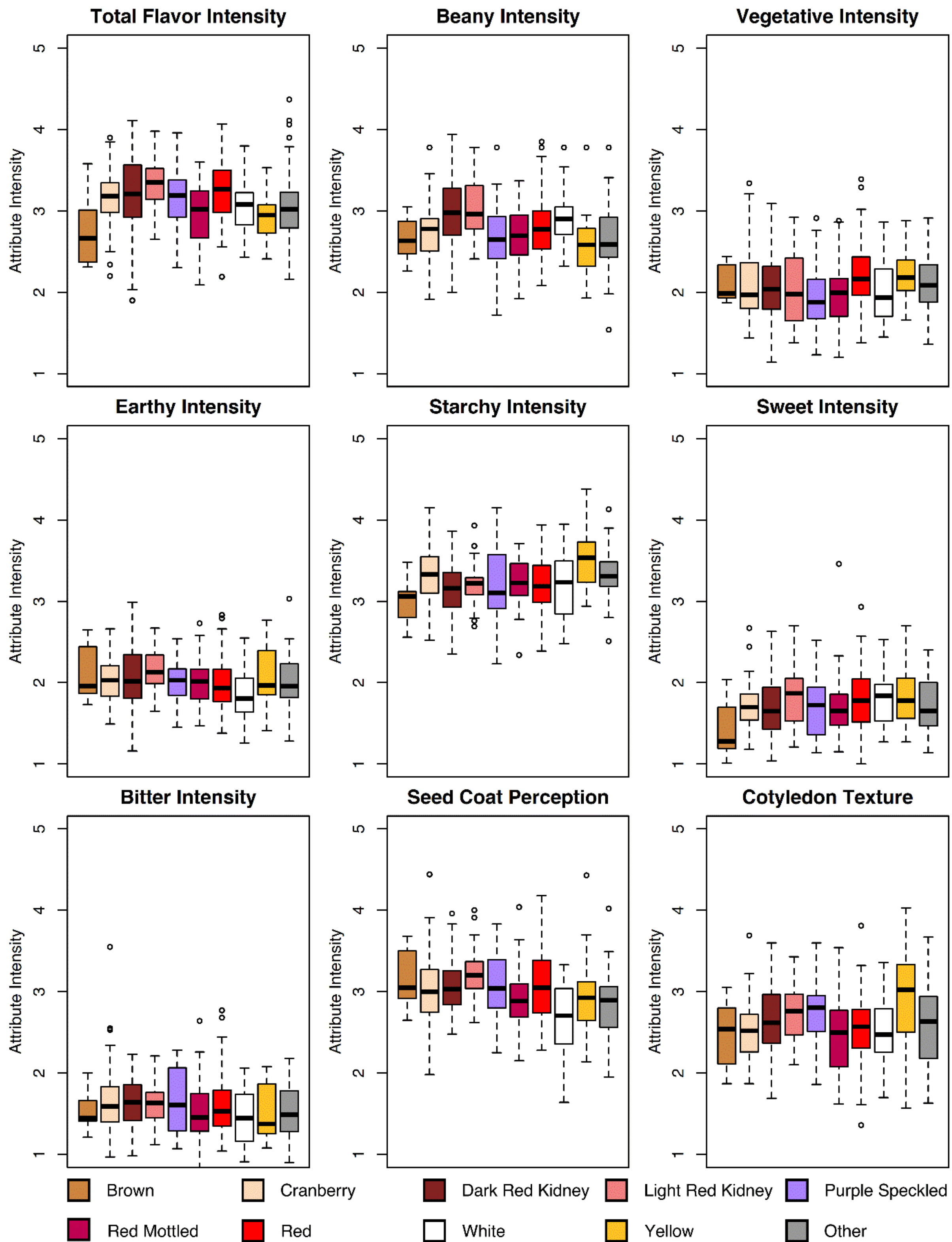


Figure 2.3 Density plots of raw seed weight, soak water uptake, cooking time, and total water uptake for the Andean Diversity Panel for all locations combined (C); Hawassa, ET (H); Kabwe, Zambia (K); and Lusaka, Zambia (L).

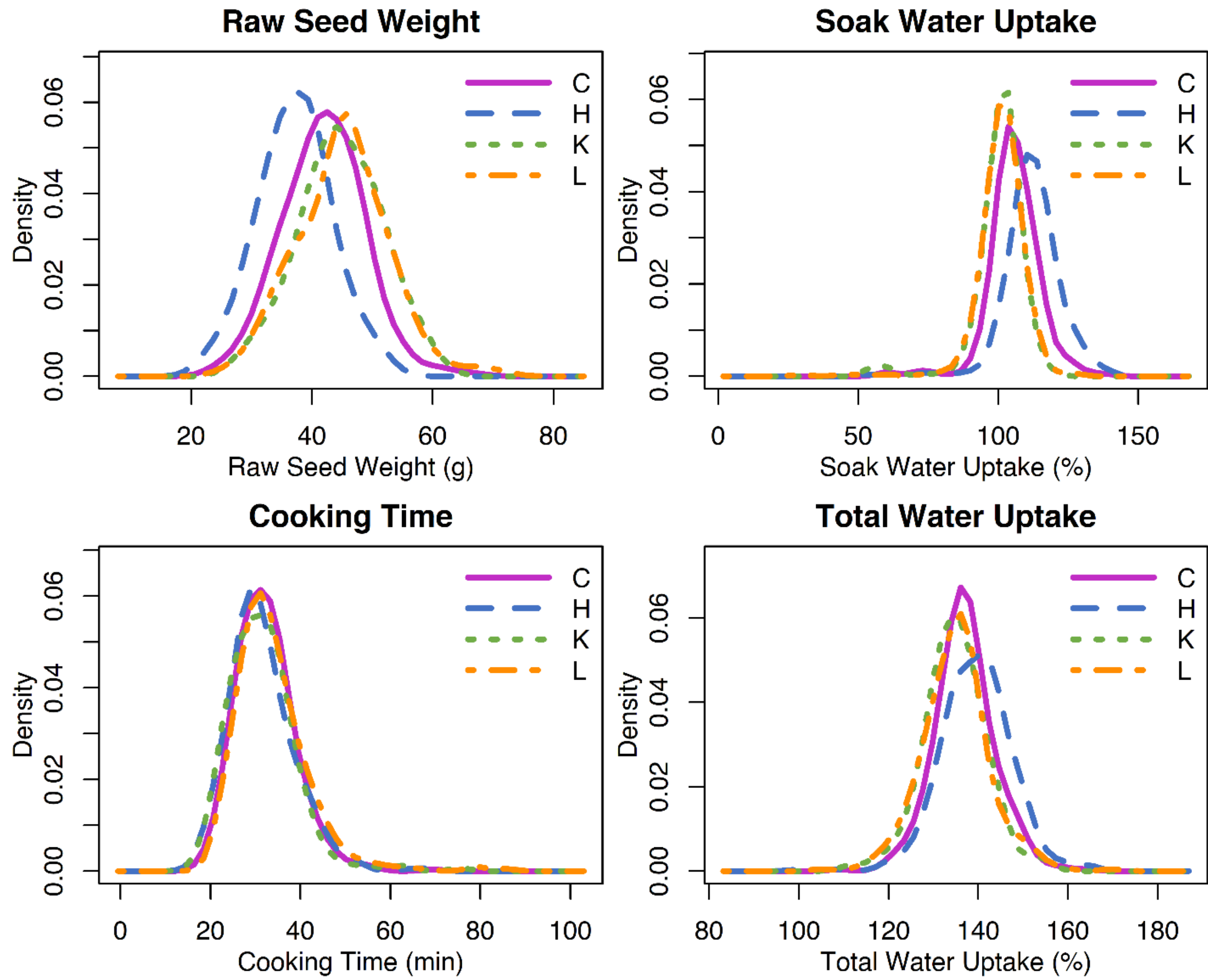


Figure 2.4 Pairwise comparison matrix of cooking time (CT), total flavor intensity (TF), beany intensity (Beany), vegetative intensity (Veg), earthy intensity (Earthy), starchy intensity (Starchy), sweet intensity (Sweet), bitter intensity (Bitter), seed coat perception (SCP), and cotyledon texture (CTex). Pearson correlation coefficients were calculated using BLUPs and are indicated in the lower left, and scatterplots for each pairwise comparison with LOWESS regression lines are shown in the upper right. *P*-values are indicated by asterisks, where *, **, and *** represent <0.05, <0.01, and <0.001 respectively.

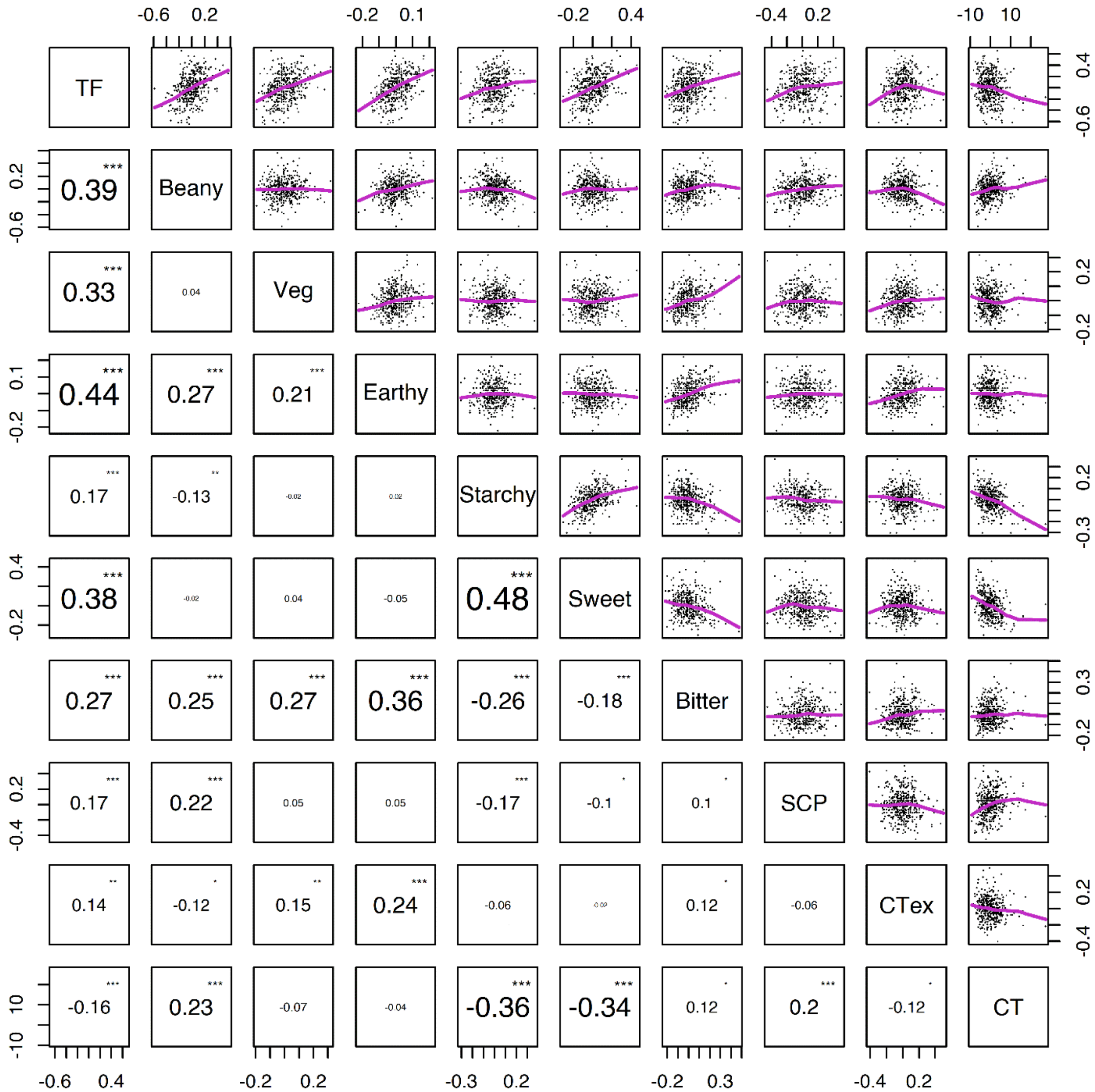


Figure 2.5 Principal component analysis biplot with each genotype colored by seed type and loadings for total flavor intensity (TF), beany intensity (Beany), vegetative intensity (Veg), earthy intensity (Earthy), starchy intensity (Starchy), sweet intensity (Sweet), bitter intensity (Bitter), seed coat perception (SCP), cotyledon texture (CTex), and cooking time (CT).

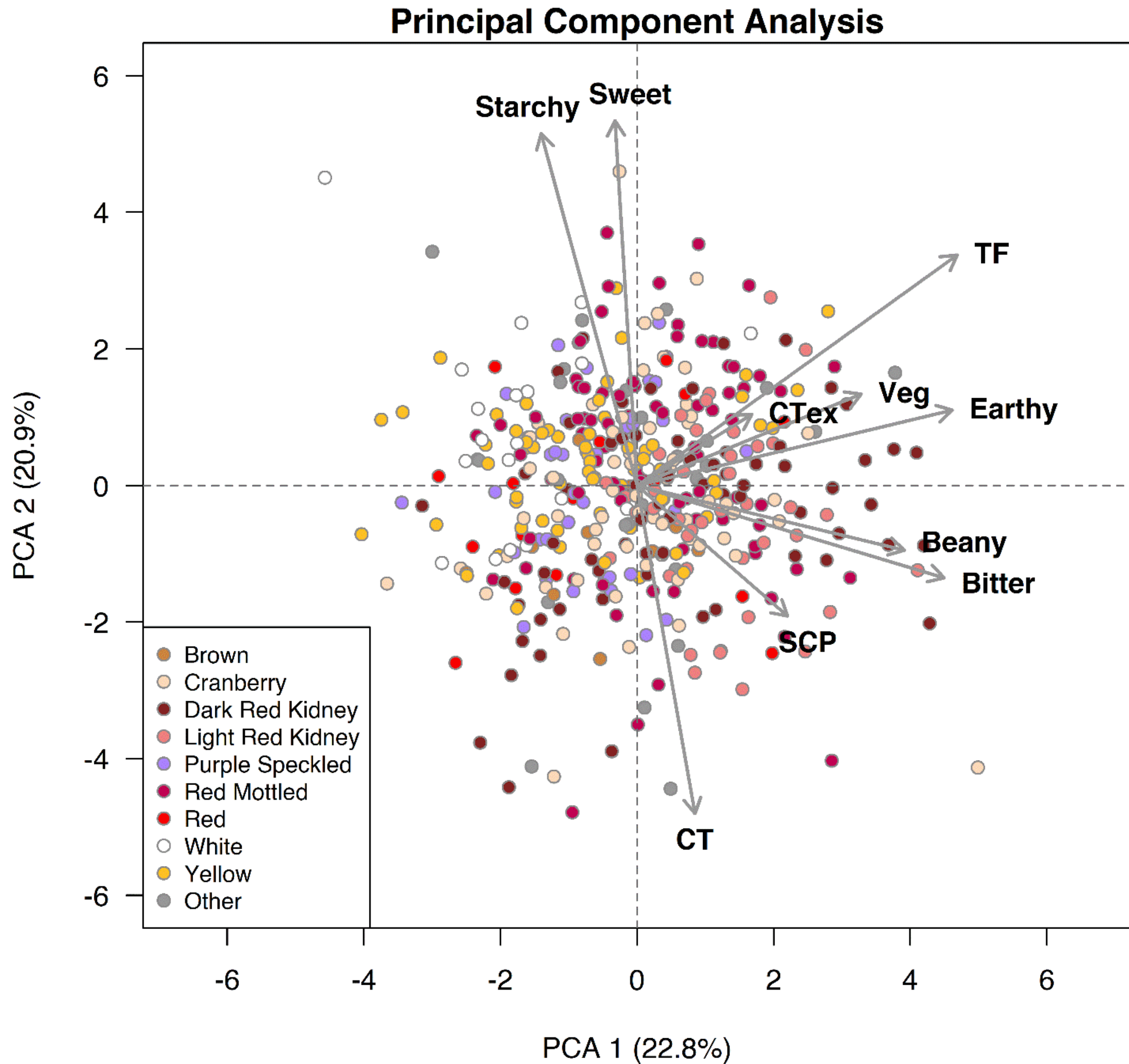


Figure 2.6 Manhattan and QQ plots for total flavor intensity, beany intensity, earthy intensity, seed coat perception, and cotyledon texture of the Andean Diversity Panel with mapping conducted using BLINK with BLUPs from all locations combined. The gray dashed line is the $\alpha = 0.05$ FDR.

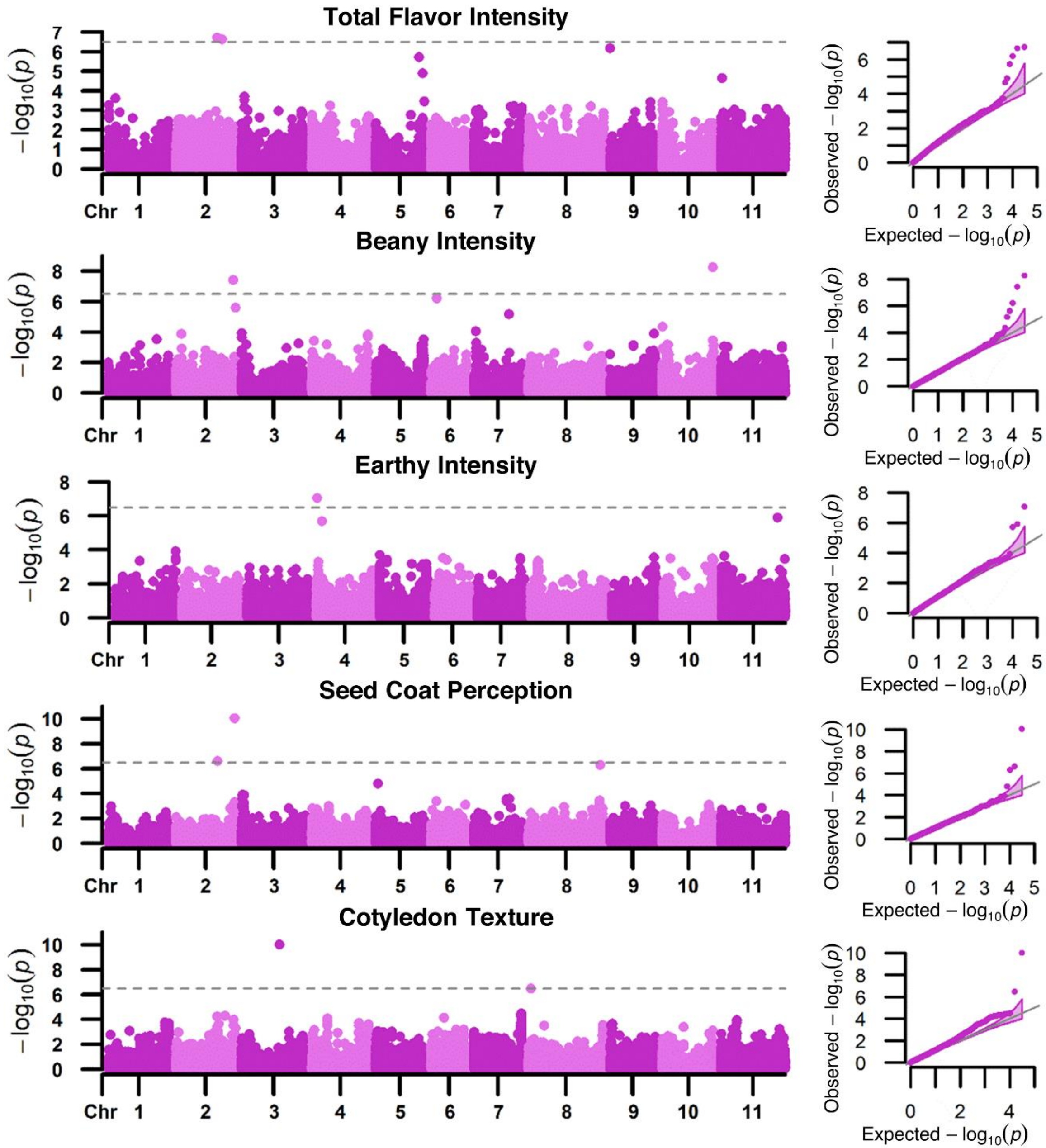
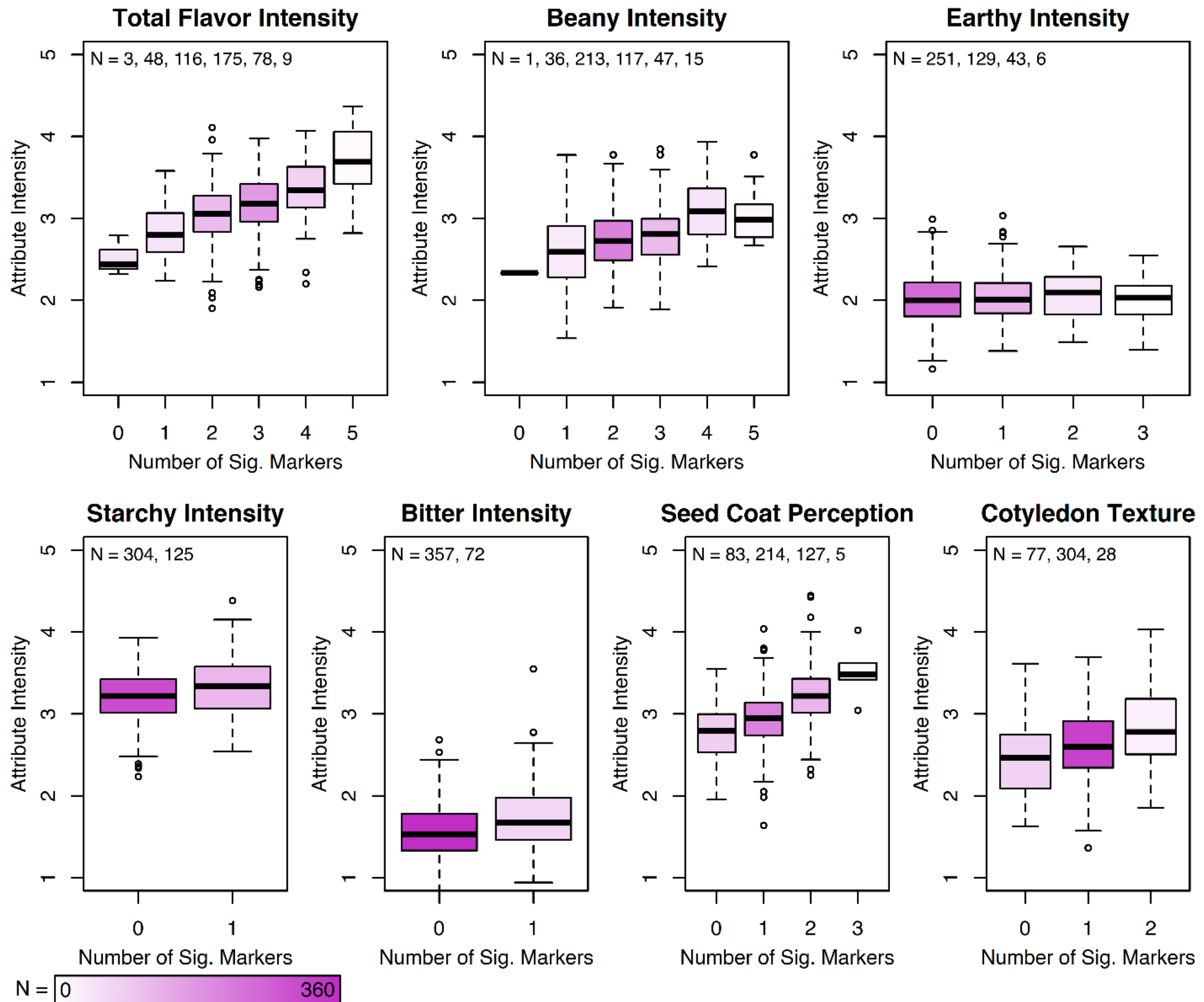


Figure 2.7 Phenotypic effects of carrying the indicated number of significant markers conferring a positive effect for each sensory attribute. Phenotypic values represent all locations combined as averages of least squares estimates from Hawassa, Ethiopia; Kabwe, Zambia; and Lusaka, Zambia. N is the number of individuals in each boxplot.



APPENDIX B:

CHAPTER 2 SUPPLEMENTAL TABLES AND FIGURES

Table S2.1 Genotype information.

ID	Genotype	Seed Type	Grown in Hawassa, ET	Kabwe, ZA	Lusaka, ZA
ADP0001	ROZIKOKO	red mottled	yes	yes	yes
ADP0002	W616444	purple speckled	no	yes	yes
ADP0003	KIDUNGU	red	yes	yes	yes
ADP0004	KILOMBERO	striped	yes	no	yes
ADP0005	KABUKU	red	yes	no	no
ADP0006	W616465	DRK	yes	no	no
ADP0007	BUKOBA	yellow	yes	no	yes
ADP0008	Nyayo	red mottled	yes	no	no
ADP0009	Maalasa	red mottled	yes	yes	yes
ADP0010	CANADA	DRK	yes	yes	yes
ADP0011	KIBOROLONI	red	yes	yes	yes
ADP0012	W616489	DRK	yes	yes	yes
ADP0013	KIBUMBULA	DRK	yes	yes	yes
ADP0014	KIANGWE	yellow	yes	yes	yes
ADP0015	W616495	DRK	yes	no	yes
ADP0016	GOLOLI	red	yes	no	no
ADP0017	W616529	DRK	yes	no	yes
ADP0018	SODAN	DRK	yes	yes	yes
ADP0019	KASUKANYWELE	striped	yes	no	yes
ADP0020	KIGOMA	yellow	no	yes	yes
ADP0021	MBULAMTWE	yellow	yes	yes	yes
ADP0022	KISAPURI	red	yes	yes	yes
ADP0023	MSHORONYLONI	red	yes	yes	yes
ADP0024	YELLOW	yellow	yes	yes	yes
ADP0025	RUHONDELA	purple speckled	yes	yes	yes
ADP0026	BlackWonder	black	yes	no	no
ADP0027	Incomparable	brown	yes	yes	yes
ADP0028	Sisi	yellow	yes	yes	yes
ADP0030	RHNo.6	black	yes	yes	yes
ADP0031	RHNo.11	DRK	yes	yes	yes
ADP0032	RHNo.21	DRK	yes	yes	yes

Table S2.1 (cont'd)

ID	Genotype	Seed Type	Grown in Hawassa, ET	Kabwe, ZA	Lusaka, ZA
ADP0033	KIJIVU	purple speckled	yes	yes	yes
ADP0034	KIJIVU	purple speckled	yes	yes	yes
ADP0035	Kokola	red mottled	yes	no	no
ADP0036	Lyamungu85	red mottled	yes	yes	yes
ADP0037	W616488	brown	no	yes	yes
ADP0038	Moono	DRK	yes	yes	yes
ADP0039	RoziKoko	red mottled	no	yes	yes
ADP0041	MRONDO	DRK	yes	no	yes
ADP0042	MKOKOLA	DRK	yes	no	no
ADP0043	BWANASHAMBA	DRK	yes	yes	yes
ADP0044	KIJIVU	purple speckled	yes	yes	yes
ADP0045	RHNo.12	purple speckled	yes	no	yes
ADP0047	MSOLINI	brown	yes	no	yes
ADP0048	W616534	red	yes	yes	yes
ADP0049	W616546	DRK	yes	no	yes
ADP0050	SALUNDE	yellow	yes	yes	yes
ADP0051	RHNo.3	purple speckled	yes	yes	yes
ADP0052	RHNo.9	purple speckled	yes	yes	yes
ADP0053	MAHARAGEMAKUBWA	DRK	yes	no	no
ADP0054	W616447	cranberry	yes	no	yes
ADP0055	KABUKU	red speckled	yes	no	no
ADP0056	SOYA	purple speckled	yes	no	no
ADP0057	KIJIVU	DRK	yes	yes	no
ADP0058	CANADA	DRK	yes	yes	yes
ADP0059	Poto	purple speckled	yes	no	no
ADP0060	CANADA	DRK	yes	yes	yes
ADP0061	Maulasi	cranberry	yes	yes	yes
ADP0062	MAULASI	red speckled	yes	yes	yes
ADP0063	Soya	purple speckled	yes	no	no
ADP0064	W616500	yellow	yes	yes	yes
ADP0065	W616501	red	yes	yes	yes
ADP0066	NJANO	yellow	yes	yes	yes
ADP0067	NJANO	yellow	yes	no	yes
ADP0068	Soya	purple speckled	no	no	yes
ADP0070	Msafiri	DRK	yes	yes	yes
ADP0071	NJANO-DOLEA	yellow	yes	yes	yes

Table S2.1 (cont'd)

ID	Genotype	Seed Type	Grown in Hawassa, ET	Kabwe, ZA	Lusaka, ZA
ADP0072	MASUSU	brown	yes	no	no
ADP0073	MASUSU	brown	no	no	yes
ADP0074	KABLANKETI	purple speckled	yes	no	no
ADP0075	MABUKU	brown	yes	no	no
ADP0076	KABLANKETI	purple speckled	yes	no	yes
ADP0077	NAMWANGA	purple speckled	yes	no	yes
ADP0080	KABLANKETI	purple speckled	yes	no	no
ADP0081	KABLANKETI	purple speckled	yes	no	yes
ADP0082	KABLANKETI	purple speckled	yes	no	yes
ADP0083	W616547	purple speckled	yes	no	no
ADP0084	KABLANKETINDEFU	purple speckled	yes	no	no
ADP0085	KABLANKETI	purple speckled	yes	yes	yes
ADP0086	NYAMHONGAMWEKUNDU	purple speckled	yes	yes	yes
ADP0087	KABLANKETI	purple speckled	yes	no	yes
ADP0088	KABLANKETI	purple speckled	yes	no	no
ADP0089	KABLANKETI	purple speckled	no	no	yes
ADP0090	KASUKANYWELE	striped	yes	no	yes
ADP0092	MORO	yellow	yes	yes	yes
ADP0093	MORO	yellow	yes	no	yes
ADP0094	LUSHALA	yellow	yes	yes	yes
ADP0095	CANADA	striped	yes	no	yes
ADP0096	Rojo	DRK	yes	yes	yes
ADP0098	Selian97	DRK	yes	yes	yes
ADP0099	BwanaShamba	DRK	yes	yes	yes
ADP0100	EG21	purple speckled	no	yes	yes
ADP0101	Witrood	white	yes	no	yes
ADP0102	Jesca	purple speckled	yes	yes	yes
ADP0103	Pesa	red	yes	yes	yes
ADP0105	Sewani97	DRK	yes	yes	yes
ADP0106	Zawadi	purple speckled	yes	yes	yes
ADP0107	Mishindi	purple speckled	yes	yes	yes
ADP0108	Njano	yellow	yes	yes	yes
ADP0109	Kablanketi	purple speckled	yes	no	yes
ADP0110	SUG-131	cranberry	yes	yes	yes
ADP0111	Uyole98	yellow	no	yes	yes
ADP0113	OPS-RS4	cranberry	no	yes	yes

Table S2.1 (cont'd)

ID	Genotype	Seed Type	Grown in Hawassa, ET	Kabwe, ZA	Lusaka, ZA
ADP0114	OPS-RS1	cranberry	yes	yes	yes
ADP0117	A483	purple mottled	yes	yes	yes
ADP0118	Werna	cranberry	yes	yes	yes
ADP0119	A193	red mottled	no	yes	yes
ADP0120	Tygerberg	cranberry	yes	yes	yes
ADP0121	KranskopHR-1	cranberry	yes	yes	yes
ADP0122	Kranskop	cranberry	no	yes	yes
ADP0123	Jenny	cranberry	yes	yes	yes
ADP0124	Mani	yellow	no	yes	yes
ADP0127	SELIAN06	pink	yes	no	no
ADP0166	NABE4	red mottled	yes	no	yes
ADP0168	KANYEBWA	red speckled	yes	no	no
ADP0180	G433	cranberry	yes	no	no
ADP0186	G1368	DRK	yes	yes	yes
ADP0190	G1678	purple speckled	yes	yes	yes
ADP0191	G1939	cranberry	no	yes	yes
ADP0196	G2875	cranberry	no	yes	yes
ADP0199	G3452	pink	yes	no	yes
ADP0205	G4494	red mottled	yes	yes	yes
ADP0206	G4499	white	yes	yes	yes
ADP0207	G4564	Jacob's cattle	yes	yes	yes
ADP0208	G4644	red mottled	yes	yes	yes
ADP0211	G4780	red mottled	yes	yes	yes
ADP0212	G4970	yellow	yes	yes	yes
ADP0213	G5034	gray	yes	yes	yes
ADP0214	G5087	black	yes	yes	yes
ADP0220	G5625	DRK	no	yes	yes
ADP0224	G6239	yellow eye	yes	yes	yes
ADP0225	G6415	LRK	yes	yes	yes
ADP0232	G7930	white	yes	no	yes
ADP0242	G9013	cranberry	yes	yes	yes
ADP0247	G9975	cranberry	yes	yes	yes
ADP0255	G10994	yellow	yes	yes	yes
ADP0267	G12689	purple cranberry	yes	no	yes
ADP0269	G13092	white	yes	no	yes
ADP0271	G13167	white	yes	no	no

Table S.1 (cont'd)

ID	Genotype	Seed Type	Grown in Hawassa, ET	Kabwe, ZA	Lusaka, ZA
ADP0272	G13336	cranberry	yes	no	no
ADP0276	G13654	yellow	no	yes	yes
ADP0277	G13778	purple mottled	no	no	yes
ADP0279	G14423	Jacob's cattle	yes	no	no
ADP0280	G14440	white	no	no	yes
ADP0303	G17913	yellow	yes	no	yes
ADP0310	G18356	cranberry	yes	yes	yes
ADP0324	G20729	DRK	yes	yes	yes
ADP0336	G21210	red mottled	no	no	yes
ADP0345	G22147	DRK	yes	no	yes
ADP0346	G22246	red mottled	yes	no	yes
ADP0350	G22365	red mottled	no	no	yes
ADP0353	G22455	brown	no	yes	yes
ADP0354	G22502	purple speckled	yes	no	yes
ADP0366	G23070	red	no	no	yes
ADP0367	G23086	cranberry	no	yes	yes
ADP0368	G23093	pink	yes	yes	yes
ADP0376	PI189408	DRK	yes	no	yes
ADP0383	PI209486	red mottled	yes	yes	yes
ADP0390	PI307808	DRK	yes	yes	yes
ADP0391	PI308894	LRK	yes	yes	yes
ADP0392	PI309701	cranberry	yes	no	no
ADP0395	PI310511	red mottled	yes	no	no
ADP0417	PI451906	DRK	yes	yes	yes
ADP0427	Badillo	LRK	yes	yes	yes
ADP0428	ColoradodelPais	red speckled	yes	yes	yes
ADP0429	PR9920-171	red speckled	yes	yes	yes
ADP0430	PR1013-3	red speckled	yes	yes	yes
ADP0431	Gurabo5	red speckled	yes	yes	yes
ADP0432	PR0637-134	red mottled	yes	yes	yes
ADP0433	PR9745-232	red mottled	yes	yes	yes
ADP0434	PR0737-1	red mottled	yes	yes	yes
ADP0436	JB-178	red mottled	no	no	no
ADP0437	PC-50	red mottled	yes	yes	yes
ADP0438	46-1	red mottled	yes	no	yes
ADP0442	LargaComercial	red mottled	yes	no	yes

Table S.1 (cont'd)

ID	Genotype	Seed Type	Grown in Hawassa, ET	Kabwe, ZA	Lusaka, ZA
ADP0449	INIAP 420	yellow	yes	no	no
ADP0450	INIAP422	white	yes	yes	yes
ADP0452	INIAP425	white	yes	no	yes
ADP0453	INIAP428	yellow	yes	no	no
ADP0454	INIAP429	red mottled	yes	no	yes
ADP0455	INIAP430	red mottled	yes	yes	yes
ADP0456	INIAP480	yellow	yes	no	yes
ADP0457	INIAP481	red mottled	yes	yes	yes
ADP0458	INIAP483	red mottled	yes	no	yes
ADP0459	PI331356-C	cranberry	yes	yes	yes
ADP0460	PI331356-B	purple mottled	yes	yes	yes
ADP0462	IZ 117	yellow	yes	no	yes
ADP0464	G39308	DRK	yes	yes	yes
ADP0465	PI321094-D	yellow	yes	yes	yes
ADP0466	PI449430	purple speckled	yes	yes	yes
ADP0467	PI209808	purple speckled	yes	no	no
ADP0468	PI527538	yellow	yes	no	yes
ADP0469	PI527521	white	yes	yes	yes
ADP0470	PI527508	red speckled	yes	yes	yes
ADP0471	IZ 102	yellow	yes	no	no
ADP0472	IZ 102	brown	yes	yes	yes
ADP0474	PI527519	red mottled	yes	yes	yes
ADP0475	PI319706	yellow	yes	yes	yes
ADP0476	Hutterite	yellow	yes	yes	yes
ADP0477	PI527512	purple speckled	no	yes	yes
ADP0478	PI353536	brown	yes	no	yes
ADP0479	PI527530	yellow	yes	no	yes
ADP0480	PI209804	purple mottled	yes	yes	yes
ADP0481	PI449428	red mottled	yes	yes	yes
ADP0482	PI209802	purple mottled	yes	no	no
ADP0483	PI209815	yellow	yes	yes	yes
ADP0508	Calembe	yellow	yes	no	yes
ADP0509	Fernando	yellow	no	yes	yes
ADP0510	Ohliodeperdiz	Jacob's cattle	yes	yes	yes
ADP0511	Canario	yellow	no	yes	yes
ADP0512	Ervilha	yellow	yes	yes	yes

Table S.1 (cont'd)

ID	Genotype	Seed Type	Grown in Hawassa, ET	Kabwe, ZA	Lusaka, ZA
ADP0513	Canario	yellow	no	yes	yes
ADP0514	MantegaAmarela	yellow	yes	no	yes
ADP0515	Katarina,Kibala	cranberry	yes	yes	yes
ADP0516	Mantega, kibala	yellow	no	no	yes
ADP0518	Mantegablanca,Kibala	yellow	yes	no	yes
ADP0519	Katarina,Cela	cranberry	yes	yes	yes
ADP0520	Chumbo,Cela	yellow	yes	no	no
ADP0521	Cebo,Cela	yellow	no	yes	yes
ADP0523	Canario,Cela	yellow	yes	no	yes
ADP0524	KATB1	yellow	yes	no	yes
ADP0525	KATB9	red	no	yes	yes
ADP0526	CAL143	red mottled	yes	yes	yes
ADP0527	POA2	red mottled	yes	yes	yes
ADP0528	LYAMUNGO85	red mottled	yes	yes	yes
ADP0530	SELIAN94	red speckled	yes	yes	yes
ADP0531	AND620	red mottled	yes	yes	yes
ADP0532	A197	yellow	yes	yes	yes
ADP0534	G22501	yellow	yes	no	no
ADP0535	ARA4	red mottled	yes	no	yes
ADP0536	CAL96	red mottled	yes	yes	yes
ADP0537	AFR619	red mottled	yes	yes	yes
ADP0538	RWR221	LRK	yes	yes	yes
ADP0540	AFR708	red mottled	yes	yes	yes
ADP0541	CIM9314-36	red mottled	no	yes	yes
ADP0543	G16157	LRK	yes	yes	yes
ADP0544	PVA773	red mottled	yes	yes	yes
ADP0546	REDCANADIANWONDER	DRK	yes	yes	no
ADP0549	RWR10	DRK	yes	yes	yes
ADP0551	AFR612	red mottled	yes	yes	yes
ADP0553	AND277	red mottled	yes	no	no
ADP0554	AND279	red mottled	yes	no	no
ADP0555	BRB191	red mottled	yes	yes	yes
ADP0556	BRB194	red	yes	no	yes
ADP0557	COS16	cranberry	yes	no	no
ADP0558	DAB528	red	yes	no	yes
ADP0559	DAB555	yellow	yes	yes	yes

Table S.1 (cont'd)

ID	Genotype	Seed Type	Grown in Hawassa, ET	Kabwe, ZA	Lusaka, ZA
ADP0560	DAB230	red mottled	yes	no	no
ADP0561	DAB246	red mottled	yes	no	yes
ADP0562	DAB387	cranberry	yes	yes	yes
ADP0564	G5164	red speckled	yes	no	yes
ADP0566	G5686	yellowmottled	yes	yes	yes
ADP0567	G4523	red mottled	yes	yes	yes
ADP0569	MDRK	DRK	no	yes	yes
ADP0570	NATALSUGAR	cranberry	yes	yes	yes
ADP0571	NUA45	red mottled	yes	no	no
ADP0572	NUA56	red mottled	yes	yes	yes
ADP0574	RADICALCERINZA	red	yes	yes	yes
ADP0575	SAB259	cranberry	yes	yes	yes
ADP0576	SAB618	red mottled	yes	no	no
ADP0577	SAB620	DRK	yes	no	no
ADP0579	SAB623	DRK	yes	no	yes
ADP0580	SAB626	cranberry	yes	yes	yes
ADP0581	SAB629	cranberry	yes	no	yes
ADP0582	SAB630	cranberry	no	yes	yes
ADP0583	SAB650	red mottled	yes	yes	yes
ADP0584	SAB659	red mottled	yes	no	yes
ADP0585	SAB686	cranberry	yes	yes	yes
ADP0586	SAB691	cranberry	yes	yes	yes
ADP0587	SAB712	white	yes	no	yes
ADP0588	SAP1	red mottled	yes	yes	yes
ADP0590	SEQ11	purple mottled	yes	yes	yes
ADP0591	VELAZCOLARGO	LRK	yes	yes	yes
ADP0592	AND1005	red mottled	yes	no	no
ADP0595	G13094	yellow	yes	no	yes
ADP0598	Charlevoix	DRK	yes	yes	yes
ADP0599	Isles	DRK	yes	yes	yes
ADP0601	Camelot	DRK	yes	yes	yes
ADP0603	Wallace773-V98	LRK	yes	yes	yes
ADP0604	1062-V98	LRK	yes	yes	yes
ADP0605	1132-V96	LRK	yes	no	no
ADP0606	NY104	LRK	yes	yes	yes
ADP0607	NY105	LRK	yes	no	no

Table S.1 (cont'd)

ID	Genotype	Seed Type	Grown in Hawassa, ET	Kabwe, ZA	Lusaka, ZA
ADP0608	UI-51	cranberry	yes	no	no
ADP0609	K-407	DRK	yes	yes	yes
ADP0610	G122	cranberry	yes	yes	yes
ADP0611	PompadourB	red mottled	yes	no	yes
ADP0612	ICAQuimbaya	DRK	yes	no	yes
ADP0615	Litekid	LRK	yes	yes	yes
ADP0616	OACLyrick	LRK	no	yes	yes
ADP0617	RedRider	cranberry	yes	yes	yes
ADP0618	ACElk	LRK	no	no	yes
ADP0619	UCD0906	Jacob's cattle	yes	yes	yes
ADP0620	UCD0405	red speckled	yes	yes	yes
ADP0621	JaloEEP558	yellow	yes	yes	yes
ADP0622	UCD0701	Jacob's cattle	yes	yes	yes
ADP0623	Drake	DRK	yes	no	no
ADP0624	Dolly	cranberry	yes	yes	yes
ADP0625	Micran	cranberry	yes	yes	yes
ADP0627	H9659-21-1	LRK	no	yes	yes
ADP0628	H9659-27-7	LRK	yes	yes	yes
ADP0629	H9659-27-10	LRK	yes	yes	yes
ADP0630	H9659-23-1	LRK	yes	yes	yes
ADP0631	OACInferno	LRK	no	no	yes
ADP0632	TARSHT1	DRK	yes	no	yes
ADP0633	TARS-HT2	LRK	yes	yes	yes
ADP0635	OACRedstar	DRK	yes	yes	yes
ADP0636	Montcalm	DRK	yes	yes	yes
ADP0637	Isabella	LRK	yes	no	yes
ADP0638	RedHawk	DRK	yes	yes	yes
ADP0639	Chinook2000	LRK	yes	yes	yes
ADP0640	Beluga	white	yes	yes	yes
ADP0641	Capri	cranberry	yes	no	no
ADP0642	TaylorHort	cranberry	yes	yes	yes
ADP0643	Cardinal	cranberry	yes	yes	yes
ADP0644	FoxFire	LRK	yes	yes	yes
ADP0645	Lassen	white	yes	yes	yes
ADP0646	Myasi	yellow	yes	no	yes
ADP0647	RedKanner	LRK	yes	no	no

Table S.1 (cont'd)

ID	Genotype	Seed Type	Grown in Hawassa, ET	Kabwe, ZA	Lusaka, ZA
ADP0648	RedKloud	LRK	yes	no	yes
ADP0649	Kamiakin	LRK	yes	yes	yes
ADP0650	K-42	LRK	yes	yes	yes
ADP0651	K-59	LRK	yes	yes	yes
ADP0652	Lisa	white	yes	no	yes
ADP0653	USDK-CBB-15	DRK	yes	yes	yes
ADP0654	USDK-4	DRK	yes	no	yes
ADP0655	Fiero	DRK	yes	no	no
ADP0656	RoyalRed	DRK	yes	no	yes
ADP0657	Kardinal	LRK	yes	yes	yes
ADP0658	Blush	LRK	yes	no	no
ADP0659	USLK-1	LRK	yes	no	no
ADP0660	Krimson	cranberry	yes	no	no
ADP0662	USCR-9	cranberry	yes	no	no
ADP0663	USCR-CBB-20	cranberry	yes	yes	yes
ADP0664	SilverCloud	white	yes	no	yes
ADP0665	USWK-CBB-17	white	yes	no	yes
ADP0666	USWK-6	white	yes	no	yes
ADP0667	VA-19	LRK	yes	no	no
ADP0668	Cran-09	cranberry	yes	yes	yes
ADP0669	OACLyrick	LRK	no	yes	yes
ADP0670	ACCalmont	DRK	no	yes	yes
ADP0671	ACElk	LRK	no	yes	yes
ADP0672	CDRK	DRK	yes	yes	yes
ADP0673	UCNichols	DRK	yes	yes	yes
ADP0674	UCD0704	white	yes	no	no
ADP0675	UCD0801	cranberry	yes	yes	yes
ADP0676	CELRK	LRK	yes	yes	yes
ADP0677	Etna	cranberry	yes	yes	yes
ADP0678	Hooter	cranberry	yes	yes	yes
ADP0679	RedRover	DRK	yes	yes	yes
ADP0680	Clouseau	LRK	yes	no	no
ADP0682	UI-686	cranberry	yes	no	no
ADP0683	IJR	red speckled	yes	yes	yes
ADP0684	Majesty	DRK	no	yes	yes
ADP0687	PinkPanther	LRK	no	yes	yes

Table S.1 (cont'd)

ID	Genotype	Seed Type	Grown in Hawassa, ET	Kabwe, ZA	Lusaka, ZA
ADP0701	1. Bola 60 Dias	yellow	no	no	yes
ADP0704	4. Canela	yellow	no	no	yes
ADP0705	5. Cerrillos	white	no	no	yes
ADP0708	8. Gordo	yellow	no	yes	no
ADP0710	10. Dore de Kirundo	yellow	no	no	yes
ADP0711	11. Lingua de Fuego	cranberry	no	no	yes
ADP0716	MW-1	cranberry	yes	no	yes
ADP0717	MW-2	cranberry	yes	yes	yes
ADP0719	MW-4	red mottled	yes	no	yes
ADP0720	MW-5	cranberry	no	yes	yes
ADP0721	MW-6	DRK	yes	no	yes
ADP0722	MW-7	cranberry	yes	yes	yes
ADP0724	MW-9	DRK	yes	yes	yes
ADP0725	MW-10	cranberry	yes	yes	yes
ADP0726	MW-11	cranberry	yes	yes	yes
ADP0727	MW-12	yellow	yes	yes	yes
ADP0728	MW-13	LRK	no	yes	yes
ADP0729	MW-14	striped	yes	yes	yes
ADP0730	MW-15	red mottled	yes	yes	yes
ADP0731	MW-16	red mottled	yes	yes	yes
ADP0732	MW-17	red mottled	yes	yes	yes
ADP0733	MW-18	red mottled	yes	no	yes
ADP0734	MW-19	red mottled	no	yes	yes
ADP0735	MW-20	red mottled	yes	yes	yes
ADP0736	MW-21	red mottled	yes	yes	yes
ADP0737	MW-22	red mottled	yes	yes	yes
ADP0738	MW-23	red	yes	no	no
ADP0739	MW-24	striped	yes	no	yes
ADP0740	MW-25	red mottled	yes	yes	yes
ADP0741	PI638823	brown	yes	no	yes
ADP0742	PI661755	DRK	no	yes	yes
ADP0743	PI638811	DRK	yes	yes	yes
ADP0744	PI638818	red speckled	yes	no	yes
ADP0745	W616496	DRK	yes	no	no
ADP0746	PI661774	DRK	yes	yes	yes
ADP0747	PI638816	DRK	no	no	yes

Table S.1 (cont'd)

ID	Genotype	Seed Type	Grown in Hawassa, ET	Kabwe, ZA	Lusaka, ZA
ADP0748	PI661756	red speckled	yes	no	no
ADP0750	W616493	DRK	yes	yes	yes
ADP0751	W616550	red mottled	yes	no	no
ADP0752	PI321119	DRK	yes	no	yes
ADP0753	PI146757	black and white	yes	yes	yes
ADP0754	PI661779	DRK	yes	no	no
ADP0757	Pasi	yellow	yes	no	no
ADP0758	Kabinima	red mottled	yes	no	yes
ADP0759	Urafiki	DRK	yes	no	no
ADP0760	Wanja	yellow	yes	yes	yes
ADP0761	Uyole-04	yellow	no	no	yes
ADP0762	Uyole-84	yellow	no	no	yes
ADP0763	Punda	purple speckled	no	yes	yes
ADP0764	Kalubungula	red	no	yes	yes
ADP0765	KK25/KK73/3/666/5-L7	DRK	no	yes	yes
ADP0767	ACUG10-D3	DRK	no	no	yes
ADP0768	ACUG12-D1	DRK	no	no	yes
ADP0769	ACUG12-C1	cranberry	yes	yes	yes
ADP0770	ACUG12-C2	cranberry	yes	no	yes
ADP0771	ACUG13-C1	cranberry	yes	yes	yes
ADP0773	ACUG13-L1	LRK	yes	no	no
ADP0774	ACUG13-L2	LRK	yes	yes	yes
ADP0775	HR202-4973	cranberry	no	no	yes
ADP0776	Dynasty	DRK	yes	no	no
ADP0777	AC-Darkid	DRK	yes	no	no
ADP0778	.	white	yes	no	yes
ADP0779	CDC-Sol	yellow	yes	no	yes
ADP0780	L11YL002	yellow	yes	no	yes
ADP0781	L11YL012	yellow	yes	no	no
ADP0782	L12LK007	LRK	yes	yes	yes
ADP0783	PS03-001-5-1-B3	cranberry	yes	no	yes
ADP0784	PS11-006C-8-B	cranberry	yes	no	no
ADP0785	PS11-006C-1-B	cranberry	yes	no	yes
ADP0788	Snowdon	white	no	no	yes
ADP0789	PR0313-3	red speckled	no	yes	yes
ADP0791	PR1146-123	yellow	yes	no	yes

Table S.1 (cont'd)

ID	Genotype	Seed Type	Grown in		
			Hawassa, ET	Kabwe, ZA	Lusaka, ZA
ADP0792	PR1146-124	yellow	yes	no	no
ADP0794	Sederberg	cranberry	no	yes	yes
ADP0796	RS 6	cranberry	no	no	yes

Table S2.2 5-point sensory attribute intensity scales.

Trait	Scale Description
Total Flavor Intensity	1-5, bland to strongly flavored
Beany Intensity	1-5, no/very little beany flavor to very strong beany flavor
Vegetative Intensity	1-5, no vegetative flavor to very strong vegetative flavor
Earthy Intensity	1-5, no earthy flavor to very strong earthy flavor
Starchy Intensity	1-5, no starchy taste to very strong starchy taste
Sweet Intensity	1-5, no sweet taste to very strong sweet taste
Bitter Intensity	1-5, no bitter taste to very strong bitter taste
Seed Coat Perception	1-5, imperceptible seed coat to very tough/lingering seed coat
Cotyledon Texture	1-5: mushy to very gritty/firm

Table S2.3 *P*-values[a] for the random effects from the sensory attribute intensity ANOVAs at the genotype level.

Trait	Rep	Panelist(Loc)	Session(Loc)
Total Flavor Intensity	NS	<0.0001	<0.0001
Beany Intensity	NS	<0.0001	<0.0001
Vegetative Intensity	NS	<0.0001	<0.0001
Earthy Intensity	NS	<0.0001	0.0004
Starchy Intensity	NS	<0.0001	<0.0001
Sweet Intensity	NS	<0.0001	<0.0001
Bitter Intensity	NS	<0.0001	0.0002
Seed Coat Perception	<0.0001	<0.0001	<0.0001
Cotyledon Texture	NS	<0.0001	<0.0001

[a] NS indicates non-significant *p*-values at $\alpha = 0.05$

Table S2.4 Mean sensory attribute intensities across the 3 locations for the genotypes exhibiting extreme sensory attribute intensities[a].

ADP ID	Total Flavor	Beany	Vegetative	Earthy	Starchy	Sweet	Bitter	Seed Coat Perception	Cotyledon Texture
ADP0106	2.64	2.96	2.00	1.65	3.10	1.25	1.74	2.80	2.86
ADP0681	3.98	3.42	2.44	2.18	3.2	2.19	1.28	3.82	2.32
ADP0654	3.75	3.61	1.67	2.22	2.93	1.71	2.06	3.02	2.56
ADP0530	3.74	3.04	3.39	2.83	3.48	2.01	1.45	2.68	2.86
ADP0057	2.51	2.74	1.97	2.99	3.49	1.85	1.68	2.53	2.44
ADP0206	3.03	2.00	2.03	2.59	4.38	2.19	1.08	2.67	3.19
ADP0190	3.60	2.59	1.20	1.82	3.63	3.46	1.03	2.34	2.82
ADP0517	3.64	2.56	2.71	2.01	2.1	1.23	3.18	3.3	2.54
ADP0005	2.43	2.90	1.69	1.54	2.79	1.37	1.13	1.64	2.64
ADP0450	2.73	2.44	2.38	1.97	2.94	1.48	1.28	4.43	3.07
ADP0791	2.16	2.17	1.98	1.83	3.35	1.40	1.28	2.53	2.00
ADP0044	2.78	2.04	1.63	1.72	2.83	1.50	2.64	2.78	3.17

[a] Extreme attributes exhibited by each genotype are indicated with boxes

Table S2.5 *P*-values[a] for the fixed and random effects from the sensory attribute intensity ANOVAs at the seed type level.

Trait	Seed Type	Loc	Seed Type x Loc	Rep	Reviewer(Loc)	Session(Loc)
Total Flavor Intensity	<.0001	0.0001	<0.0001	0.0008	<0.0001	<0.0001
Beany Intensity	<.0001	NS	0.0002	0.0408	<0.0001	<0.0001
Vegetative Intensity	<.0001	NS	0.0006	NS	<0.0001	<0.0001
Earthy Intensity	<.0001	NS	0.0009	NS	<0.0001	<0.0001
Starchy Intensity	<.0001	NS	NS	NS	<0.0001	<0.0001
Sweet Intensity	<.0001	NS	<0.0001	0.0314	<0.0001	<0.0001
Bitter Intensity	<.0001	NS	0.0032	NS	<0.0001	<0.0001
Seed Coat Perception	<.0001	NS	0.0011	<0.0001	<0.0001	<0.0001
Cotyledon Texture	<.0001	0.0009	<0.0001	NS	<0.0001	<0.0001

[a] NS indicates non-significant *p*-values at $\alpha = 0.05$

Table S2.6 GWAS significant markers associated with sensory attribute intensities determined via BLINK with marker, chromosome (Chr), position, *P*-value, minor allele frequency (MAF), major and minor alleles (Maj/Min), significance (Sig), and location indicated.

Trait	Marker	Chr	Position[a]	*P*-value	MAF	Maj/Min[b]	Sig[c]	Loc[d]
Total Flavor Intensity								
	S02_37932341	2	37932341	1.04E-08	0.25	G/**A**	***	H
	S03_36213088	3	36213088	4.32E-06	0.18	T/**A**	**	K
	S03_51252684	3	51252684	1.57E-07	0.45	G/**A**	***	H
	S04_47465212	4	47465212	8.95E-09	0.12	**T**/C	***	H
	S05_8530078	5	8530078	1.84E-06	0.19	**T**/A	***	H
	S05_35951411	5	35951411	8.83E-08	0.09	**G**/A	***	H
	S05_40598752	5	40598752	7.14E-06	0.10	G/**T**	*	L
	S06_6583452	6	6583452	1.92E-07	0.42	**T**/A	***	L
	S08_4550936	8	4550936	9.09E-09	0.06	**C**/T	***	K
	S09_10273671	9	10273671	3.39E-06	0.32	**G**/A	**	K
	S10_42515259	10	42515259	2.38E-08	0.06	**T**/A	***	K
	S10_42798266	10	42798266	4.04E-06	0.11	**A**/G	*	L
	S11_726776	11	726776	6.73E-07	0.25	G/**A**	***	H
	S11_1465049	11	1465049	1.12E-07	0.24	**C**/T	***	K
	S11_46750806	11	46750806	1.72E-06	0.25	G/**T**	**	K
Beany Intensity								
	S02_48688740	2	48688740	1.29E-05	0.07	A/**G**	*	L
	S06_5391064	6	5391064	1.93E-06	0.07	**T**/G	**	L
	S10_42528848	10	42528848	6.12E-09	0.12	**G**/A	***	K
	S10_44117615	10	44117615	4.69E-06	0.06	**C**/T	**	L
	S11_44125952	11	44125952	2.57E-09	0.07	C/**A**	***	K
	S11_46580267	11	46580267	2.21E-08	0.16	**A**/C	***	L
Earthy Intensity								
	S02_48899330	2	48899330	4.00E-11	0.12	A/**C**	***	L
	S04_448769	4	448769	1.56E-09	0.08	C/**T**	***	K
	S11_8151131	11	8151131	7.34E-07	0.09	A/**G**	**	L
Seed Coat Perception								
	S02_34387999	2	34387999	6.35E-07	0.06	G/**T**	***	L
	S02_49203869	2	49203869	5.40E-10	0.28	G/**A**	***	H
	S05_1034657	5	1034657	7.00E-09	0.37	**T**/A	***	H
	S05_2198768	5	2198768	2.29E-06	0.44	**C**/G	*	K
	S07_3664145	7	3664145	2.50E-07	0.49	G/**C**	***	L

[a] Position is based on the P. vulgaris v2.1 reference genome (DOE-JGI and USDA-NIFA, http://phytozome.jgi.doe.gov/)

Table S2.6 (cont'd)

[b] Alleles in bold confer a positive effect on the indicated trait
[c] Significance is indicated by asterisks, such that *, **, *** indicate significance at $\alpha = 0.1$, $\alpha = 0.05$, $\alpha = 0.01$ using the false discovery rate
[d] H is Hawassa, Ethiopia; K is Kabwe, Zambia; and L is Lusaka, Zambia

Table S2.7 GWAS significant markers associated with cooking time, soak water uptake, raw seed weight, and total water uptake, with chromosome (Chr), position, R2, effect associated with the minor allele, major and minor alleles, minor allele frequency (MAF), major and minor alleles (Maj/Min), significance (Sig), and method indicated.

Trait	Marker	Chr	Position[a]	*P*-value	MAF	Maj/Min[b]	Sig[c]	Method
Raw Seed Weight								
	S01_47840887	1	47840887	9.04E-07	0.14	**G**/A	***	BLINK
	S01_49584124	1	49584124	3.14E-06	0.47	C/**G**	***	BLINK
	S02_33254640	2	33254640	5.72E-18	0.06	**T**/A	***	BLINK
	S03_40318649	3	40318649	6.57E-06	0.30	**G**/A	**	BLINK
	S03_41895570	3	41895570	3.55E-07	0.31	A/**C**	***	BLINK, MLM
	S04_1769598	4	1769598	1.36E-06	0.12	G/**A**	***	BLINK
	S05_1069847	5	1069847	1.79E-15	0.49	**A**/G	***	BLINK
	S05_1138961	5	1138961	6.10E-07	0.36	C/**T**	**	MLM
	S05_36225413	5	36225413	1.05E-11	0.15	T/**C**	***	BLINK
	S06_18456447	6	18456447	2.59E-09	0.08	G/**A**	***	BLINK
	S07_1842933	7	1842933	1.91E-06	0.18	**C**/A	***	BLINK
	S07_25513414	7	25513414	8.08E-06	0.18	T/**C**	**	BLINK
	S08_61954787	8	61954787	7.27E-12	0.39	A/**G**	***	BLINK
	S09_33770475	9	33770475	1.27E-06	0.07	**A**/G	***	BLINK
	S11_46634045	11	46634045	4.36E-07	0.13	G/**A**	***	BLINK
Soak Water Uptake								
	S02_47837868	2	47837868	8.17E-08	0.25	**G**/A	***	BLINK
	S03_25546920	3	25546920	5.30E-06	0.05	**C**/T	*	MLM
	S03_50652595	3	50652595	3.36E-06	0.07	**A**/T	**	MLM
	S03_51140861	3	51140861	1.89E-11	0.07	**G**/A	***	BLINK
	S04_30764016	4	30764016	1.41E-05	0.24	C/**T**	**	BLINK
	S04_47654443	4	47654443	1.26E-05	0.08	**G**/A	*	MLM
	S05_37924556	5	37924556	4.53E-06	0.07	**C**/G	**	MLM
	S07_3919560	7	3919560	2.66E-06	0.06	**C**/G	***	BLINK
	S07_18212326	7	18212326	1.34E-06	0.09	**A**/G	***	BLINK
	S07_27774103	7	27774103	4.22E-10	0.19	C/**T**	***	BLINK
	S07_38497123	7	38497123	3.20E-06	0.38	**A**/G	***	BLINK
	S07_39390008	7	39390008	1.10E-09	0.42	A/**G**	***	BLINK
	S08_59981977	8	59981977	1.49E-06	0.05	**T**/A	***	BLINK
	S08_60478317	8	60478317	1.14E-07	0.34	C/**T**	***	BLINK
	S10_37637761	10	37637761	8.68E-08	0.12	**T**/C	***	BLINK,MLM
	S10_43391440	10	43391440	3.25E-07	0.06	**A**/G	**	MLM
	S11_5714496	11	5714496	1.83E-11	0.05	**C**/A	***	BLINK

Table S2.7 (cont'd)

Cooking Time							
S03_4885990	3	4885990	1.03E-07	0.05	T/**G**	***	BLINK
S03_5243893	3	5243893	2.04E-06	0.07	A/**G**	*	BLINK
S03_51292502	3	51292502	2.07E-05	0.06	A/**T**	*	BLINK
S04_3957256	4	3957256	2.24E-05	0.24	**C**/G	*	MLM
S04_47068842	4	47068842	2.93E-08	0.08	A/**G**	***	BLINK
S06_19636517	6	19636517	2.02E-05	0.08	**T**/G	*	BLINK
S07_3009718	7	3009718	6.05E-08	0.07	T/**C**	***	BLINK
S07_30919254	7	30919254	1.54E-05	0.35	**T**/C	*	BLINK
S08_60104796	8	60104796	2.31E-06	0.27	**C**/A	**	BLINK
S08_62659170	8	62659170	3.52E-06	0.16	A/**G**	**	MLM
S11_10805992	11	10805992	2.22E-07	0.10	**C**/T	***	BLINK
Total Water Uptake							
S03_2580077	3	2580077	1.11E-06	0.07	G/**A**	**	BLINK
S03_7619818	3	7619818	1.17E-06	0.27	**T**/C	**	BLINK
S04_30764016	4	30764016	4.25E-07	0.24	C/**T**	**	BLINK
S09_37046204	9	37046204	8.29E-06	0.08	**C**/T	*	BLINK
S11_48753729	11	48753729	3.44E-06	0.12	**T**/A	**	BLINK

[a] Position is based on the P. vulgaris v2.1 reference genome (DOE-JGI and USDA-NIFA, http://phytozome.jgi.doe.gov/)
[b] Alleles in bold confer a positive effect on the indicated trait
[c] Significance is indicated by asterisks, such that *, **, *** indicate significance at $\alpha = 0.1$, $\alpha = 0.05$, $\alpha = 0.01$ using the false discovery rate for the BLINK method and a Bonferroni correction based on the effective number of markers determined using the SimpleM algorithm for the MLM method

Figure S2.1 Images of the genotypes exhibiting extreme sensory attribute intensities identified from screening accessions of the Andean Diversity Panel grown in Hawassa, Ethiopia.

Figure S2.2 Manhattan and QQ plots for total flavor intensity, beany intensity, earthy intensity, seed coat perception, and cotyledon texture of the Andean Diversity Panel with mapping conducted using MLM with BLUPs from all locations combined. The gray dashed line is the $\alpha = 0.05$ Bonferroni correction based on the effective number of markers determined using the SimpleM algorithm.

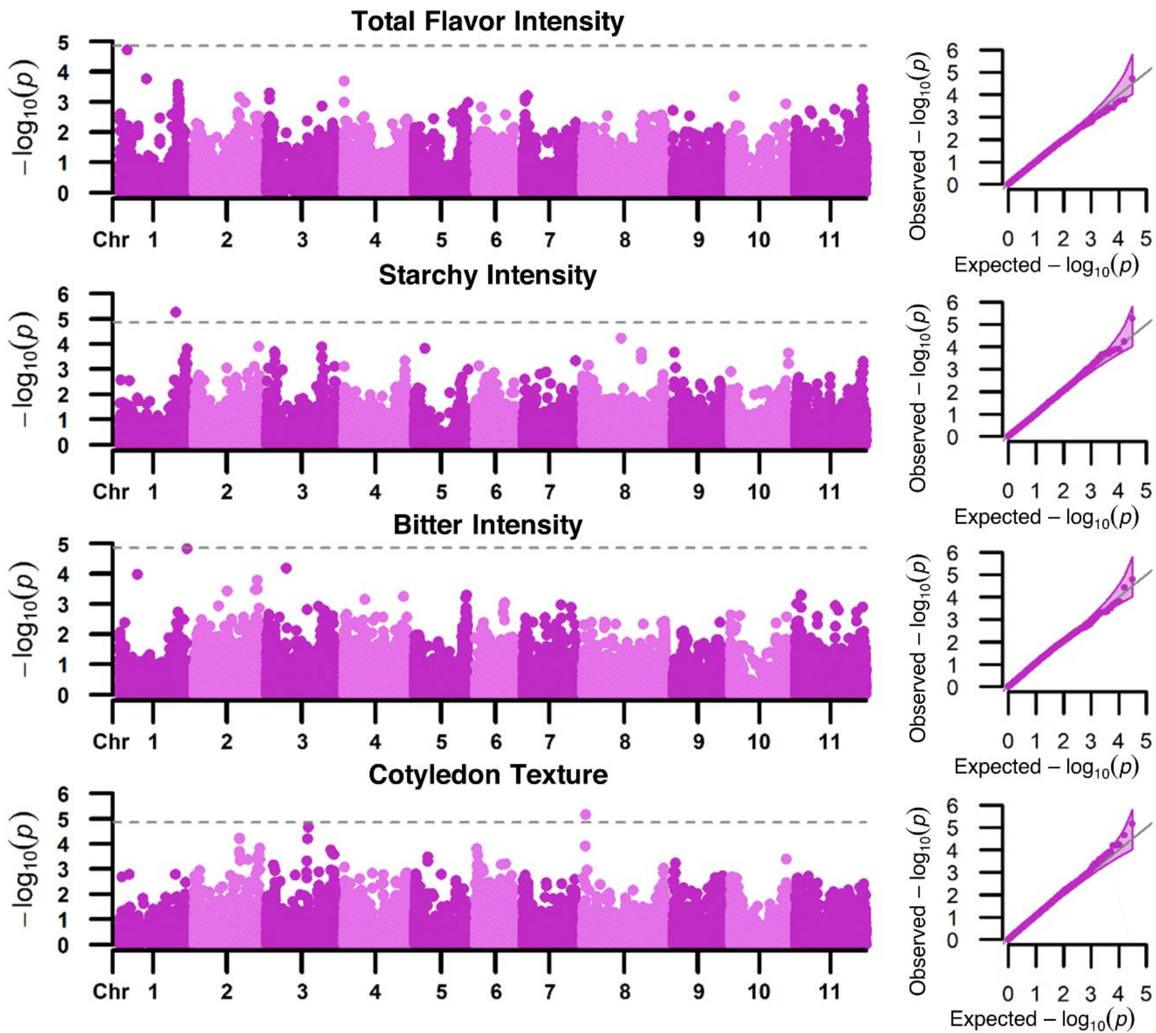


Figure S2.3 Manhattan and QQ plots for total flavor intensity of the Andean Diversity Panel with mapping conducted using BLINK with BLUPs for Hawassa, Ethiopia (H); Kabwe, Zambia (K); and Lusaka, Zambia (L). The gray dashed line is the α = 0.05 FDR.

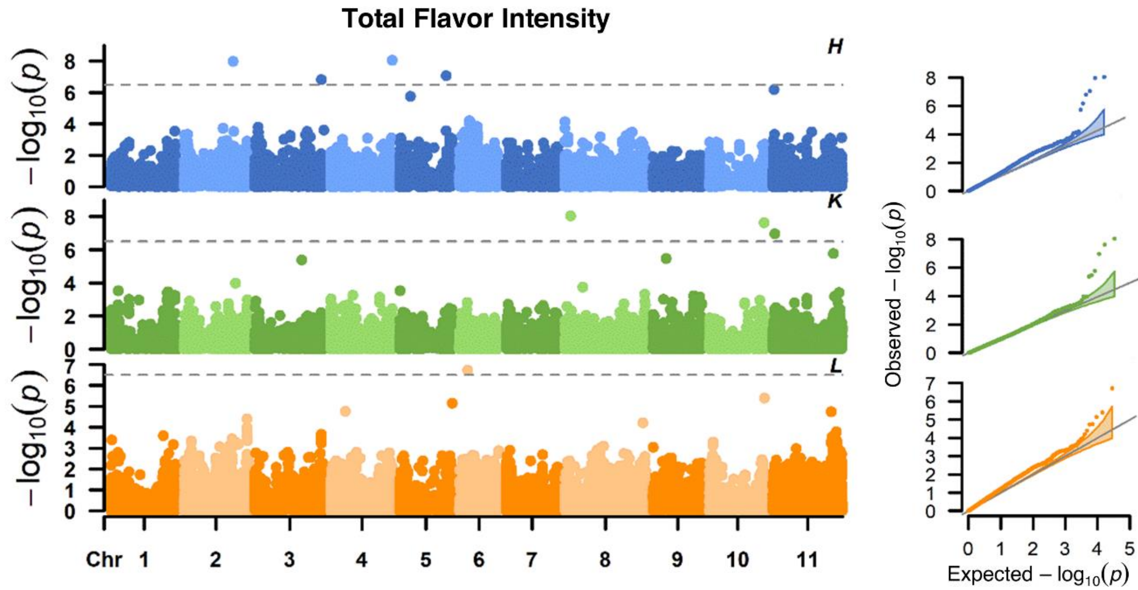


Figure S2.4 Manhattan and QQ plots for beany intensity of the Andean Diversity Panel with mapping conducted using BLINK with BLUPs for Hawassa, Ethiopia (H); Kabwe, Zambia (K); and Lusaka, Zambia (L). The gray dashed line is the α = 0.05 FDR.

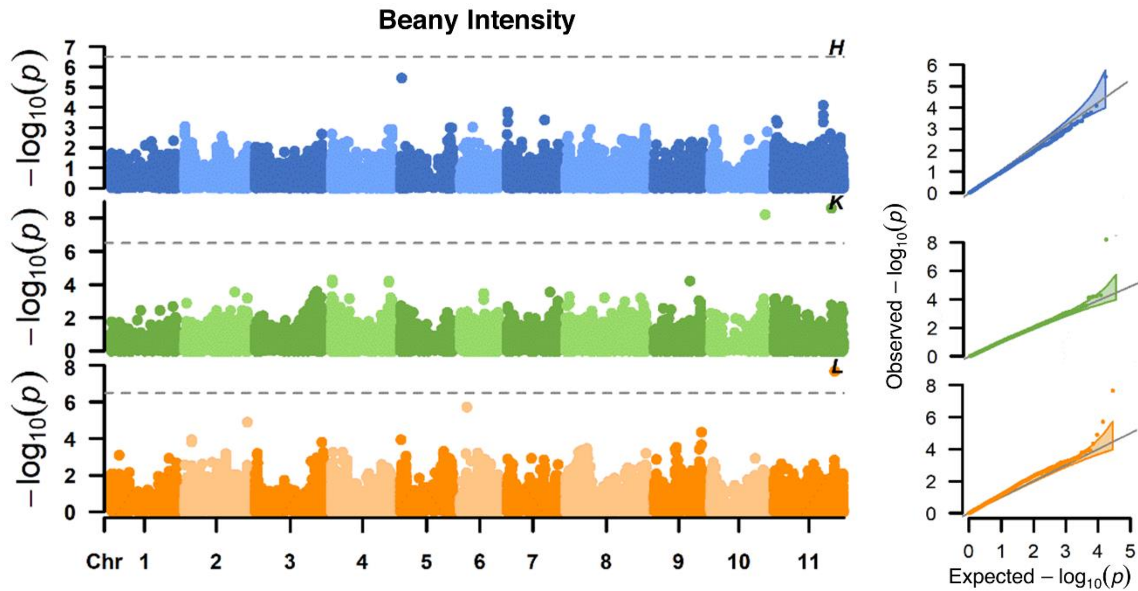


Figure S2.5 Manhattan and QQ plots for earthy intensity of the Andean Diversity Panel with mapping conducted using BLINK with BLUPs for Hawassa, Ethiopia (H); Kabwe, Zambia (K); and Lusaka, Zambia (L). The gray dashed line is the α = 0.05 FDR.

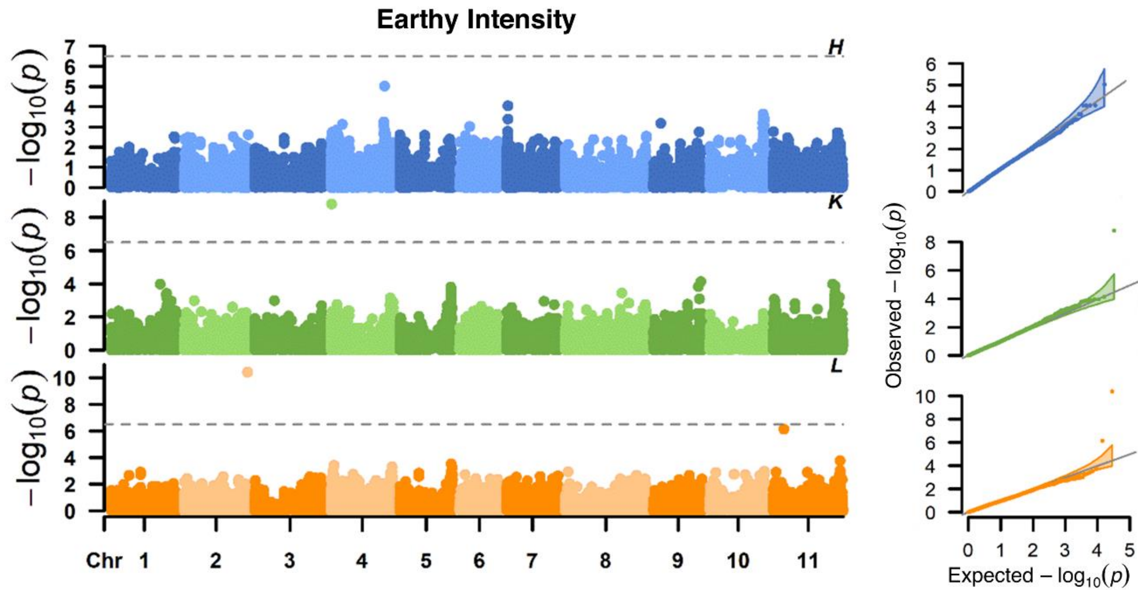


Figure S2.6 Manhattan and QQ plots for seed coat perception of the Andean Diversity Panel with mapping conducted using BLINK with BLUPs for Hawassa, Ethiopia (H); Kabwe, Zambia (K); and Lusaka, Zambia (L). The gray dashed line is the α = 0.05 FDR.

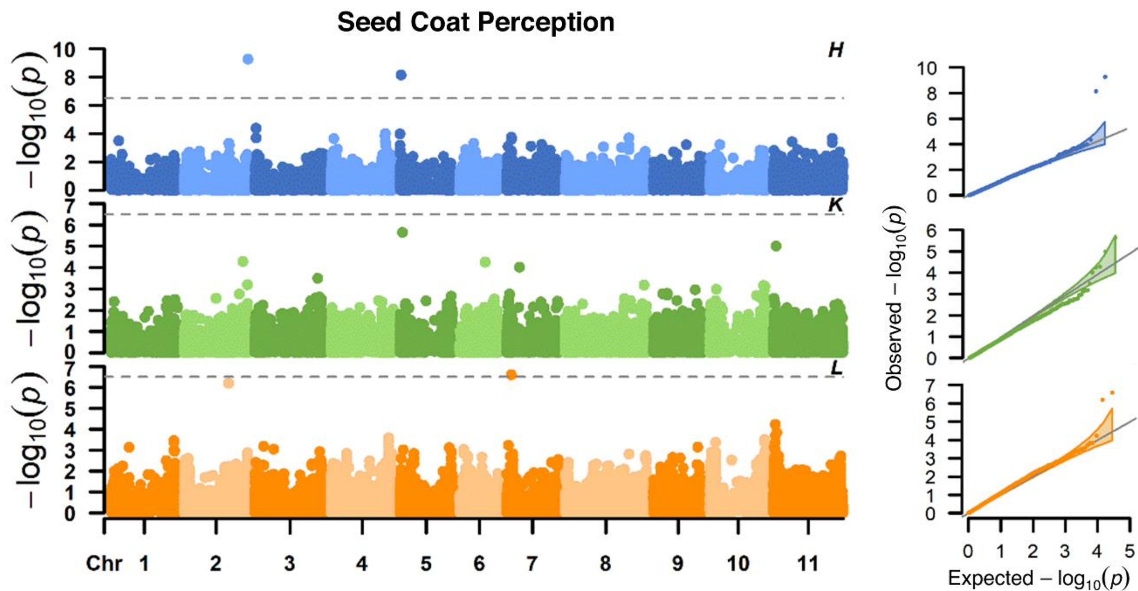


Figure S2.7 Manhattan and QQ plots for raw seed weight, soak water uptake, cooking time, and total water uptake of the Andean Diversity Panel with mapping conducted using BLINK with BLUPs from all locations combined. The gray dashed line is the α = 0.05 FDR.

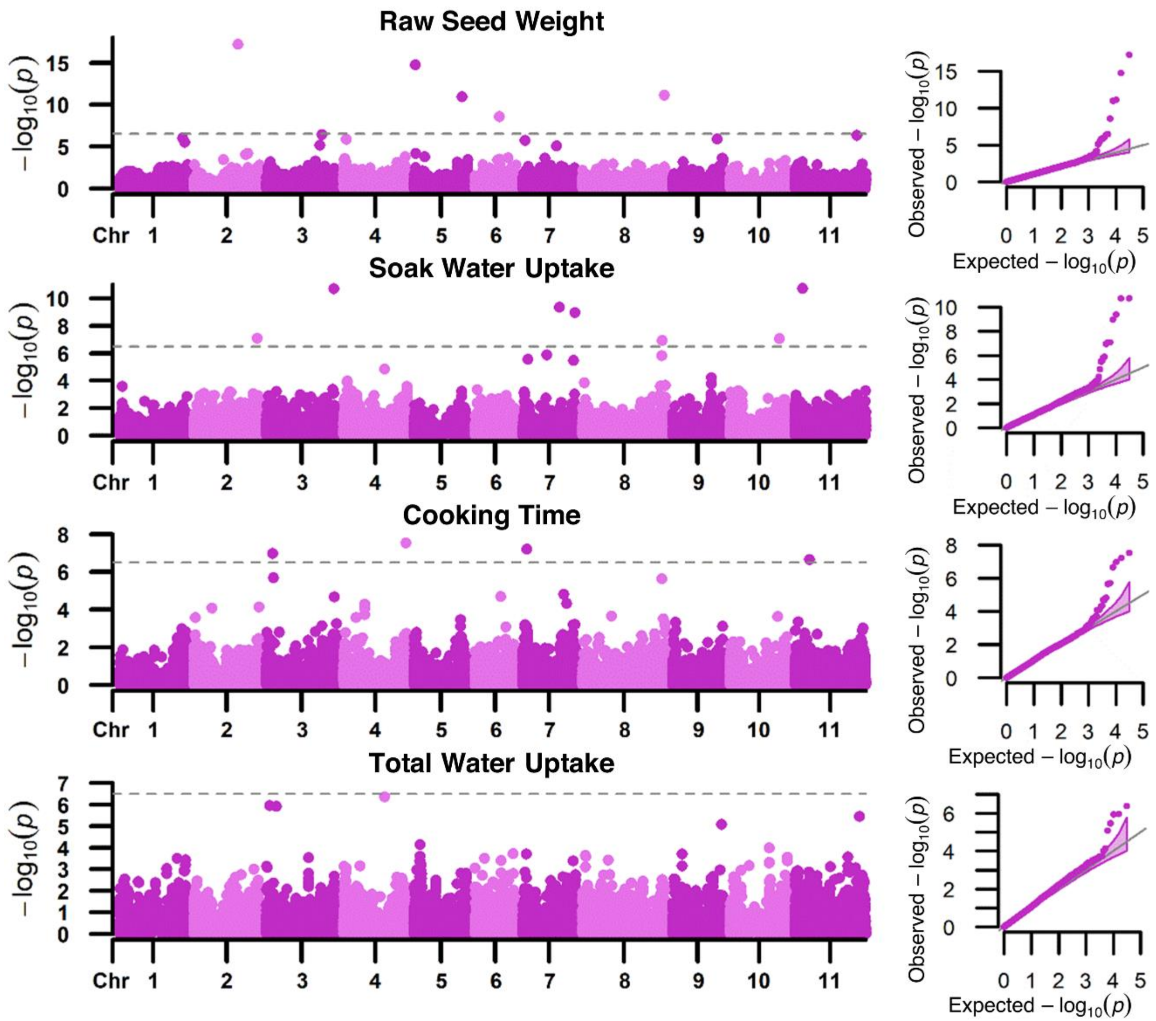


Figure S2.8 Manhattan and QQ plots for raw seed weight, soak water uptake, cooking time, and total water uptake of the Andean Diversity Panel with mapping conducted using MLM with BLUPs from all locations combined. The gray dashed line is the α = 0.05 Bonferroni correction based on the effective number of markers determined using the SimpleM algorithm.

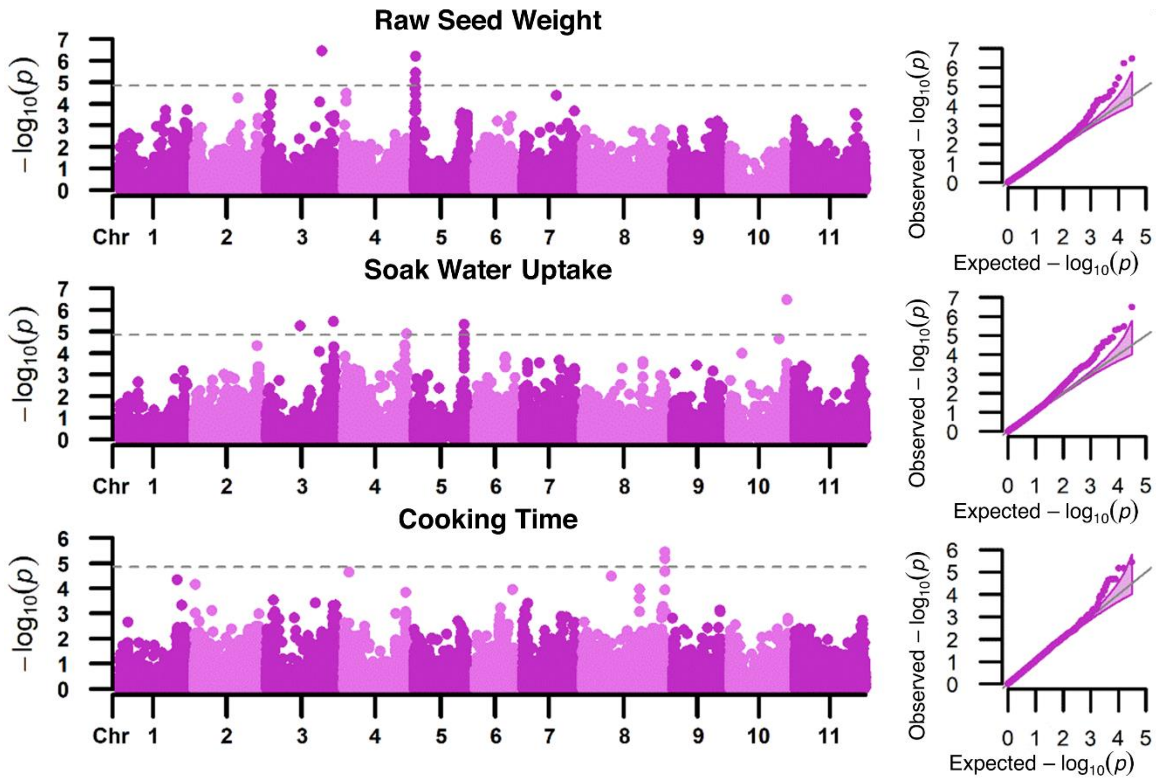
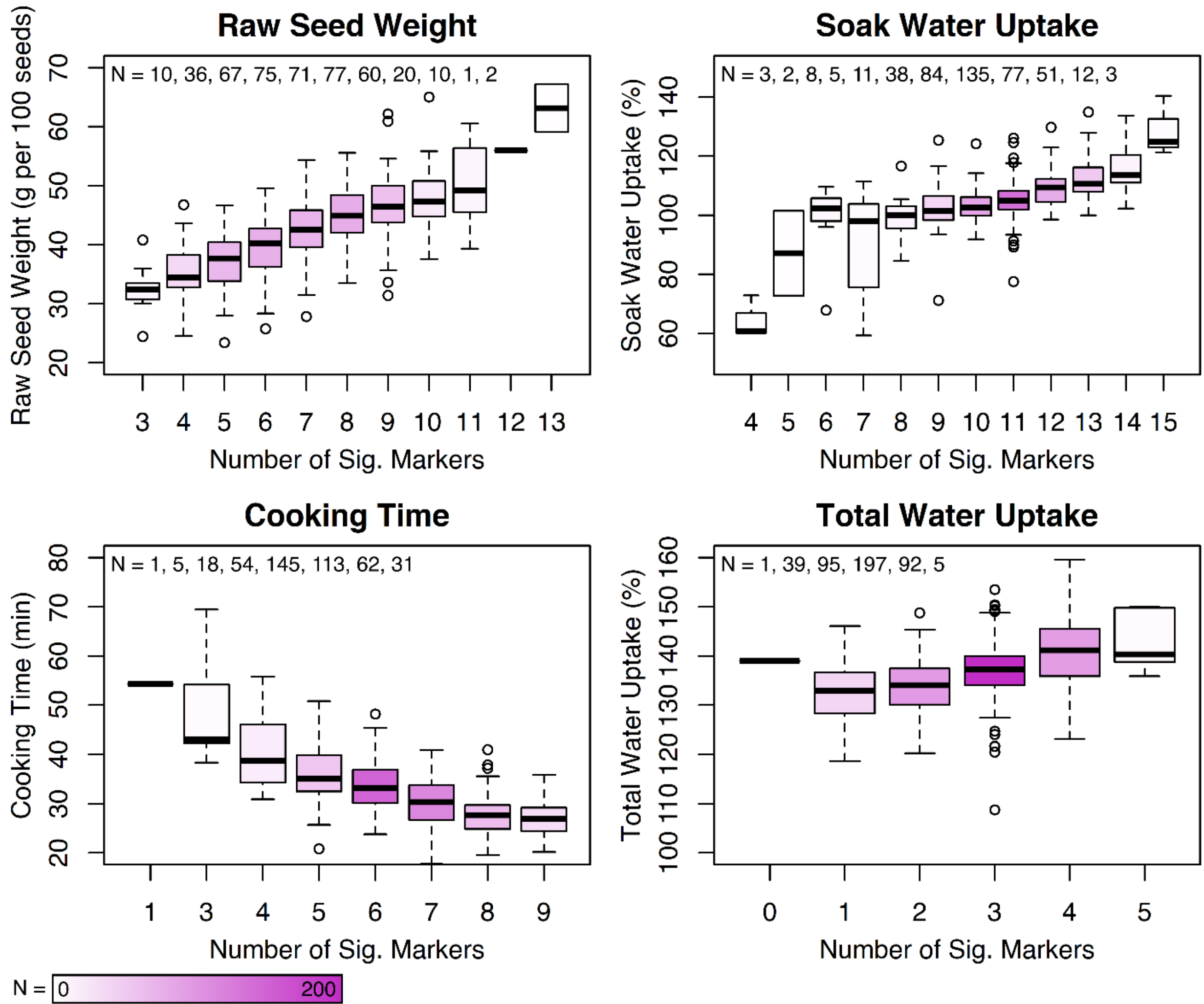


Figure S2.9 Phenotypic effects of carrying the indicated number of significant markers conferring a positive effect for raw seed weight, soak water uptake, and total water uptake and a negative effect for cooking time. Phenotypic values represent all locations combined as averages from Hawassa, Ethiopia; Kabwe, Zambia; and Lusaka, Zambia. N is the number of individuals in each boxplot.



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CHAPTER 3:

QTL MAPPING OF SEED QUALITY TRAITS INCLUDING COOKING TIME, FLAVOR, AND TEXTURE IN YELLOW DRY BEANS (*Phaseolus vulgaris* L.)

QTL mapping of seed quality traits including cooking time, flavor, and texture in yellow dry beans (*Phaseolus vulgaris* L.)

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ABSTRACT

Manteca yellow beans have many quality traits that appeal to consumers, including fast cooking times, creamy texture, and sweet, buttery flavor. These beans are native to Chile and consumed in regions in South America and Africa, but are largely unfamiliar to U.S. consumers. While cooking time, flavor, and texture have not been a focus in U.S. dry bean breeding programs, genetic variability exists, which could allow consumer preferences for these traits to be addressed through breeding. In this study, a recombinant inbred line (RIL) population was developed from Ervilha and PI527538, Manteca and Njano yellow beans with contrasting cooking time and sensory attributes. The population and parents were grown for two years in Michigan and evaluated for cooking time and sensory attribute intensities, including total flavor, beany, vegetative, earthy, starchy, sweet, bitter, seed coat perception, and cotyledon texture. Cooking time ranged from 19 to 34 minutes and exhibited a high broad-sense heritability of 0.76. Sensory attribute intensities also exhibited variation among the RILs, although broad-sense heritability was low, with beany and total flavor intensity exhibiting the highest (0.33 and 0.27). A linkage map of 973 SNP markers was developed for QTL mapping, which revealed important loci for soak water uptake, cooking time, sensory attribute intensities, color, seed coat postharvest non-darkening, seed weight, total

water uptake, and seed yield. Co-localization was identified for total flavor, beany, starchy, bitter, seed coat perception, cotyledon texture, and color (Pv03); vegetative, earthy, sweet, and cotyledon texture (Pv07); and color and non-darkening (Pv10).

INTRODUCTION

Dry beans (*Phaseolus vulgaris* L.) are widely regarded as a nutritious and affordable food (Akibode and Maredia, 2011). The species encompasses many different market classes grown and consumed around the world with many regional preferences (Siddiq and Uebersax, 2012). There is variability not just for seed size, color, and shape, but also end-use quality attributes, including cooking time, color, and flavor (Bassett et al., 2020). Some market classes may be of particular interest to modern consumers looking to incorporate beans into their diets for their nutritional benefits and also looking for convenience not typically associated with dry beans considering their often long cooking times (Sloan, 2015).

The Manteca yellow bean market class has multiple quality traits of value to consumers (Leakey, 2000; Wiesinger et al., 2016, 2018). Manteca are pale yellow with a grey hilum. They are Andean beans native to Chile (Leakey, 1992) and currently consumed in South America and Africa (Wiesinger et al., 2018). Manteca are appreciated for their sweet, buttery flavor (Leakey, 2000) as well as fast cooking time and high iron bioavailability (Wiesinger et al., 2016, 2018). U.S. American consumers are largely unfamiliar with this yellow market class, making it easy to set apart from familiar market classes and highlight its positive attributes in new varieties.

Current dietary guidelines in the US recommend ¼ cup of pulse per day, but less than 50% of the population meets that recommendation (Britten et al., 2012). There is an opportunity to increase utilization of dry beans by addressing consumer preferences for convenience and flavor

as well as developing bean products to reach new consumers (IPSOS, 2010; Karlsen et al., 2016; Hooper et al., 2019; Winham et al., 2019). While U.S. dry bean breeders have always prioritized quality traits, they primarily have focused on seed size, shape, color, and canning quality and production-related traits with minor if any consideration for cooking time and flavor (Kelly and Cichy, 2012). As a result, genetic variability exists for cooking time, flavor, and texture in modern cultivars as well as the breeding lines used for their development (Bassett et al., 2020b). There is an opportunity to address these consumer-valued traits through breeding to increase dry bean consumption, and Manteca beans make a prime target for this effort, as they already excel in these traits and provide additional novelty to those unfamiliar with them.

Cooking time has been reported to be controlled by few genes and have moderate to high heritability, with narrow sense heritability values estimated between 0.74 and 0.90 (Elia et al., 1997; Jacinto-Hernandez et al., 2003). Genotypic cooking time patterns are stable across environments (Cichy et al., 2019). Following screening of 206 accessions of the Andean Diversity Panel (ADP), several significant single nucleotide polymorphisms (SNPs) associated with cooking time were identified on Pv02, Pv03 and Pv06 (Cichy et al., 2015b). A more recent screening of 430 accessions of the ADP revealed additional significant SNPs on Pv03, Pv04, Pv06, Pv07, Pv08, and Pv11 (Bassett et al., 2020). In addition, a recent quantitative trait loci (QTL) mapping study using a recombinant inbred line (RIL) population developed from two ADP accessions revealed QTL for cooking time on Pv01, Pv02, Pv03, Pv05, Pv06, Pv10, and Pv11 (Berry et al., 2020). With further study, marker-assisted selection may be a feasible method for breeding faster cooking beans, which could reduce the need to phenotype for cooking time and allow greater incorporation of the fast cooking trait in breeding programs.

Flavor is a major influence on consumer food choices (Glanz, Basil et al. 1998), but evaluating flavor and texture is time consuming and requires trained panelists. As it stands, little is understood about consumer preference in regard to flavor and texture in dry beans apart from a general preference for beans that are sweet and soft and for bean products without a beany "off" flavor (Kinsella, 1979; Bott and Chambers, 2006; Mkanda et al., 2007; Hooper et al., 2019). A few studies have identified genetic variability for sensory attributes, including flavor and texture acceptability, seed coat perception, seed coat roughness, cotyledon mealiness, and beany flavor intensity (Koehler et al., 1987; Rivera et al., 2013). A recent study identified genetic variability in the Andean Diversity Panel (ADP) for total, beany, vegetative, earthy, starchy, bitter, and sweet flavor intensities as well as seed coat perception and cotyledon texture (Bassett et al., 2020b). Using a genome-wide association approach, significant SNPs were identified for many of these traits. As for cooking time, the potential for marker-assisted selection could reduce the need for extensive phenotyping and allow breeders to incorporate flavor and texture into their breeding programs more easily. With a greater understanding of consumer preference for flavor and texture, new varieties could be developed that appeal to consumers and are suitable for use as ingredients in products.

In this study, a yellow bean RIL population developed from two ADP accessions with contrasting cooking time and sensory characteristics was screened for cooking time and sensory attribute intensities to elucidate their genetic control and aid in the development of molecular markers for these traits.

MATERIALS AND METHODS

Germplasm

A RIL population of 240 F5:F7-F8 lines was developed from two yellow bean genotypes of the Andean gene pool: Ervilha (ADP0512) and PI527538 (ADP0468) (Figure 3.1) (Bassett and Cichy, 2020). The RILs were developed by advancing F2 seed via single seed descent to the F5 generation and then bulking seeds from individual plants to form RILs.

Ervilha is a pale yellow Manteca seed type with a gray hilum that was collected at a marketplace in Angola in 2010 (Cichy et al., 2015a). PI527538 is a yellow-green Njano seed type with hints of purple and a black hilum that was collected in Burundi in 1985 (Cichy et al., 2015a). Both genotypes are likely members of race Nueva Granada. These genotypes were selected to develop a RIL population after a screening of 206 lines of the Andean Diversity Panel (ADP) for cooking time (Cichy et al., 2015b). Ervilha cooks faster than PI527538, and this relative difference in cooking time is stable across environments (Cichy et al., 2015b; Katuuramu et al., 2020).

The genotypes were grown at the Montcalm Research Farm in MI in 2016 and 2017. The soil type is Eutric Glossoboralfs (coarse-loamy, mixed) and Alfic Fragiorthods (coarse-loamy, mixed, frigid). Two row plots 4.75 m long with 0.5 m spacing between rows were arranged in a randomized complete block design with two replications per genotype. In 2016, 100 seeds were planted per plot due to limited seed, and in 2017, 160 seeds were planted per plot. Standard agronomic practices were followed as described in the MSU SVREC 2017 Farm Research Report (Kelly et al., 2017). Plants were hand-pulled at maturity and threshed with a Hege 140 plot harvester (Wintersteiger, Utah, USA). Following harvest, seeds were cleaned by hand to remove field debris, off types, and damaged seed. Seed weights (g per 100 seeds) and seed yield (kg per ha) were recorded for each field replicate.

CIELAB Analysis and Seed Coat Postharvest Darkening

For both years, images were collected for one field replicate of each genotype using a custom machine vision system as described in Mendoza et al., 2017. For each image, a 60 x 15 mm petri dish was filled with representative seeds cleaned of debris and damaged seeds. The EOS Rebel T3i software settings were consistent across each image as follows: lens aperture f = 5.6, shutter speed 1/125, white balanced, and ISO = 100. Following image collection, each image was cropped to center the petri dish and minimize background. To examine the relationship among color, cooking time, and sensory attributes, CIELAB values were obtained using a custom batch macro in ImageJ that applies a gamma correction of 0.5, excludes background pixels outside the petri dish, and measures each slice of the LAB stack. CIELAB uses three values to describe color: L* for black (0) to white (100), a* for green (−) to red (+), and b* for blue (−) to yellow (+). These values were collected relative to the imaging conditions and reflect average color of seeds without calibration for the purpose of observing differences among lines rather than determining absolute color.

Variability in seed coat postharvest darkening among genotypes was observed after the first year, so the potential presence of the non-darkening trait in this population was explored. Genotypes grown in 2017 were stored for approximately two years in opaque paper bags in a cool, dry barn prior to evaluation for seed coat postharvest darkening in January 2020. Samples that appeared visibly darkened after this storage period were given a score of 1 and those that remained light were given a score of 0.

Cooking Time Evaluation

For each year, two field replicates of 30 seed per genotype were equilibrated to 10-14% moisture in a 4 °C humidity chamber prior to evaluating for cooking time. Each 30 seed sample

was soaked for 12 hours in distilled water prior to cooking time evaluation using an automated Mattson cooker method (Wang and Daun, 2005). Genotypes were cooked in a random order to minimize seed aging effects. Seed weights after soaking were recorded for each sample to determine soak water uptake. Mattson cookers loaded with soaked seeds were placed into 4 L stainless steel beakers with 1.8 L of boiling distilled water on Cuisinart CB-30 Countertop Single Burners to cook. The Mattson cookers (Michigan State University Machine Shop, East Lansing, MI) use twenty-five 65g stainless steel rods with 2mm diameter pins to pierce beans as they finish cooking in each well. As the pins drop, a custom software reports the cooking time associated with each pin. A low boil was maintained during cooking, and the 80% cooking times were recorded and regarded as the time required to fully cook each sample. Final cooked seed weights were recorded, and the total water uptake following cooking was calculated.

Sensory Evaluation

Ervilha, PI527538, and the RILs were evaluated in duplicate using a modified Quantitative Descriptive Analysis (QDA) approach (Stone et al., 1974), in which four panelists per session independently evaluated samples using a non-consensus approach to limit group bias. For the purposes of this study, the QDA approach was modified as described by Bassett et al. (2020b) to increase suitability for implementation in public breeding programs with limited resources. In brief, seeds from each field replicate were prepared for sensory evaluation in the same order that they were cooked for cooking time evaluation to minimize seed aging effects. Sensory evaluation sessions were held daily with four panelists per session until each genotype had been evaluated twice for each year. Twelve genotypes were evaluated at each session including Ervilha and PI527538 as controls. Each sample was evaluated using 5-point attribute intensity scales (low → high intensity) for total, beany, vegetative, earthy, starchy, bitter, and sweet flavor intensities as

well as seed coat perception and cotyledon texture. The scale for seed coat perception ranged from imperceptible (1) to tough and lingering (5). For cotyledon texture, the scale ranged from mushy (1) to very gritty/firm (5) (Bassett et al., 2020b). This sensory evaluation protocol was approved by the Institutional Review Board of Michigan State University (IRB# x16-763e Category: Exempt 6).

Panel Training

Panelists were recruited from the USDA (East Lansing, MI) and Michigan State University dry bean breeding programs due to their familiarly with dry beans and their availability for long term sensory evaluation projects. Initially, seven panelists were trained using a diverse set of dry bean genotypes selected from the USDA and MSU dry bean programs with the intention of exposing panelists to a wide range of attribute intensities. This initial set included dark red kidney, Jacob's cattle, white kidney, and yellow beans. A training set of genotypes exhibiting extreme attribute intensities identified in the ADP (Bassett et al., 2020b) was used to train eleven panelists for the second year. This training set was grown at the MSU Montcalm Research Center in Lakeview, MI alongside the RIL population.

Panelists were trained over multiple sessions using a non-consensus approach to improve their familiarity with the selected scales and their sensory evaluation skills. Panelist performance was assessed via ANOVA with FGenotype (p-value < 0.05) indicating ability to discriminate and Frep (p-value > 0.05) indicating consistency (Meilgaard et al., 1999; Armelim et al., 2006). Sensory evaluation commenced after successful training of each panelist. Following screening of the parents and RILs from both years, panel performance was assessed as during training.

Sample Preparation for Sensory Evaluation

Samples were prepared as described in Bassett et al. (2020b). Prior to each session, 4 seeds per panelist of each genotype scheduled for evaluation were soaked for 12 hours in distilled water prior to cooking. Large tea bags filled with the soaked samples were boiled in distilled water for the cooking time determined by the Mattson cooker method, timed so they all finished cooking together. The cooked samples were poured into preheated (105 °C) ceramic ramekins, covered with aluminum foil, and placed in a chafing dish to maintain temperature prior to evaluation. Samples were given a random letter code to mask their identity. Panelists were asked to refrain from wearing strong scents or eating during the hour before each session. Samples were served out of the ceramic ramekins with a plastic spoon onto paper plates. Lemon water was made available as a palette cleanser, and panelists were asked to drink water between samples.

Statistics

PROC MIXED in SAS version 9.4 of the SAS System for Windows (SAS Institute Inc. Cary, NC, USA) was used to conduct analyses of variance (ANOVAs) for each recorded trait. For seed weight, soak water uptake, cooking time, and total water uptake, the fixed effects were genotype, year, and genotype by year with replicate as a random effect. For L*, a*, and b* color values, the fixed effects were genotype and year with no random effects. For the sensory attribute intensity traits, the fixed effects were genotype, year, and genotype by year with replicate, panelist(year), and session(year) as random effects. Least squares estimates for sensory traits were calculated via the LSMeans statement in PROC MIXED for visualization of trait distributions. Mean separation of parents was determined using pdiff in PROC MIXED.

To analyze both years combined while minimizing environmental effects, best linear unbiased predictors (BLUPs) were generated for each trait using the lme4 package (Bates et al.,

2015) in R (R Core Team, 2017) with genotype, year, genotype by year, and rep nested in year as random effects. For sensory traits, panelist nested in year and session nested in year were also included as random effects. For analysis within individual years, BLUPs were calculated for sensory traits with genotype, rep, panelist, and session included as random effects.

Broad sense heritability (H2) was calculated on a family mean basis for each trait using the equation var(G)/(var(G) + (var (G*Y)/no. Y) + (var(error)/no. Y * rep), where var is variance, G is genotype, and G*Y is genotype by year, and no. Y is number of years. Variance components were calculated using PROC VARCOMP in SAS version 9.4 with method = restricted maximum likelihood method (reml) (Holland et al., 2003). Principle component analysis among traits was conducted with BLUPs from both years combined using the Prcomp function in R.

Genotyping

DNA was extracted from young trifoliate leaf tissue from three plants each for the 240 RILs and the two parental lines (Ervilha and PI527538) using a Macherey-Nagel NucleoSpin Plant II kit. Three genotyping-by-sequencing (GBS) libraries were constructed at 96-plex as described by Elshire et al. (2011) with the parental lines prepared in quintuplicate. Fragment sizes were evaluated using the Agilent Bioanalyzer High Sensitivity DNA Kit (Bioanalyzer 2100, Agilent). Single-end sequencing (50 bp reads) of one 96-plex library per flowcell channel was performed on an Illumina HiSeq 4000. The raw sequence data were cleaned of adapters and trimmed for quality score ≥ 30 and minimum length ≥ 30 via Cutadapt (Martin, 2011) and evaluated via FastQC (Andrews, 2010). Cleaned reads were demultiplexed using the Next Generation Sequencing Eclipse Plugin (NGSEP) pipeline with NGSEP version 3.0.2 (Duitama et al., 2014; Perea et al., 2016), aligned to the Phaseolus vulgaris v2.1 genome (DOE-JGI and USDA-NIFA, http://phytozome.jgi.doe.gov/) using Bowtie 2 (Langmead and Salzberg, 2012), and then sorted

using Picard (http://broadinstitute.github.io/picard). Variant calling and annotation were performed via NGSEP. Raw SNPs were filtered to eliminate repetitive regions, markers with more than 50% missing data, and markers that were not polymorphic in the parents.

Linkage and QTL Mapping

Linkage mapping was performed using MapDisto version 2.1.7 (Heffelfinger et al., 2017). Genotyping error candidates meeting the 1e-4 threshold were replaced with missing data, and missing data was filled with flanking genotypes. Markers exhibiting segregation distortion (p-value < 1e-10) or causing excessive map length were excluded. A fixed order genetic map of 1567 cM was generated using the Kosambi function with 973 markers.

QTL mapping was performed using QTL Cartographer version 2.5 (Wang et al., 2005). The composite interval mapping (CIM) procedure was performed with the parameters set to 10 cM window size and 1 cM walkspeed with forward and backward regression. BLUPs were used in QTL mapping for both years combined and for sensory traits for individual years, and means were used for analyses of all other traits for individual years. The LOD thresholds for each trait in each year and across years were determined using 1000 permutations in scanone from rQTL with the extended Haley-Knott method (p-value < 0.05) (Broman et al., 2003; Feenstra et al., 2006). The constructed linkage maps with QTL overlaid were visualized using Mapchart 2.32 (Voorrips, 2002).

RESULTS

Cooking Time Evaluation

Genotype significantly affected soak water uptake, cooking time, and total water uptake (p-value < 0.05) (Table 3.1, S3.1). Year significantly affected soak water uptake and cooking time

(p-value < 0.05), and genotype by year significantly affected cooking time (p-value < 0.05). For the parents Ervilha and PI527538 respectively averaged across both years, the soak water uptakes were 109.3 and 98.8 percent; the cooking times were 21.0 and 29.7 min; and the total water uptakes were 138.2 and 146.3 percent.

Soak water uptake, cooking time, and total water uptake for the RILs varied minimally across years and exhibited approximately normal distributions (Table S3.2; Figures 3.1, S3.1). Averaged across both years, soak water uptake ranged 69.2 – 117.4%; cooking time ranged 19.1 – 33.9 min; and total water uptake ranged 109.9 – 148.0% (Table 3.1).

Broad-sense heritability varied greatly across traits, with cooking time (0.76) exhibiting high heritability and soak water uptake (0.34) and total water uptake (0.23) exhibiting low heritability.

Sensory Evaluation

Genotype significantly affected all sensory attributes (p-value < 0.05) (Table 3.1). Year did not significantly affect any sensory attributes, and genotype by year only significantly affected cotyledon texture (p-value < 0.05). Rep effects were insignificant for all sensory attributes, which indicates panelists were consistent across reps, although significant panelist and session effects were observed (Table S3.3). For the parents Ervilha and PI527538 respectively with least squares estimates averaged across both years, the total flavor intensities were 3.1 and 3.2; beany intensities were 2.2 and 3.3; vegetative intensities were 2.7 and 2.5; earthy intensities were 2.0 and 2.2; starchy intensities were 3.6 and 3.0; sweet intensities were 2.3 and 1.8; bitter intensities were 1.4 and 1.9; seed coat perceptions were 2.8 and 3.4; and cotyledon textures were 2.4 and 2.0 (Table 3.1).

Least squares estimates for all sensory attribute intensities varied minimally across years and exhibited approximately normal distributions (Table S3.2, Figure 3.3). Across both years, least

squares estimates ranged 2.2 – 4.1 for total flavor intensity, 1.5 – 3.9 for beany intensity, 1.7 – 3.4 for vegetative intensity, 1.5 – 3.1 for earthy intensity, 2.5 – 3.9 for starchy intensity, 1.3 – 3.2 for sweet intensity, 1.1 – 2.3 for bitter intensity, 2.4 – 3.9 for seed coat perception, and 1.4 – 3.0 for cotyledon texture (Table 3.1). While panelists were able to differentiate among genotypes using 5-point scales, sensory attribute ranges did not exceed 2.4, suggesting panelists did not make full use of the scales. This could reflect the limited differences in sensory attribute intensities observed between the parents.

Broad-sense heritability for sensory attribute intensities were low, ranging from 0.05 to 0.33 (Table 3.1). Beany intensity and total flavor intensity exhibited the highest broad-sense heritability (0.33 and 0.27), while vegetative intensity, earthy intensity, and cotyledon texture exhibited the lowest (0.05, 0.06, and 0.06).

Color and Seed Coat Postharvest Darkening

Genotype significantly affected L*, a*, b*, and seed coat postharvest darkening (p-value < 0.05) (Table 3.1). Year significantly affected L*, a*, and b* (p-value < 0.05). For the parents Ervilha and PI527538 respectively averaged across both years, L* values were 64.8 and 54.1; a* values were -0.7 and 3.5; b* values were 22.3 and 14.6; and seed coat postharvest darkening values were 0 (non-darkening) and 1 (darkening).

The L*, a*, and b* for the RILs varied minimally across years and exhibited approximately normal distributions (Table S3.2, Figure 3.4). Averaged across both years, L* ranged from 40.3 – 67.3; a* ranged from -3.2 – 5.9; and b* ranged from 8.5 – 34.4 (Table 3.1). Seed coat postharvest darkening was only determined for seeds from one year (2017), and progeny exhibiting both non-darkening and darkening were observed. Broad-sense heritability was high for L* (0.86), a* (0.86), b* (0.78), and seed coat postharvest darkening (1.00).

Seed Yield and Seed Weight

Genotype, year, and genotype by year significantly affected seed weight and seed yield (p-value < 0.05) (Table S3.1). For the parents Ervilha and PI527538 respectively averaged across both years, the seed weights were 52.8 and 48.0 g per 100 seeds. Seed yield data for Ervilha is not available for 2016 (Table S3.2), and fewer seeds were planted per plot in 2016, making averages across years misleading. In 2017, the seed yields for Ervilha and PI527538 respectively were 1731.4 and 2384.4 kg/ha.

The seed weight for the RILs varied minimally across years and exhibited approximately normal distributions (Table S3.2, Figure S3.1). Seed yield for the RILs varied substantially across years due to reduced seeds planted per plot in 2016 but exhibited approximately normal distributions. Averaged across both years, seed weight ranged 39.1 – 68.4 g per 100 seeds and seed yield ranged 751.0 – 3283.9 kg per ha (Table S3.2).

Broad-sense heritability for seed weight (0.89) was high and for seed yield was moderate (0.57) (Table S3.1).

PCA

For the PCA, the first two principal components (PCs) explained approximately 52% of the variance (Figure 3.5). The first PC separates the genotypes approximately by beany, earthy, and bitter intensities as well as L*, a*, b*, and seed coat postharvest non-darkening and represents over a third of the variation (38.5%). The second PC separates the genotypes approximately by cooking time; total flavor, vegetative, starchy, and sweet intensities; and cotyledon texture and seed coat perception. The second PC represents over an eighth of the variance (13.0%). The remaining PCs accounted for 11.1, 7.4, 6.0, 5.4, 4.2, 3.2, 2.9, 2.5, 2.2, 1.6, 1.1, 0.9% of the variance respectively (data not shown).

The PCA biplot highlights distinct groupings of traits that tend to be observed together. Loadings that group together highlight strong positive relationships within each group, and groups of loadings opposite of each other highlight strong negative relationships between groups. Loadings for starchy intensity, sweet intensity, and cotyledon texture are positioned close to each other and opposite cooking time and seed coat perception. Loadings for beany intensity and bitter intensity also group together and are somewhat opposite starchy intensity, sweet intensity, and cotyledon texture. The loadings for total flavor intensity earthy intensity, a*, and seed coat postharvest non-darkening group together, opposite of loadings for L* and b*. The loading for vegetative intensity does not appear to group with or opposite of other loadings, but lies in between loadings for total flavor intensity and sweet intensity. The genotypes are fairly evenly spread across the biplot, with Ervilha and PI527538 positioned opposite each other.

QTL Mapping

A linkage map was developed with 973 SNPs spread across eleven chromosomes for a total map length of 1,567 cM with a marker density of 1.61 cM per SNP (Table 3.2). Significant QTL were identified for soak water uptake, cooking time, total flavor intensity, beany intensity, vegetative intensity, earthy intensity, starchy intensity, sweet intensity, bitter intensity, seed coat perception, cotyledon texture, L*, a*, b*, seed coat postharvest darkening, seed weight, total water uptake, and seed yield (Tables 3.3-5, S3.4; Figure 3.5, S3.2-13).

For soak water uptake, four QTL were identified on Pv03, Pv06, Pv10, and Pv11 (Table 3.3, Figures 3.6, S3.5, S3.8, S3.12-13). WU.3.1 and WU.10.1 were identified in both years combined; WU.6.1 was only identified in 2016; and WU.11.1 was only identified in 2017 (Table 3.3). The total proportion of variance explained by the two QTL identified in both years combined

was 11.0%. For WU3.1 and WU.10.1, alleles contributed by Ervilha conferred positive effects (Tables 3.3).

For cooking time, two QTL were identified on Pv03 and Pv11 (Table 3.3, Figures 3.6, S3.5, S3.13). CT.3.1 and CT.11.1 were identified in both years combined (Table 3.3). The total proportion of variance explained by the two QTL identified in both years combined was 17.5%. For CT.3.1 and CT.11.1, alleles contributed by Ervilha conferred both negative and positive effects, respectively, despite Ervilha cooking significantly faster cooking than PI527538 (Tables 3.1, 3.3).

For total flavor intensity, one QTL was identified on Pv03 (Table 3.4, Figures 3.6, S3.5). TFI.3.1 was only identified in 2016 (Table 3.4). The proportion of variance explained by TFI.3.1 was 5.7%, and Ervilha contributed an allele conferring a negative effect, reflecting its lower total flavor intensity compared to PI527538 (Tables 3.1, 3.4).

For beany intensity, one QTL was identified on Pv03 (Table 3.4, Figures 3.6, S3.5). BFI.3.1 was identified in both years combined (Table 3.4). The proportion of the variance explained by BFI.3.1 in both years combined was 6.8%, and Ervilha contributed an allele conferring a negative effect, reflecting its lower beany intensity compared to PI527538 (Tables 3.1, 3.4).

For vegetative intensity, three QTL were identified on Pv02, and Pv07 (Table 3.4, Figures 3.6, S3.4, S3.9). VFI.7.1 was identified in both years combined; VF.7.2 was only identified in 2016, and VF.2.1 was only identified in 2017 (Table 3.4). The proportion of variance explained by VFI.7.1 in both years combined was 5.6%. Across all vegetative intensity QTL, most alleles contributed by Ervilha conferred negative effects despite its higher vegetative intensity compared to PI527538. For VFI.7.1, the allele contributed by Ervilha conferred a positive effect.

For earthy intensity, three QTL were identified on Pv07 and PV10 (Table 3.4, Figures 3.6, S3.9, S3.12). EFI.10.1 was identified in both years combined; EFI.7.1 was only identified in 2016; and EFI.10.2 was only identified in 2017 (Table 3.4). The proportion of variance explained by EFI.10.1 in both years combined was 12.3%. Across all earthy intensity QTL, most alleles contributed by Ervilha conferred positive effects despite its lower earthy intensity compared to PI527538.

For starchy intensity, two QTL were identified on Pv03 and Pv11 (Table 3.4, Figures 3.6, S3.5, S3.13). STI.3.1 was identified in both years combined, and STI.11.1 was only identified in 2016 (Table 3.4). The proportion of variance explained by STI.3.1 in both years combined was 6.9%. Across both starchy intensity QTL, alleles contributed by Ervilha conferred positive effects, reflecting its higher starchy intensity as compared to PI527538 (Tables 3.1, 3.4).

For sweet intensity, two QTL were identified on Pv02 and Pv07 (Table 3.4, Figures 3.6, S3.4, S3.9). SWI.2.1 was identified in both years combined, and SWI.7.1 was only identified in 2016 (Table 3.4). The proportion of variance explained by SWI.2.1 in both years combined was 5.9%. Across both sweet intensity QTL, alleles contributed by Ervilha conferred positive effects, reflecting its higher sweet intensity as compared to PI527538 (Tables 3.1, 3.4).

For bitter intensity, two QTL were identified on Pv01 and Pv03 (Table 3.4, Figures 3.6, S3.3, S3.5). BI.1.1 was identified in both years combined, and BI.3.1 was only identified in 2017 (Table 3.4). The proportion of variance explained by BI.1.1 in both years combined was 5.9%. Across both bitter intensity QTL, alleles contributed by Ervilha conferred negative effects, reflecting its lower bitter intensity as compared to PI527538 (Tables 3.1, 3.4).

For seed coat perception, one QTL was identified on Pv03 (Table 3.4, Figures 3.6, S3.5). SPE.3.1 was identified in both years combined (Table 3.4). The proportion of variance explained

by SPE.3.1 in both years combined was 6.8%, and Ervilha contributed an allele conferring a negative effect, reflecting its lower seed coat perception compared to PI527538 (Tables 3.1, 3.4).

For cotyledon texture, two QTL were identified on Pv05 and Pv07 (Table 3.4, Figures 3.6, S3.7, S3.9). CTX.7.1 was only identified in 2016, and CTX.5.1 was only identified in 2017 (Table 3.4). Across both bitter intensity QTL, alleles contributed by Ervilha conferred negative effects despite its higher cotyledon texture as compared to PI527538 (Tables 3.1, 3.4).

For L*, two QTL were identified on Pv03 and Pv10 (Table 3.5, Figures 3.6, S3.5, S3.12). SL*.3.1 and SL*.10.1 were identified in both years combined (Table 3.5). The total proportion of variance explained by two QTL identified in both years combined was 12.1%. Across both QTL, Ervilha contributed alleles conferring positive effects, reflecting its higher L* as compared to PI527538 (Tables 3.1, 3.5).

For a*, two QTL were identified on Pv03 and Pv10 (Table 3.5, Figures 3.6, S3.5, S3.12). Sa*.3.1 and Sa*.10.1 were identified in both years combined (Table 3.5). The total proportion of variance explained by the two QTL identified in both years combined was 15%. Across both QTL, Ervilha contributed alleles conferring negative effects, reflecting its lower a* as compared to PI527538 (Tables 3.1, 3.5).

For b*, four QTL were identified on Pv01, Pv05, and Pv10 (Table 3.5, Figures 3.6, S3.3, S3.7, S3.12). Sb*.5.1, Sb*.5.2, and Sb*.10.1 were identified in both years combined, and Sb*.1.1 was only identified in 2017 (Table 3.5). The total proportion of variance explained by the three QTL identified in both years combined was 21%. Across the b* QTL, Ervilha contributed alleles conferring mostly positive effects, reflecting its higher b* as compared to PI527538 (Tables 3.1, 3.5).

For seed coat postharvest darkening, one QTL was identified on Pv10 (Table 3.5, Figures 3.6, S3.12). Seed coat postharvest darkening was only evaluated for 2017 seeds. The proportion of variance explained by ND.10.1 was 10.4%, and Ervilha contributed an allele conferring a negative effect, reflecting its lack of darkening over time (Tables 3.1, 3.5).

While seed weight, total water uptake, and seed yield were not central to this study, several QTL were identified for these traits as well. Additional information is available in the supplemental material (Figures S3.2, Table S3.4).

Several QTL co-localized on Pv03, Pv07, and Pv10. On Pv03, QTL for soak water uptake (WU.3.1), beany intensity (BFI.3.1), bitter intensity (BI.3.1), seed coat perception (SPE.3.1), L* (SL*.3.1), and a* (Sa*.3.1) co-localized. Alleles from Ervilha conferred positive effects for WU.3.1 and SL*.3.1 and negative effects for BFI.3.1, SPE.3.1, and Sa*.3.1. QTL for cooking time (CT.3.1), total flavor intensity (TFI.3.1), and starchy intensity (STI.3.1) also co-localized on Pv03. Alleles from Ervilha conferred a positive effect for STI.3.1 and negative effects for CT.3.1 and TFI.3.1. On Pv07, QTL for vegetative intensity (VFI.7.1) and sweet intensity (SWI.7.1) co-localized. Alleles from Ervilha conferred positive effects for both VFI.7.1 and SWI.7.1. QTL for vegetative intensity (VFI.7.2), earthy intensity (EFI.7.1), and cotyledon texture (CTX.7.1) also co-localized on Pv07. Alleles from Ervilha conferred a positive effect for EFI.7.1 and negative effects for VFI.7.2 and CTX.7.1. On Pv10, QTL for L*, a*, b*, and seed coat postharvest darkening (ND.10.1) co-localized. Alleles from Ervilha conferred positive effects for SL*.10.1 and Sb*.10.1 and negative effects for Sa*.10.1 and ND.10.1.

DISCUSSION

The broad-sense heritability for cooking time was moderately high in this study, as was the case for previous reports looking at both broad-sense and narrow-sense heritability (Elia et al., 1997; Jacinto-Hernandez et al., 2003; Cichy et al., 2019; Bassett et al., 2020b). This supports the idea that marker-assisted selection for fast cooking time may be feasible with few molecular markers. Using marker-assisted selection as opposed to phenotyping could save breeding programs time and prevent the need to purchase specialized machinery specific for the evaluation of cooking time. It could also allow for early generation screening that would otherwise not be feasible due to limited seed and the large number of lines to be evaluated for cooking time.

Differences in sensory attribute intensities among genotypes were successfully detected, allowing the relationship among attributes in this population to be determined and for significant QTL to be identified for the evaluated sensory attributes. While significant panelist and session effects were identified (Table S3.2), QDA does not rely on consensus among panelists, and these effects can be accounted for by using least squares estimates and BLUPs where appropriate. Although broad-sense heritability for sensory attributes tended to be low to very low, it is clear that genotype is important for flavor and texture. In the context of a breeding program, heritability can be improved by screening fewer lines with greater replication to better account for panelist and session effects while managing limited seed and personnel resources. As has been previously noted, panelists tend not to use the full range of the rating scales, which prevents detection of small differences between samples (Bassett et al., 2020b). In the case of this population, it is unlikely that this RIL population exhibited a full range of sensory attribute intensities, especially for traits with limited differences in the parents, so incomplete use of the scales likely reflects a lack of extreme differences among genotypes. However, increasing the size of the scales or using line

scales that allow for continuous ratings may better reflect the diversity of attribute intensities exhibited in a population in future studies, which might return higher heritability for sensory traits. Year and genotype by year effects were not significant for sensory traits, apart from cotyledon texture, which had a significant genotype by year effect. This is encouraging because location of production and crop management practices have previously been identified as factors affecting sensory quality (Mkanda et al., 2007; Ferreira et al., 2012). This indicates that flavor and texture traits do not change across years in the same production environment, which is useful for meeting expectations of consistency for consumers and for product developers, who need consistent ingredients over time for their products to be successful.

There did not appear to be distinct groupings of genotypes based on cooking time and attribute intensity in the PCA biplot, indicating that there was a general mixing of these traits in the progeny (Figure 3.4). This suggests that extensive efforts at breaking linkages among traits are not needed to combine desired traits and achieve a target cooking time and sensory profile. Developing new yellow bean varieties with both fast cooking time and desirable flavor and texture would address two major factors influencing consumer purchasing decisions regarding dry beans and provide novelty for the many consumers unfamiliar with the yellow seed type (Leterme and Carmenza Muñoz, 2002; Eihusen and Albrecht, 2007; Winham et al., 2019).

Many QTL were identified in this study, with those for cooking time and sensory attribute intensities of particular interest. Both cooking time QTL (CT.3.1 and CT.11.1) were located in close proximity to significant SNPs previously identified via genome-wide association in the ADP (Bassett et al., 2020b). While the LODs and R2 values were not particularly high for CT.3.1 and CT.11.1, they have potential for use in marker-assisted selection due to their consistently detectable effects in this study and their support in a previous study. Other recent studies have

identified QTL or significant SNPs related to cooking time on Pv03 and Pv11 as well, but the physical positions were not proximal to CT.3.1 or CT.11.1 (Cichy et al., 2015b; Berry et al., 2020). The genetic control of sensory attributes is a new area of research in dry beans with limited study (Bassett et al., 2020b). For total flavor intensity, TFI.3.1 was located in relatively close proximity to SNP S03_51252684, which was identified in association with total flavor intensity for the ADP grown in Hawassa, Ethiopia. Otherwise, the QTL identified for sensory attributes in this study were novel. While several QTL including BFI.3.1, VFI.7.1, EFI.10.1, STI.3.1, SWI.2.1, BI.1.1, and SPE.3.1 were consistent across environments, further validation would be beneficial before use in marker-assisted selection. For certain traits, including vegetative intensity, earthy intensity, and cotyledon texture, the alleles contributed by Ervilha conferred effects that would seem more likely to come from PI527538. These traits also had the lowest heritability in this study, indicating that evaluating them was particularly challenging.

Many QTL were identified for soak water uptake and CIELAB values. Some soak water uptake and CIELAB QTL were proximal to QTL and genetic markers identified in previous studies (Cichy et al., 2014, 2015b; Erfatpour et al., 2018; Bassett et al., 2020b; Berry et al., 2020). WU.3.1 was near SNPs for water uptake identified by Cichy et al. (2015b) and a QTL identified by Berry et al. (2020) for water uptake. SL*.10.1 and Sa*.10.1 overlapped with the J-locus associated with postharvest non-darkening (Erfatpour et al., 2018). Sb*5.1 and Sb*5.2 were near QTL identified for anthocyanin content, L*, and b* of canned beans (Cichy et al., 2014). Most of the QTL identified for these traits were novel and may be useful for research central to these traits.

Seed coat postharvest darkening was detected in PI527538 and half of the RILs. Seed coat postharvest darkening describes the tendency of some genotypes to darken in color over time due to the presence of proanthocyanidin precursors in the seed coat (Beninger et al., 2005; Chen et al.,

2015). This phenomenon has been most studied in pinto and cranberry beans but can be observed in other market classes. Lighter seed coats are perceived by consumers as indications of freshness or quality, so seeds exhibiting postharvest darkening have reduced market value (Nasar-Abbas et al., 2009; Erfatpour and Pauls, 2020). The J locus was previously identified on Pv10, and genotypes that are homozygous recessive at J do not exhibit postharvest darkening (Bassett, 2007; Elsadr et al., 2011; Erfatpour et al., 2018). The QTL identified for the non-darkening trait in this study overlaps with a previously identified QTL for non-darkening located between 40,164,667 bp and 40,295,580 bp on Pv10 (Table S3.5) (Erfatpour et al., 2018). Flavan-3-ols, which include proanthocyandidins, have been previously associated with bitterness and astringency depending on their degree of polymerization (Robichaud and Noble, 1990; Peleg et al., 1999), so seed coat postharvest darkening may alter flavor over time. The relationship between seed coat postharvest darkening and flavor after beans have darkened was not examined in this study, but it remains practical to select against darkening when developing new varieties to ensure greater visual appeal to consumers, which would bypass flavor changes caused by darkening altogether. A SNP-based marker has been developed to allow marker-assisted selection for this trait (Erfatpour and Pauls, 2020).

As there is still much to be understood regarding flavor and texture in dry beans, other methods for assessing these sensory traits like GC-MS and texture measurements should be explored. Volatile concentrations and texture measurements have been used successfully as proxies for flavor and texture in studies looking at genetic control of sensory traits in other crops, and these measurements can be cheaper and easier to obtain than those generated by a descriptive panel (Zhang et al., 2015; Amyotte et al., 2017; Bauchet et al., 2017; Zhao et al., 2019). Apart from beany intensity (Vara-Ubol et al., 2004; Bott and Chambers, 2006), however, the contribution

of volatiles to perceived flavors in dry beans is not well understood, and texture measurements have not been well explored outside of their use in the evaluation of firmness in canned samples (Kelly and Cichy, 2012). In addition, research assessing consumer preference for flavor and texture in dry beans is needed to define breeding targets for sensory attributes. Understanding which traits are most important for consumer preference and what the expectations are for different seed types will help breeders address flavor and texture with a focused, efficient approach.

Dry beans in the U.S. are sold as market classes rather than variety preserved. Variation exists within market classes for consumer-valued traits like cooking time, flavor, and texture so consumers are not able to make informed purchasing decisions taking these traits into account (Cichy et al., 2015b; Bassett et al., 2020b; Berry et al., 2020). In addition, the canning industry cannot receive the benefits of reduced energy costs and higher efficiency associated with fast-cooking genotypes if slow-cooking genotypes are present in the same cans (Bassett et al., 2020a). Because yellow beans are largely unfamiliar to U.S. consumers, there is an opportunity to develop new yellow bean varieties that prioritize these traits so that the yellow color can serve as a marker for convenience and culinary quality to consumers and the canning industry can produce quality canned products with yellow beans while benefitting from shorter processing times. Consumers are already seeking out unique flavors, textures, seed patterns, and colors from heirloom beans (Bullard, 2016), but heirlooms are not suited to modern farming practices, which makes them more expensive and less widely available than more familiar market classes. Yellow beans, the Manteca market class in particular, could serve this consumer interest while addressing grower needs.

CONCLUSION

This work adds to the currently limited pool of resources available for dry bean breeders to target fast cooking time, flavor, and texture in their breeding programs. The QTL identified in this work, in particular CT.3.1 and CT.11.1, can be used to develop molecular markers for the incorporation of fast cooking time into new bean varieties to benefit both consumers and the canning industry. For sensory attributes, several QTL including BFI.3.1, VFI.7.1, EFI.10.1, STI.3.1, SWI.2.1, BI.1.1, and SPE.3.1 were consistent across years and show potential for use in marker-assisted selection following identification of breeding targets for sensory attributes informed by consumer preference. Consumers are seeking bean products with improved culinary characteristics and unique appearance. Yellow beans like those used in this study are unfamiliar to U.S. consumers, but they tend to be fast cooking with desirable sensory attributes. With the recent increased interest in plant-based proteins, now is an opportune time to address consumer preference in dry beans to remain competitive with other pulses, and yellow beans might be an ideal vehicle to a fast-cooking, flavorful, and flourishing future of dry beans.

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APPENDICES

APPENDIX A:

CHAPTER 3 TABLES AND FIGURES

Table 3.1 Parental phenotypes, means[a], ranges, and broad-sense heritability (H2) for the RILs for both years combined with ANOVA *p*-values[b] for genotype, year, and genotype by year indicated.

Trait	Ervilha	PI527538	Mean	Range	H^2	Genotype	Year	Genotype x Year
Soak Water Uptake (%)								
	$109.3^a \pm 3.5$	$98.8^a \pm 1.2$	101.64 ± 0.3	69.2 - 117.4	0.25	< 0.0001	< 0.0001	NS
Cooking Time (min)								
	$21.0^b \pm 1.5$	$29.7^a \pm 2.4$	25.25 ± 0.2	19.1 - 33.9	0.68	< 0.0001	< 0.0001	0.0054
Total Flavor Intensity								
	$3.1^b \pm 0.1$	$3.2^a \pm 0.1$	3.28 ± 0.0	2.2 - 4.1	0.27	< 0.0001	NS	NS
Beany Intensity								
	$2.2^b \pm 0.2$	$3.3^a \pm 0.1$	2.87 ± 0.0	1.5 - 3.9	0.33	< 0.0001	NS	NS
Vegetative Intensity								
	$2.7^a \pm 0.1$	$2.5^b \pm 0.1$	2.59 ± 0.0	1.7 - 3.4	0.05	0.0020	NS	NS
Earthy Intensity								
	$2.0^b \pm 0.0$	$2.2^a \pm 0.0$	2.23 ± 0.0	1.5 - 3.1	0.06	0.0010	NS	NS
Starchy Intensity								
	$3.6^a \pm 0.0$	$3.0^b \pm 0.1$	3.17 ± 0.0	2.5 - 3.9	0.13	< 0.0001	NS	NS
Sweet Intensity								
	$2.3^a \pm 0.1$	$1.8^b \pm 0.1$	2.05 ± 0.0	1.3 - 3.2	0.19	< 0.0001	NS	NS
Bitter Intensity								
	$1.4^b \pm 0.0$	$1.9^a \pm 0.1$	1.7 ± 0.0	1.1 - 2.3	0.14	< 0.0001	NS	NS
Seed Coat Perception								
	$2.8^b \pm 0.1$	$3.4^a \pm 0.0$	3.05 ± 0.0	2.4 - 3.9	0.21	< 0.0001	NS	NS
Cotyledon Texture								
	$2.4^a \pm 0.1$	$2.0^b \pm 0.1$	2.29 ± 0.0	1.4 - 3.0	0.06	< 0.0001	NS	< 0.0001
L*								
	64.8a ± 0.2	54.1b ± 1.8	58.8 ± 0.3	40.3 - 67.3	0.86	< 0.0001	< 0.0001	.
a*								
	-0.7b ± 0.6	3.5a ± 0.2	1.4 ± 0.1	-3.2 - 5.9	0.86	< 0.0001	< 0.0001	.
b*								
	22.3a ± 0.9	14.6b ± 2.3	20.2 ± 0.3	8.5 - 34.4	0.78	< 0.0001	< 0.0001	.
Seed Coat Postharvest Darkening (0 = non-darkening; 1 = darkening)								
	$0^b \pm 0$	$1^a \pm 0$	0.5 ± 0.0	0 - 1	1.00	< 0.0001	.	.

[a] Mean separation is indicated by letter superscript. Least squares estimates are presented for sensory attribute intensities instead of means

Table 3.1 (cont'd)

[b] NS indicates non-significant *p*-values at $\alpha = 0.05$

Table 3.2 Linkage map information for the 240 RILs.

Chromosome	Number of Markers	Size (cM)	Marker Density (cM)
1	41	112.88	2.75
2	104	138.56	1.33
3	115	112.46	0.98
4	141	158.81	1.13
5	64	115.83	1.81
6	46	103.26	2.24
7	70	163.81	2.34
8	105	159.35	1.52
9	51	104.61	2.05
10	182	188.83	1.04
11	54	208.80	3.87
Total:	973	1567.20	1.61

Table 3.3 Quantitative trait loci identified in the RIL population (N = 240) grown in Entrican, MI in 2016 and 2017 for soak water uptake and cooking time. Linkage group (LG), peak position (Pos), year[a], logarithm of odds (LOD), R^2, QTL effect[b] (a), flanking markers[c], QTL range[d], and significance[e] of the QTL are indicated.

Trait	QTL Name	LG	Pos (cM)	Year	LOD	R^2 (%)	a	Flanking Markers	QTL Range (cM)	Sig
Soak Water Uptake										
	WU.3.1	Pv03	48.6	C	2.92	4.8	+	33177106 - 33854971	48.36 - 49.56	**
	WU.6.1	Pv06	43.8	2016	3.26	6.9	-	19164538 - 19553914	40.77 - 44.77	**
	WU.10.1	Pv10	93.4	C	3.26	6.2	+	30125056 - 31195987	92.85 - 95.67	**
		Pv10	95.4	2016	3.47	6.7	+	31195987 - 31195987	93.37 - 95.67	**
	WU.11.1	Pv11	151.7	2017	2.82	4.8	-	46682849 - 47215098	150.81 - 152.65	*
Cooking Time										
	CT.3.1	Pv03	111.1	C	4.07	7.7	-	51423691 - 51934861	101.05 - 112.07	**
		Pv03	102.1	2016	3.18	5.6	-	51291118 - 51934861	95.46 - 112.07	*
	CT.11.1	Pv11	37.8	C	3.46	9.8	+	11733856 - 16663857	36.84 - 40.15	**
		Pv11	38.8	2016	3.64	9.8	+	11733856 - 16663857	35.84 - 41.15	**

The largest LOD and R^2 within the QTL are reported
[a] "C" indicates both years combined
[b] + and – indicate positive and negative effects on the mean as conferred by alleles from Ervilha in the QTL region.
[c] Flanking markers indicate the physical positions of the nearest markers upstream and downstream
[d] Region where LOD scores are significant at the indicated significance level
[e] Significance at $\alpha = 0.1$ and $\alpha = 0.05$ are indicated by * and **, respectively, based on 1000 permutations

Table 3.4 Quantitative trait loci identified in the RIL population (N = 240) grown in Entrican, MI in 2016 and 2017 for sensory attributes. Linkage group (LG), peak position (Pos), year[a], logarithm of odds (LOD), R^2, QTL effect[b] (a), flanking markers[c], QTL range[d], and significance[e] of the QTL are indicated.

Trait	QTL Name	LG	Pos (cM)	Year	LOD	R^2 (%)	a	Flanking Markers	QTL Range (cM)	Sig
Total Flavor Intensity										
	TFI.3.1	Pv03	102.9	2016	3	5.7	-	51433552 - 51495521	102.06 - 103.80	**
Beany Intensity										
	BFI.3.1	Pv03	27.3	C	4.3	6.8	-	5101738 - 11872699	26.67 - 29.81	**
		Pv03	41.8	2017	5.52	8.8	-	12630923 - 33254421	30.30 – 48.56	**
Vegetative Intensity										
	VFI.2.1	Pv02	86.0	2017	3.91	6.7	-	41385822 - 41401290	85.88 - 86.48	**
	VFI.7.1	Pv07	47.2	C	3.2	5.6	+	7714725 - 8753083	46.96 - 48.21	**
	VFI.7.2	Pv07	132.4	2016	3.39	8.1	-	30492292 - 38224460	130.75 - 132.84	**
Earthy Intensity										
	EFI.7.1	Pv07	136.8	2016	3.12	5.9	+	39018976 - 39082640	136.29 - 137.80	*
	EFI.10.1	Pv10	72.3	C	4.41	12.3	+	9922603 - 28143113	68.12 - 77.33	**
		Pv10	71.3	2016	2.95	7.9	+	28038978 - 28143113	70.33 - 72.33	*
		Pv10	71.3	2017	3.7	9.9	+	9922603 - 28143113	69.12 - 74.33	**
	EFI.10.2	Pv10	180.6	2017	4.68	7.7	-	42840998 - 43205231	180.04 - 186.99	**
Starchy Intensity										
	STI.3.1	Pv03	89.0	C	4.0	6.9	+	51161323 - 51220644	88.07 - 89.30	**
		Pv03	88.1	2016	3.7	6.6	+	51140633 - 51171573	87.24 - 89.00	**
		Pv03	89.3	2017	3.3	5.4	+	51171573 - 51224571	89.00 - 90.12	**
	STI.11.1	Pv11	100.5	2016	3.5	9.1	+	32258466 - 40017638	99.48 - 103.25	**
Sweet Intensity										
	SWI.2.1	Pv02	55.4	C	3.2	5.9	+	31959417 - 33225006	54.53 - 55.72	*
		Pv02	55.4	2016	2.8	4.9	+	31959417 - 33225006	54.53 - 55.72	*
	SWI.7.1	Pv07	37.4	2016	3.9	7.2	+	7175486 - 7438454	37.03 - 37.9	**
Bitter Intensity										
	BI.1.1	Pv01	44.8	C	3.3	5.9	-	14308851 - 32577126	44.51 - 45.76	**
	BI.3.1	Pv03	37.8	2017	3.9	7.9	-	18090483 - 21241672	36.84 - 38.43	**
Seed Coat Perception										
	SPE.3.1	Pv03	46.1	C	3.6	6.8	-	32484452 - 32983892	46.02 - 48.07	**
		Pv03	46.1	2016	4.4	8.1	-	29498694 - 32983892	45.33 - 47.12	**
		Pv03	39.2	2017	4.5	8.7	-	18090483 - 33254421	35.84 - 48.56	**
Cotyledon Texture										
	CTX.5.1	Pv05	79.4	2017	3.1	6.5	-	25938962 - 39102354	77.60 - 81.43	*
	CTX.7.1	Pv07	140.4	2016	3.0	5.9	-	39116608 - 39166109	139.37 - 140.94	*

Table 3.4 (cont'd)

The largest LOD and R^2 within the QTL are reported
[a] "C" indicates both years combined
[b] + and – indicate positive and negative effects on the mean as conferred by alleles from Ervilha in the QTL region.
[c] Flanking markers indicate the physical positions of the nearest markers upstream and downstream
[d] Region where LOD scores are significant at the indicated significance level
[e] Significance at $\alpha = 0.1$ and $\alpha = 0.05$ are indicated by * and **, respectively, based on 1000 permutations

Table 3.5 Quantitative trait loci identified in the RIL population (N = 240) grown in Entrican, MI in 2017 for color and seed coat postharvest darkening. Linkage group (LG), peak position (Pos), year, logarithm of odds (LOD), R^2, QTL effect[a] (a), flanking markers[b], QTL range[c], and significance[d] of the QTL are indicated.

Trait	QTL Name	LG	Pos (cM)	Year	LOD	R^2 (%)	a	Flanking Markers	QTL Range (cM)	Sig
L*										
	SL*.3.1	Pv03	28.2	C	3.3	5.5	+	6264322 - 7619818	27.33 - 28.57	**
		Pv03	28.2	2016	3.3	5.4	+	6264322 - 7619818	27.33 - 28.57	**
		Pv03	37.8	2017	3.0	5.8	+	1872699 - 18827421	29.81 - 37.84	*
	SL*.10.1	Pv10	114.7	C	4.2	6.6	+	40970486 - 41609109	112.42 - 116.97	**
		Pv10	114.7	2016	4.2	6.6	+	40970486 - 41609109	112.42 - 116.97	**
a*										
	Sa*.3.1	Pv03	29.6	C	3.4	5.3	-	7619818 - 11872699	29.57 - 29.81	**
		Pv03	29.6	2016	3.4	5.2	-	7619818 - 11872699	28.57 - 29.81	**
		Pv03	29.6	2017	4.2	6.6	-	6514180 - 11872699	28.17 - 29.81	**
	Sa*.10.1	Pv10	114.47	C	6.2	9.7	-	35799233 - 41965681	105.38 - 123.1	**
		Pv10	114.47	2016	6.2	9.6	-	35799233 - 41965681	105.38 - 123.1	**
		Pv10	114.47	2017	4.6	7.1	-	40970486 - 41965681	111.42 - 123.1	**
b*										
	Sb*.1.1	Pv01	0.01	2017	3	4.8	-	703233 - 787996	0.01 - 1.01	*
	Sb*.5.1	Pv05	1.21	C	4.1	6.7	-	1077531 - 1091909	0.91 - 1.21	**
	Sb*.5.2	Pv05	12.96	C	3.7	6.3	+	1960475 - 1963046	12.96 - 13.96	**
		Pv05	12.96	2016	3.7	6.3	+	1960475 - 1963046	12.96 - 13.96	**
	Sb*.10.1	Pv10	114.69	C	4.6	8	+	35799233 - 41088499	105.38 - 115.97	**
		Pv10	109.38	2016	4.2	8.9	+	35799233 - 41070183	104.38 - 115.28	**
		Pv10	114.69	2017	6.3	10.2	+	35799233 - 41922512	104.38 - 121.95	**
Seed Coat Postharvest Darkening										
	ND.10.1	Pv10	115.3	2017	5.9	10.4	-	35799233 - 41931454	108.38 - 122.23	**

The largest LOD and R^2 within the QTL are reported
[a] "C" indicates both years combined
[b] + and – indicate positive and negative effects on the mean as conferred by alleles from Ervilha in the QTL region.
[c] Flanking markers indicate the physical positions of the nearest markers upstream and downstream
[d] Region where LOD scores are significant at the indicated significance level
[e] Significance at α = 0.1 and α = 0.05 are indicated by * and **, respectively, based on 1000 permutations

Figure 3.1 Images of Ervilha and PI527538 raw seeds.

Figure 3.2 Density plots of soak water uptake and cooking time for the RILs from 2016, 2017, and both years combined (C). Means for Ervilha and PI527538 from both years combined are indicated in yellow and brown, respectively.

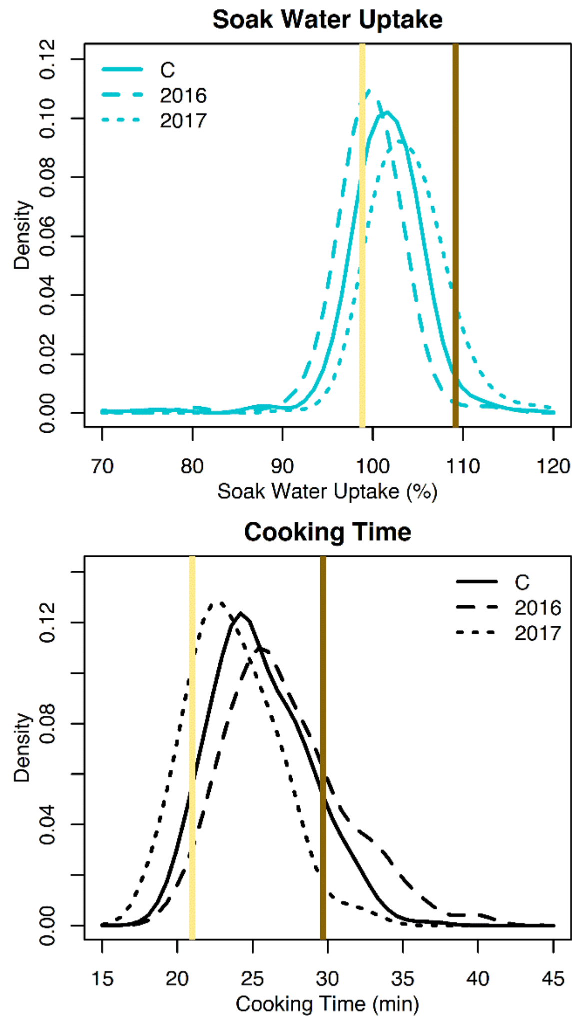


Figure 3.3 Density plots of least squares estimates of sensory attribute intensities for the RILs from 2016, 2017, and both years combined (C). Attribute intensities for Ervilha and PI527538 from both years combined are indicated in yellow and brown, respectively.

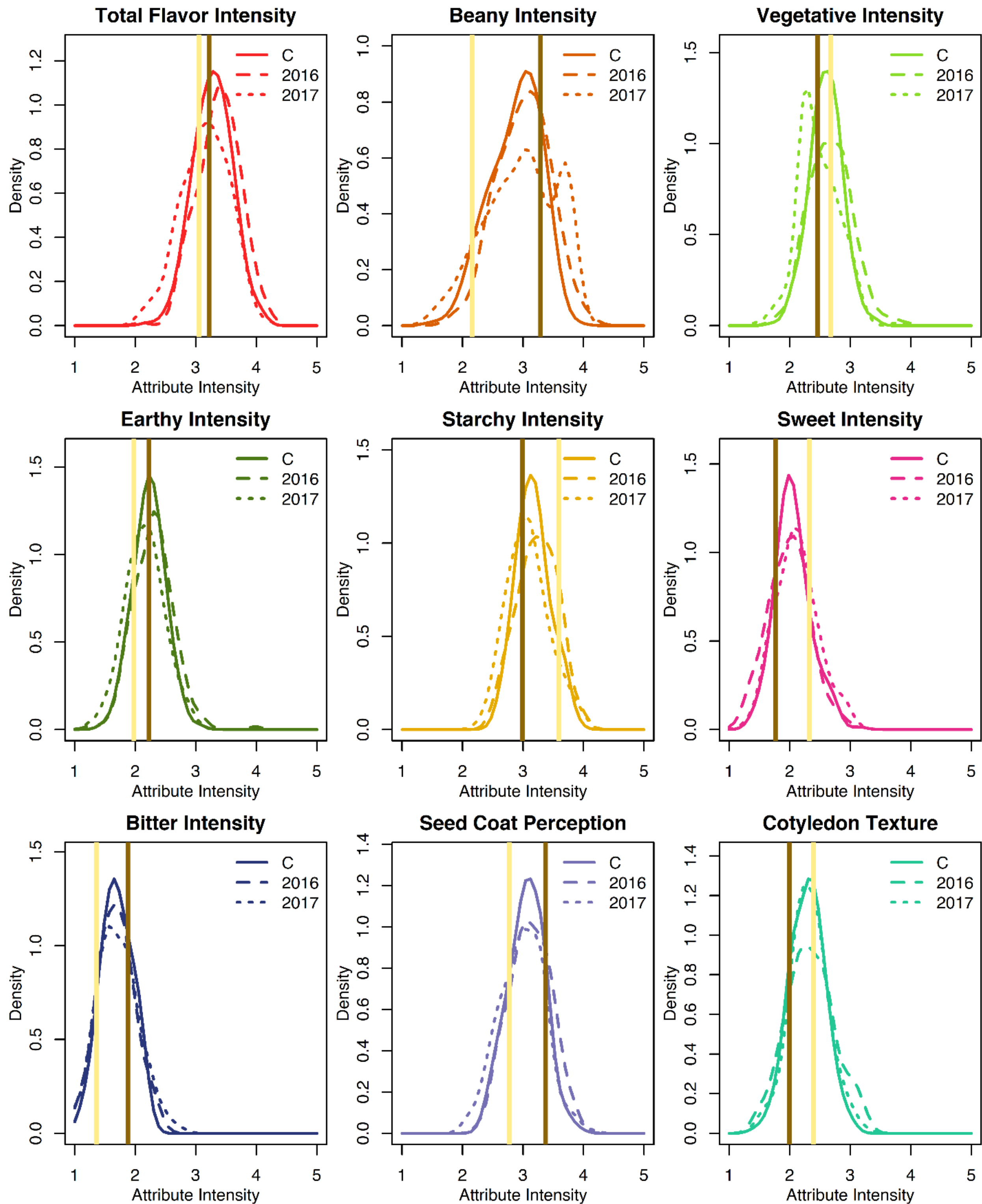


Figure 3.4 Density plots of CIELAB values for the RILs from 2016, 2017, and both years combined (C). Attribute intensities for Ervilha and PI527538 from both years combined are indicated in yellow and brown, respectively.

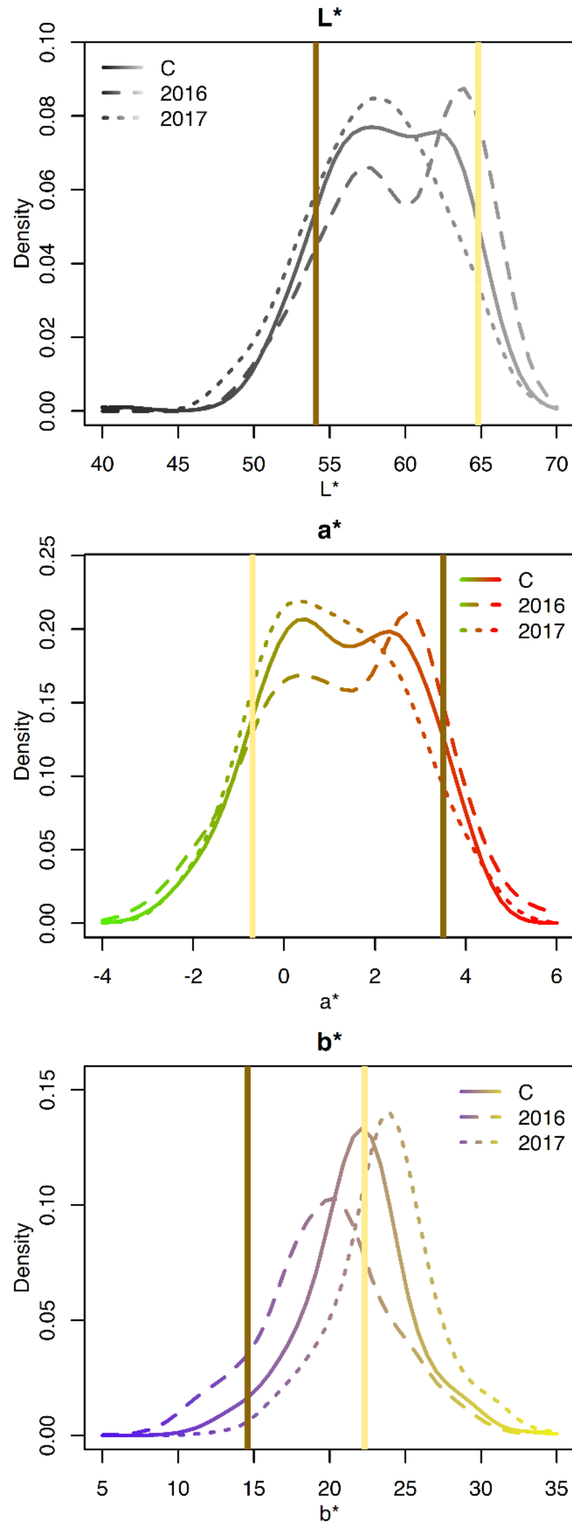


Figure 3.5 Principal component analysis biplot with loadings for cooking time (CT), total flavor intensity (TFI), beany intensity (BFI), vegetative intensity (VFI), earthy intensity (EFI), starchy intensity (STI), sweet intensity (SWI), bitter intensity (BI), seed coat perception (SPE), and cotyledon texture (CTX). Ervilha and PI527538 are indicated in yellow and brown, respectively.

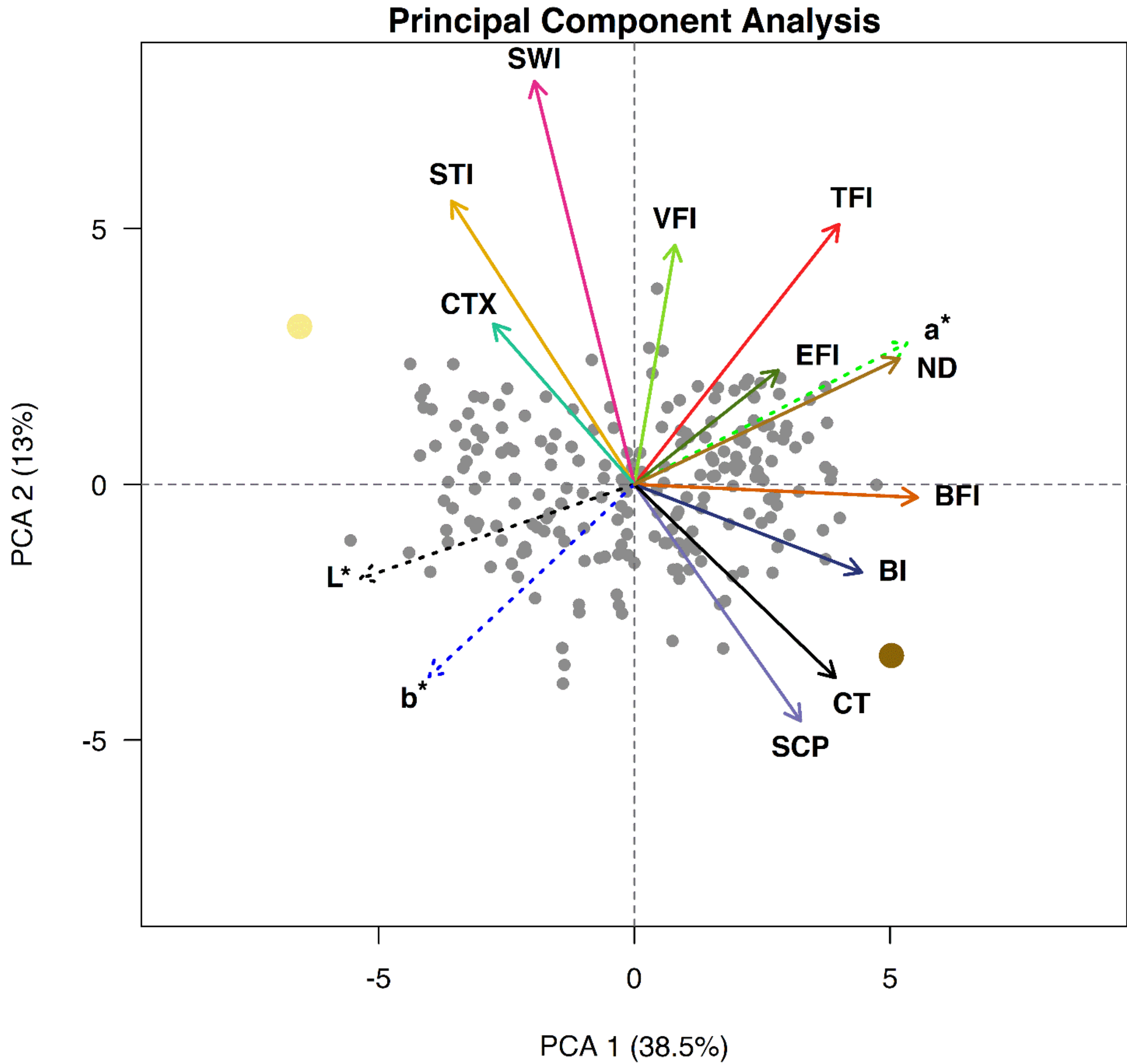
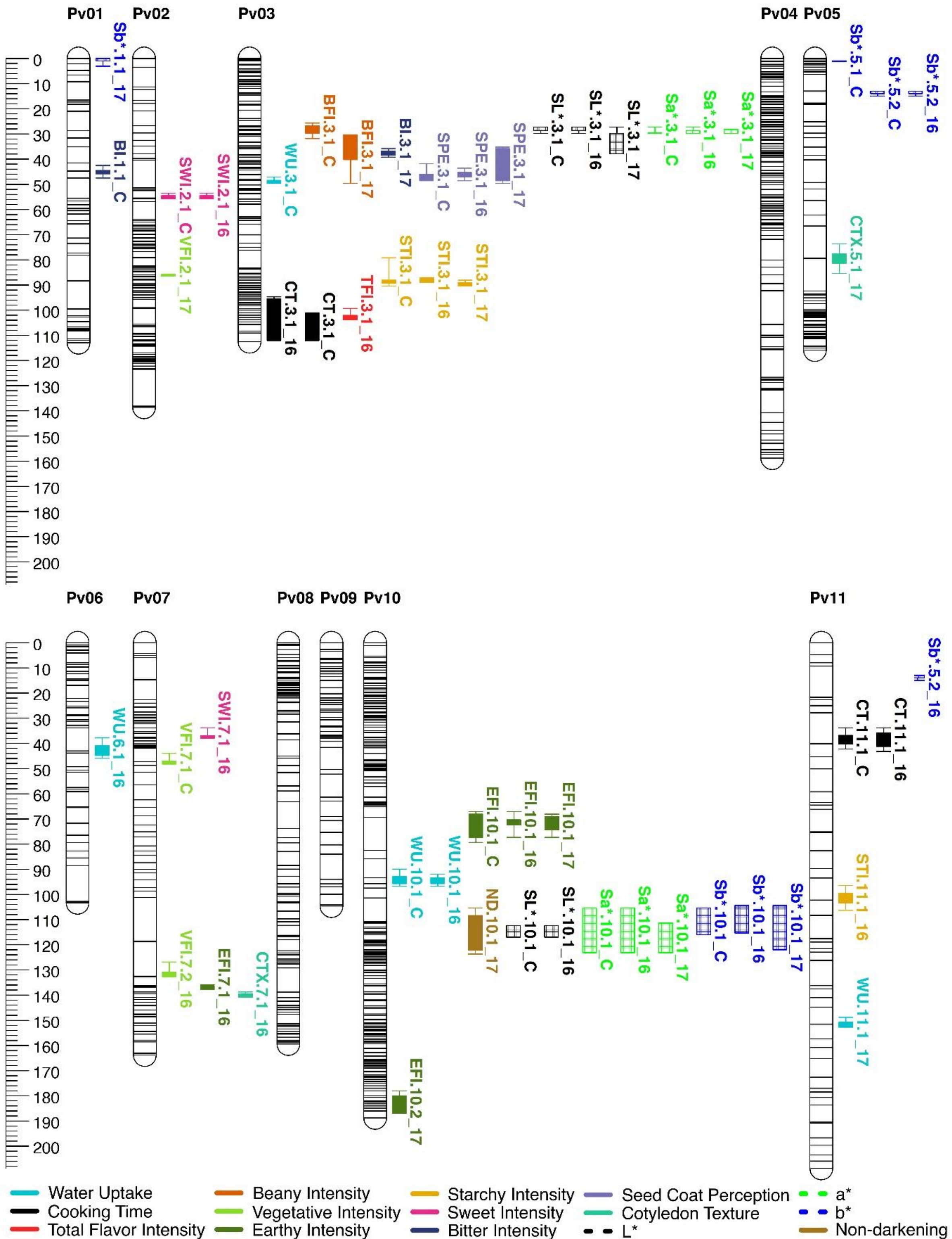


Figure 3.6 QTL map for soak water uptake, cooking time, total flavor intensity, beany intensity, vegetative intensity, earthy intensity, starchy intensity, sweet intensity, bitter intensity, seed coat perception, cotyledon texture, L*, a*, b*, and seed coat postharvest non-darkening in the RIL population. Size is in cM. Year is indicated for each QTL, where "C" is both years combined.



APPENDIX B:

CHAPTER 3 SUPPLEMENTAL TABLES AND FIGURES

Table S3.1 Parental phenotypes, means[a], ranges, and broad-sense heritability (H2) for the RILs for both years combined with ANOVA *p*-values for genotype, year, and genotype by year indicated.

Trait	Ervilha	PI527538	Mean	Range	H^2	Genotype	Year	Genotype x Year
Seed Weight(g per 100 seeds)								
	52.8[a] ± 0.1	48.0[b] ± 1.2	51.35 ± 0.3	39.1 - 68.4	0.84	< 0.0001	< 0.0001	< 0.0001
Total Water Uptake (%)								
	138.2[a] ± 7.7	146.3[a] ± 9.0	131.85 ± 0.3	109.9 – 148.0	0.16	0.0012	NS	NS
Seed Yield (kg/ha)								
	1891.6[a] ± 403.9	1731.4[a] ± 639.2	2072.9 ± 23.8	751.0 – 3283.9	0.57	< 0.0001	< 0.0001	< 0.0001

[a] Mean separation is indicated by letter superscript.

Table S3.2 Parental phenotypes and means and ranges for the RILs for 2016 and 2017.

Trait	Year	Ervilha	PI527538	Mean	Range
Soak Water Uptake (%)					
	2016	104.3 ± 1.9	97.0 ± 0.1	99.0 ± 0.5	38.6 - 114.9
	2017	114.2 ± 4.8	100.5 ± 4.8	103.9 ± 0.3	90.2 - 135.7
Cooking Time (min)					
	2016	23.1 ± 0.6	33.2 ± 1.4	27.2 ± 0.3	19.7 - 40.3
	2017	18.8 ± 0.3	26.3 ± 1.1	23.6 ± 0.2	17.8 - 33.0
Total Flavor Intensity					
	2016	3.2 ± 0.1	3.4 ± 0.1	3.4 ± 0.0	2.1 - 4.2
	2017	2.9 ± 0.0	3.1 ± 0.0	3.2 ± 0.0	2.1 - 4.2
Beany Flavor Intensity					
	2016	2.4 ± 0.2	3.5 ± 0.1	3.0 ± 0.0	1.8 - 4.1
	2017	1.9 ± 0.1	3.1 ± 0.1	3.0 ± 0.0	1.5 - 3.9
Vegetative Flavor Intensity					
	2016	2.9 ± 0.1	2.6 ± 0.0	2.7 ± 0.0	1.7 - 3.9
	2017	2.5 ± 0.2	2.4 ± 0.2	2.5 ± 0.0	1.5 - 3.8
Earthy Flavor Intensity					
	2016	2.0 ± 0.0	2.2 ± 0.1	2.3 ± 0.0	1.2 - 4.0
	2017	2.0 ± 0.0	2.2 ± 0.0	2.2 ± 0.0	1.3 - 3.0
Starchy Flavor Intensity					
	2016	3.6 ± 0.1	3.1 ± 0.0	3.2 ± 0.0	2.5 - 4.1
	2017	3.6 ± 0.0	2.9 ± 0.1	3.1 ± 0.0	2.2 - 4.0
Sweet Flavor Intensity					
	2016	2.2 ± 0.2	1.7 ± 0.1	2.0 ± 0.0	1.1 - 3.1
	2017	2.5 ± 0.0	1.9 ± 0.1	2.1 ± 0.0	1.2 - 3.1
Bitter Flavor Intensity					
	2016	1.4 ± 0.1	2.0 ± 0.1	1.7 ± 0.0	0.9 - 2.5
	2017	1.3 ± 0.0	1.8 ± 0.0	1.7 ± 0.0	0.9 - 2.8
Seed Coat Perception					
	2016	2.9 ± 0.0	3.4 ± 0.1	3.1 ± 0.0	2.3 - 3.9
	2017	2.6 ± 0.0	3.4 ± 0.1	3.0 ± 0.0	2.0 4.1
Cotyledon Texture					
	2016	2.3 ± 0.0	1.8 ± 0.1	2.3 ± 0.0	1.4 - 3.3
	2017	2.5 ± 0.2	2.2 ± 0.0	2.3 ± 0.0	1.4 - 3.3
L*					
	2016	65.2	51.6	59.9 ± 0.3	49.3 - 68.0
	2017	64.5	56.6	58.0 ± 0.3	40.3 - 68.8

Table S3.2 (cont'd)

a*				
2016	-1.5	3.7	1.4 ± 0.1	-3.2 - 5.9
2017	0.1	3.2	1.1 ± 0.1	-2.4 - 4.7
b*				
2016	21.0	11.4	19.9 ± 0.3	8.4 - 34.4
2017	23.5	17.8	23.6 ± 0.2	12.8 - 34.6
Darkening (0 = Nondarkening; 1 = Darkening)				
2016	.	.	.	.
2017	0	1	0.5 - 0.0	0.0 - 1.0
Seed Weight(g)				
2016	53.0 ± 1.8	46.3 ± 1.4	49.9 ± 0.3	39.5 - 64.7
2017	52.7 ± 2.3	49.7 ± 0.9	52.7 ± 0.3	39.1 - 72.1
Total Water Uptake (%)				
2016	127.3 ± 9.0	133.6 ± 0.4	131.4 ± 0.5	89.8 - 160.6
2017	149.0 ± 11.7	159.0 ± 15.6	132.3 ± 0.4	102.0 ± 159.0
Seed Yield (kg/ha)				
2016	.	1399.2 ± 23.1	1448.3 ± 32.0	301.0 - 2876.5
2017	1731.5 ± 639.2	2384.4 ± 591.0	2623.2 ± 32.4	592.1 - 3912.0

Table S3.3 *P*-values[a] for the random effects from the sensory attribute intensity ANOVAs at the genotype level.

Trait	Rep	Panelist(Year)	Session(Year)
Total Flavor Intensity	NS	<0.0001	<0.0001
Beany Intensity	NS	<0.0001	0.0145
Vegetative Intensity	NS	<0.0001	<0.0001
Earthy Intensity	NS	<0.0001	NS
Starchy Intensity	NS	<0.0001	0.0136
Sweet Intensity	NS	<0.0001	<0.0001
Bitter Intensity	NS	<0.0001	0.005
Seed Coat Perception	NS	<0.0001	<0.0001
Cotyledon Texture	NS	<0.0001	<0.0001

[a] NS indicates non-significant *p*-values at $\alpha = 0.05$

Table S3.4 Quantitative trait loci identified in the RIL population (N = 240) grown in Entrican, MI in 2017 for seed weight, total water uptake, and yield. Linkage group (LG), peak position (Pos), year, logarithm of odds (LOD), R^2, QTL effect[a] (a), flanking markers[b], QTL range[c], and significance[d] of the QTL are indicated.

Trait	QTL Name	LG	Pos (cM)	Year	LOD	R^2 (%)	a	Flanking Markers	QTL Range (cM)	Sig
Seed Weight (g per 100 seeds)										
	SW.6.1	Pv06	6.1	2017	2.89	4.8	+	5685389 - 6728096	5.1 - 7.1	*
	SW.8.1	Pv08	5.9	C	4.10	6.7	+	5255874 - 7162048	5.73 - 14.04	**
		Pv08	5.9	2016	3.40	5.7	+	5255874 - 7162048	4.73 - 14.04	**
		Pv08	5.9	2017	4.36	7.1	+	5255874 - 7162048	5.73 - 14.14	**
	SW.10.1	Pv10	11.8	2016	3.34	5.5	-	861914 - 881899	10.78 - 11.79	**
	SW.10.2	Pv10	42.7	2016	3.43	6.5	+	2459869 - 2484767	41.73 - 43.73	**
	SW.10.3	Pv10	114.7	C	5.54	9.2	-	40970486 - 41070183	112.42 - 115.28	**
		Pv10	114.7	2016	3.42	5.7	-	40984982 - 41070183	114.47 - 115.28	**
		Pv10	114.7	2017	4.48	7.5	-	40971012 - 41070183	113.47 - 115.28	**
	SW.10.4	Pv10	174.6	2016	3.95	6.1	+	42732517 - 42758310	174.16 - 175.05	**
Total Water Uptake										
	TWU.3.1	Pv03	28.6	C	3.79	5.8	+	6514180 - 11872699	28.17 - 29.57	**
		Pv03	28.6	2016	3.56	5.5	+	6514180 - 11872699	28.17 - 29.57	**
	TWU.8.1	Pv08	95.9	2017	4.66	8.0	-	41088373 - 44547580	94.79 - 96.85	**
	TWU.9.1	Pv09	25.5	C	4.01	6.6	+	10763996 - 12942768	22.98 - 26.39	**
		Pv09	25.5	2016	4.17	7.0	+	10449643 - 12942768	22.37 - 26.39	**
	TWU.10.1	Pv10	114.7	C	3.09	4.8	+	40971012 - 41070183	114.47 - 115.28	*
		Pv10	114.7	2016	3.10	4.8	+	40971012 - 41070183	114.47 - 115.28	*
	TWU.10.2	Pv10	159.3	2017	3.91	6.7	+	42515259 - 42521192	158.68 - 160.27	**
	TWU.10.3	Pv10	176.0	2017	4.30	7.2	+	42758310 - 42791961	175.05 - 177.01	**
	TWU.10.4	Pv10	184.9	2017	5.11	8.7	+	42853098 - 42872742	183.21 - 185.99	**
Seed Yield (kg/ha)										
	YLD.2.1	Pv02	12.5	C	3.35	5.8	+	3095899 - 5589384	12.37 - 13.45	**
		Pv02	12.5	2016	3.21	6.1	+	3095899 - 5589384	12.37 - 13.45	*
	YLD.3.1	Pv03	98.3	2016	4.23	7.2	-	51291118 - 51376970	95.46 - 99.42	**
	YLD.7.1	Pv07	152.1	2017	3.79	6.3	-	39309171 - 39372454	151.07 - 153.07	**
	YLD.10.1	Pv10	118.9	2016	4.6	8.2	-	41088499 - 41965681	116.97 - 123.1	**
	YLD.11.1	Pv11	64.5	2017	3.26	5.9	+	21357068 - 21564226	64.37 - 64.49	**
	YLD.11.2	Pv11	112.3	2017	4.11	9.4	-	38773018 - 41950354	107.25 - 116.31	**

The largest LOD and R^2 within the QTL are reported

[a] + and – indicate positive and negative effects on the mean as conferred by alleles from Ervilha in the QTL region.

[b] Flanking markers indicate the physical positions of the nearest markers upstream and downstream

Table 3.4 (cont'd)

[c] Region where LOD scores are significant at the indicated significance level
[d] Significance at $\alpha = 0.1$ and $\alpha = 0.05$ are indicated by * and **, respectively, based on 1000 permutations

Figure S3.1 Density plots of seed weight, total water uptake, and yield for the RILs from 2016, 2017, and both years combined (C). Means for Ervilha and PI527538 from both years combined (2017 for seed yield) are indicated in yellow and brown, respectively.

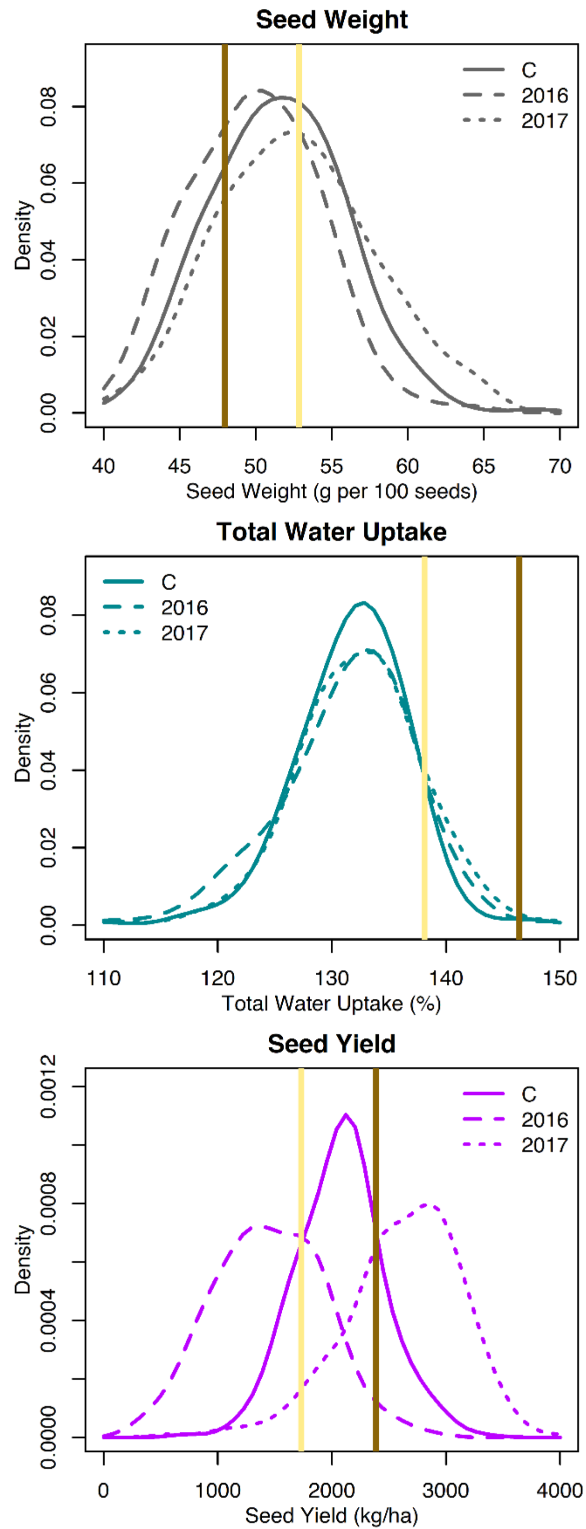


Figure S3.2 QTL map for seed weight (SW), total water uptake (TWU), and seed yield (YLD) in the RIL population. Size is in cM. Year is indicated for each QTL, where "C" is both years combined.

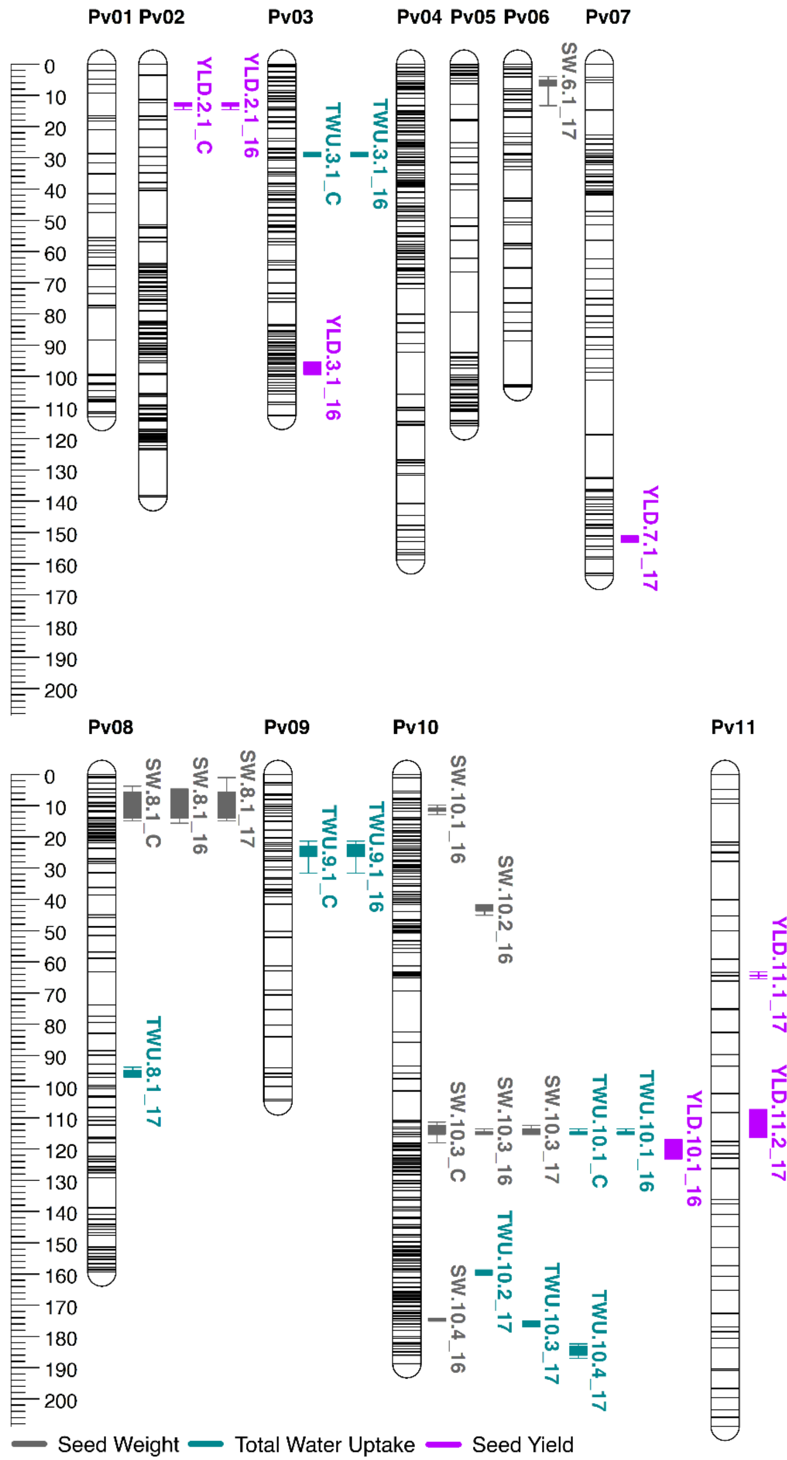


Figure S3.3 Line graphs of LOD by Pv01 position for both years combined, 2016, and 2017 for soak water uptake (WU), cooking time (CT), total flavor intensity (TFI), beany intensity (BFI), vegetative intensity (VFI), earthy intensity (EFI), starchy intensity (STI), sweet intensity (SWI), bitter intensity (BI), seed coat perception (SPE), cotyledon texture (CTX), L*, a*, b*, seed coat postharvest non-darkening (ND), seed weight (SW), total water uptake (TWU), and seed yield (YLD). The gray dashed line approximates the LOD threshold (α = 0.05) for all traits.

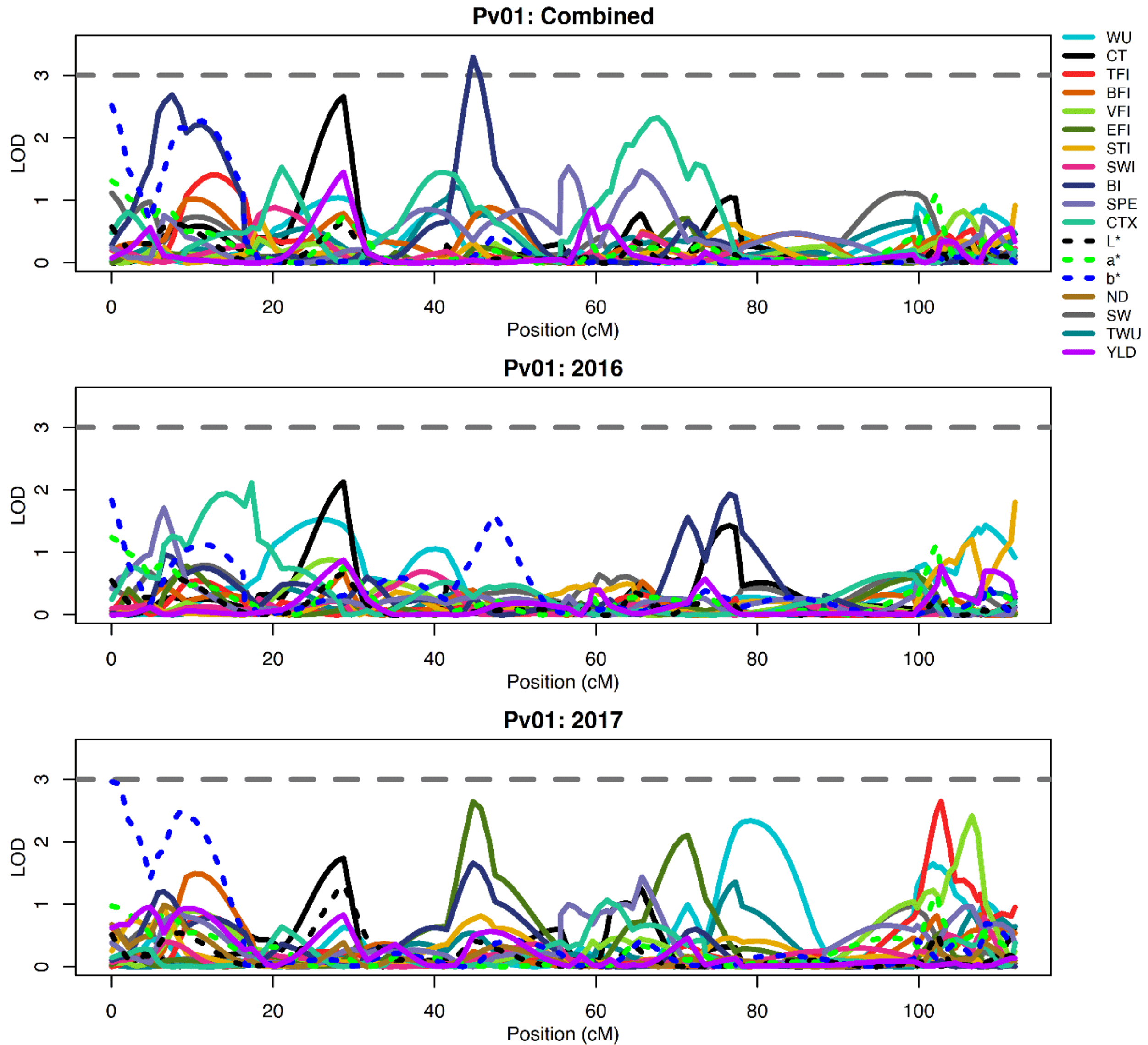


Figure S3.4 Line graphs of LOD by Pv02 position for both years combined, 2016, and 2017 for soak water uptake (WU), cooking time (CT), total flavor intensity (TFI), beany intensity (BFI), vegetative intensity (VFI), earthy intensity (EFI), starchy intensity (STI), sweet intensity (SWI), bitter intensity (BI), seed coat perception (SPE), cotyledon texture (CTX), L*, a*, b*, seed coat postharvest non-darkening (ND), seed weight (SW), total water uptake (TWU), and seed yield (YLD). The gray dashed line approximates the LOD threshold (α = 0.05) for all traits.

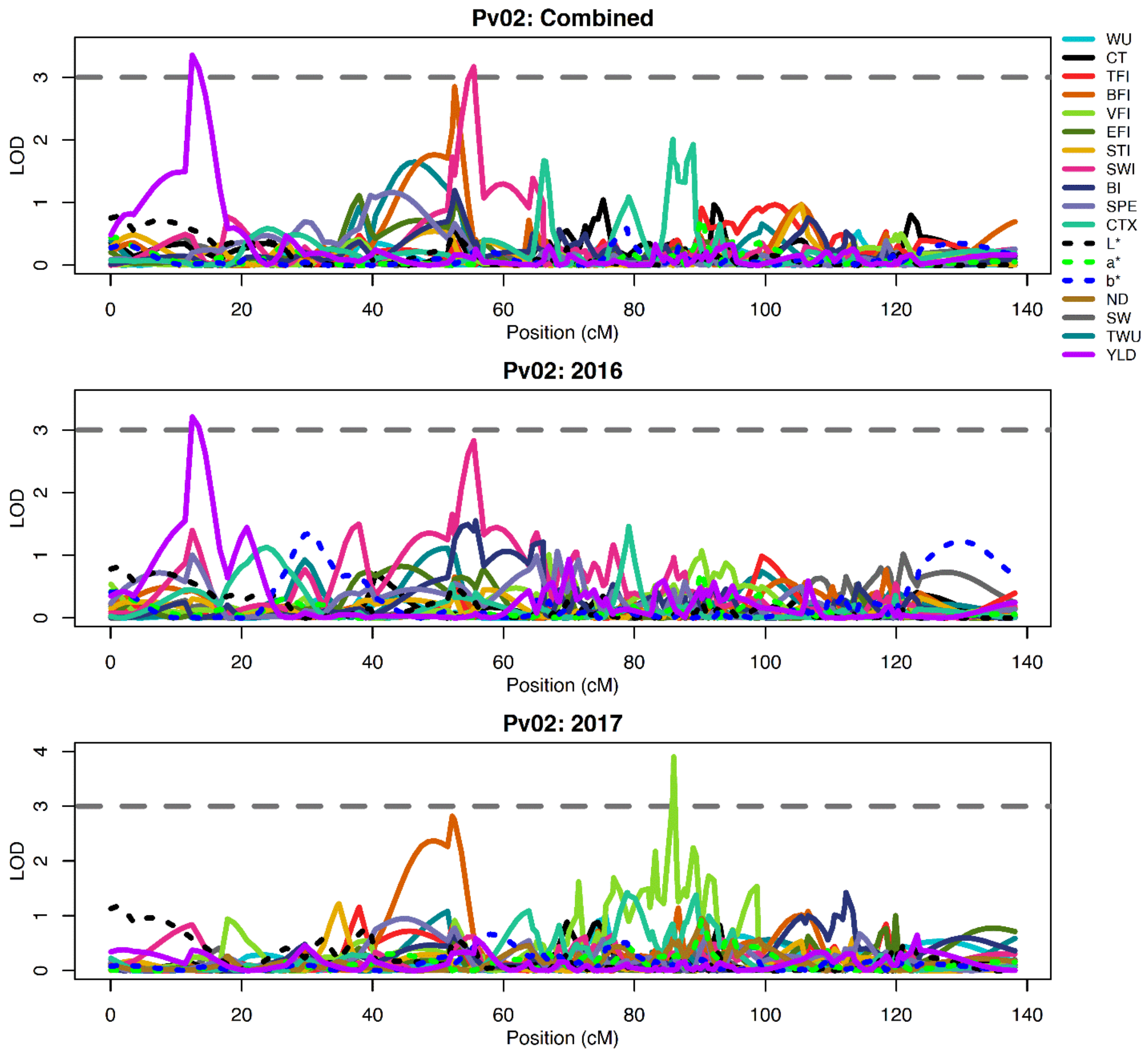


Figure S3.5 Line graphs of LOD by Pv03 position for both years combined, 2016, and 2017 for soak water uptake (WU), cooking time (CT), total flavor intensity (TFI), beany intensity (BFI), vegetative intensity (VFI), earthy intensity (EFI), starchy intensity (STI), sweet intensity (SWI), bitter intensity (BI), seed coat perception (SPE), cotyledon texture (CTX), L*, a*, b*, seed coat postharvest non-darkening (ND), seed weight (SW), total water uptake (TWU), and seed yield (YLD). The gray dashed line approximates the LOD threshold ($\alpha = 0.05$) for all traits.

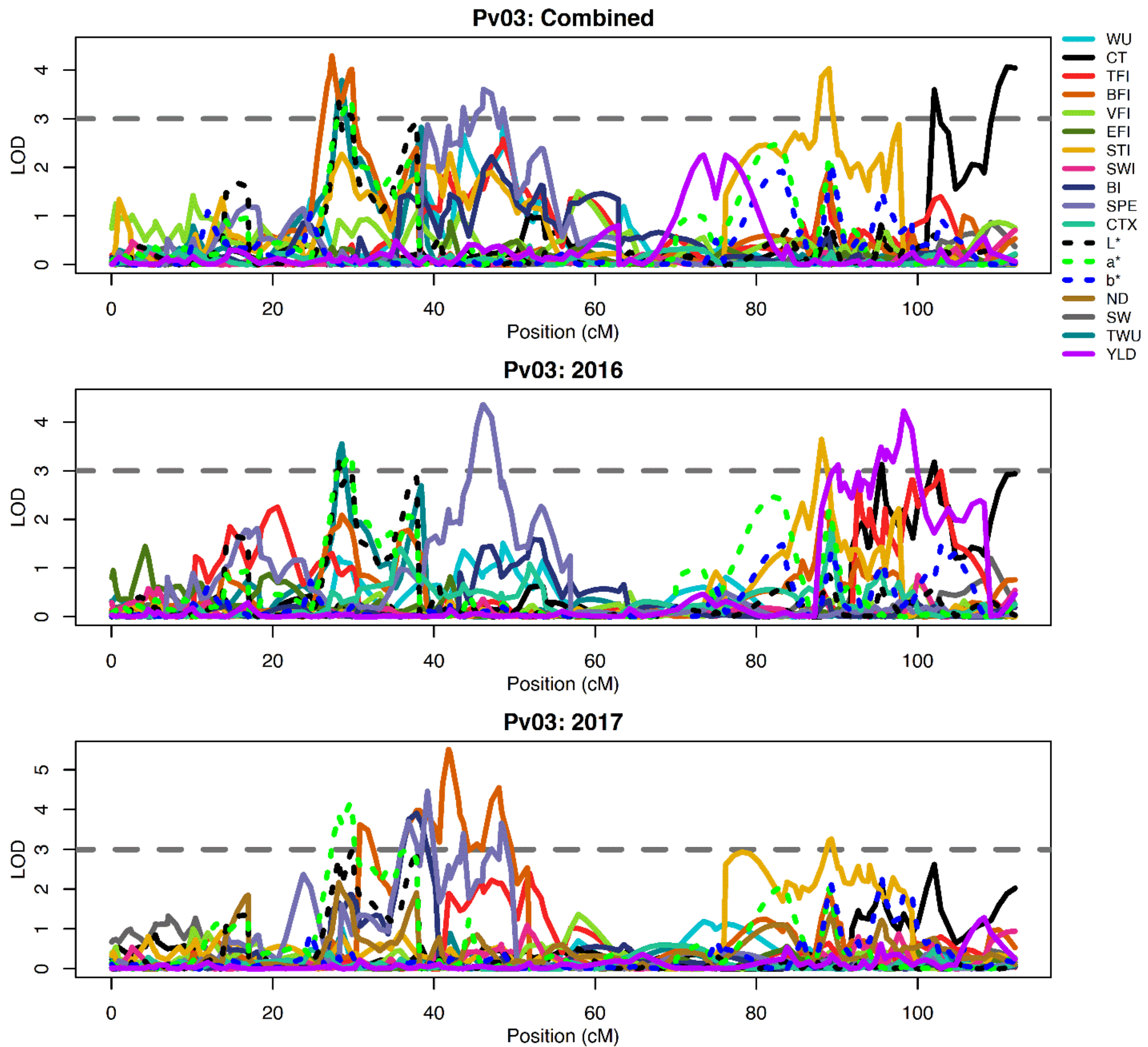


Figure S3.6 Line graphs of LOD by Pv04 position for both years combined, 2016, and 2017 for soak water uptake (WU), cooking time (CT), total flavor intensity (TFI), beany intensity (BFI), vegetative intensity (VFI), earthy intensity (EFI), starchy intensity (STI), sweet intensity (SWI), bitter intensity (BI), seed coat perception (SPE), cotyledon texture (CTX), L*, a*, b*, seed coat postharvest non-darkening (ND), seed weight (SW), total water uptake (TWU), and seed yield (YLD). The gray dashed line approximates the LOD threshold ($\alpha = 0.05$) for all traits.

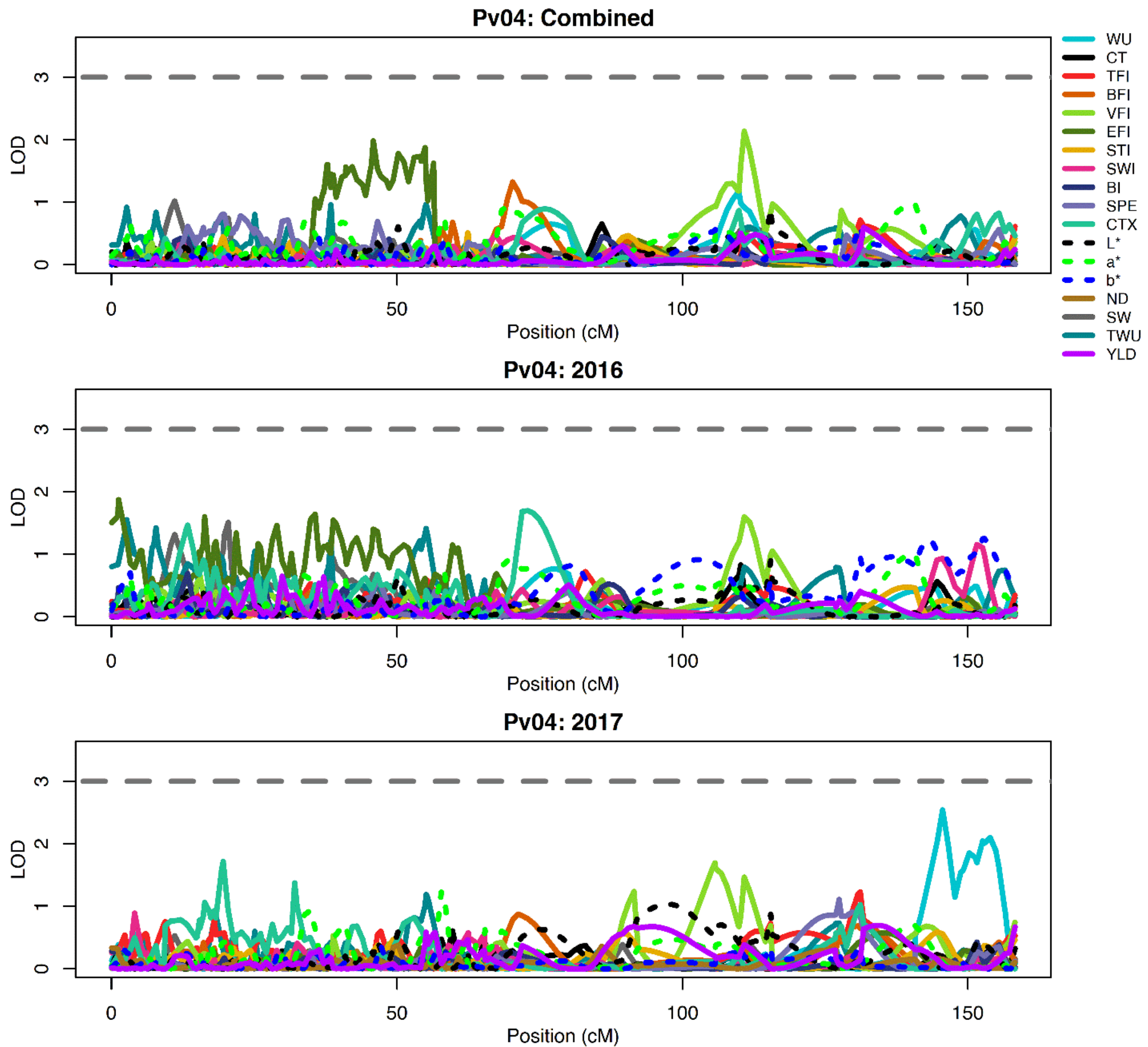


Figure S3.7 Line graphs of LOD by Pv05 position for both years combined, 2016, and 2017 for soak water uptake (WU), cooking time (CT), total flavor intensity (TFI), beany intensity (BFI), vegetative intensity (VFI), earthy intensity (EFI), starchy intensity (STI), sweet intensity (SWI), bitter intensity (BI), seed coat perception (SPE), cotyledon texture (CTX), L*, a*, b*, seed coat postharvest non-darkening (ND), seed weight (SW), total water uptake (TWU), and seed yield (YLD). The gray dashed line approximates the LOD threshold (α = 0.05) for all traits.

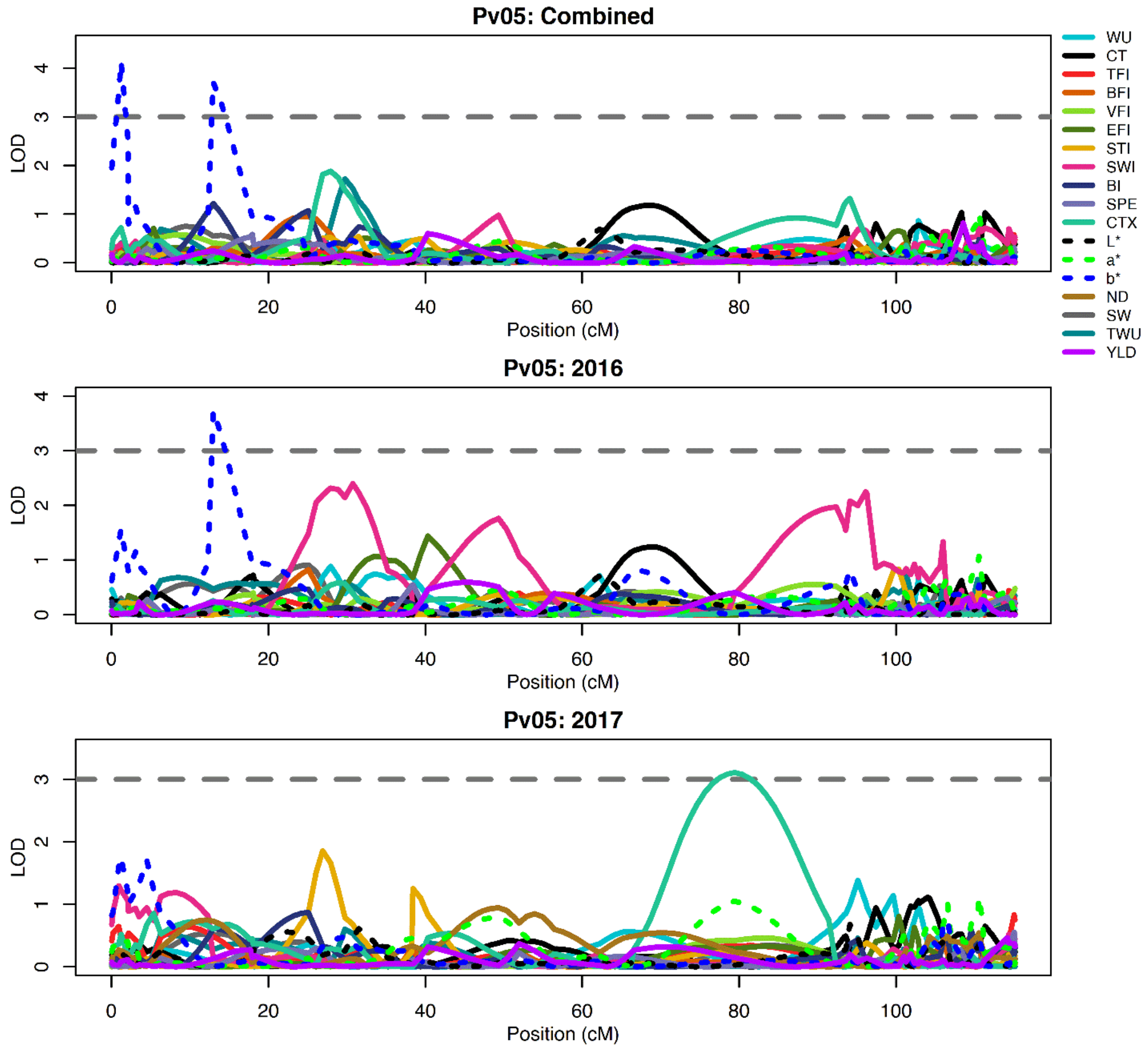


Figure S3.8 Line graphs of LOD by Pv06 position for both years combined, 2016, and 2017 for soak water uptake (WU), cooking time (CT), total flavor intensity (TFI), beany intensity (BFI), vegetative intensity (VFI), earthy intensity (EFI), starchy intensity (STI), sweet intensity (SWI), bitter intensity (BI), seed coat perception (SPE), cotyledon texture (CTX), L*, a*, b*, seed coat postharvest non-darkening (ND), seed weight (SW), total water uptake (TWU), and seed yield (YLD). The gray dashed line approximates the LOD threshold (α = 0.05) for all traits.

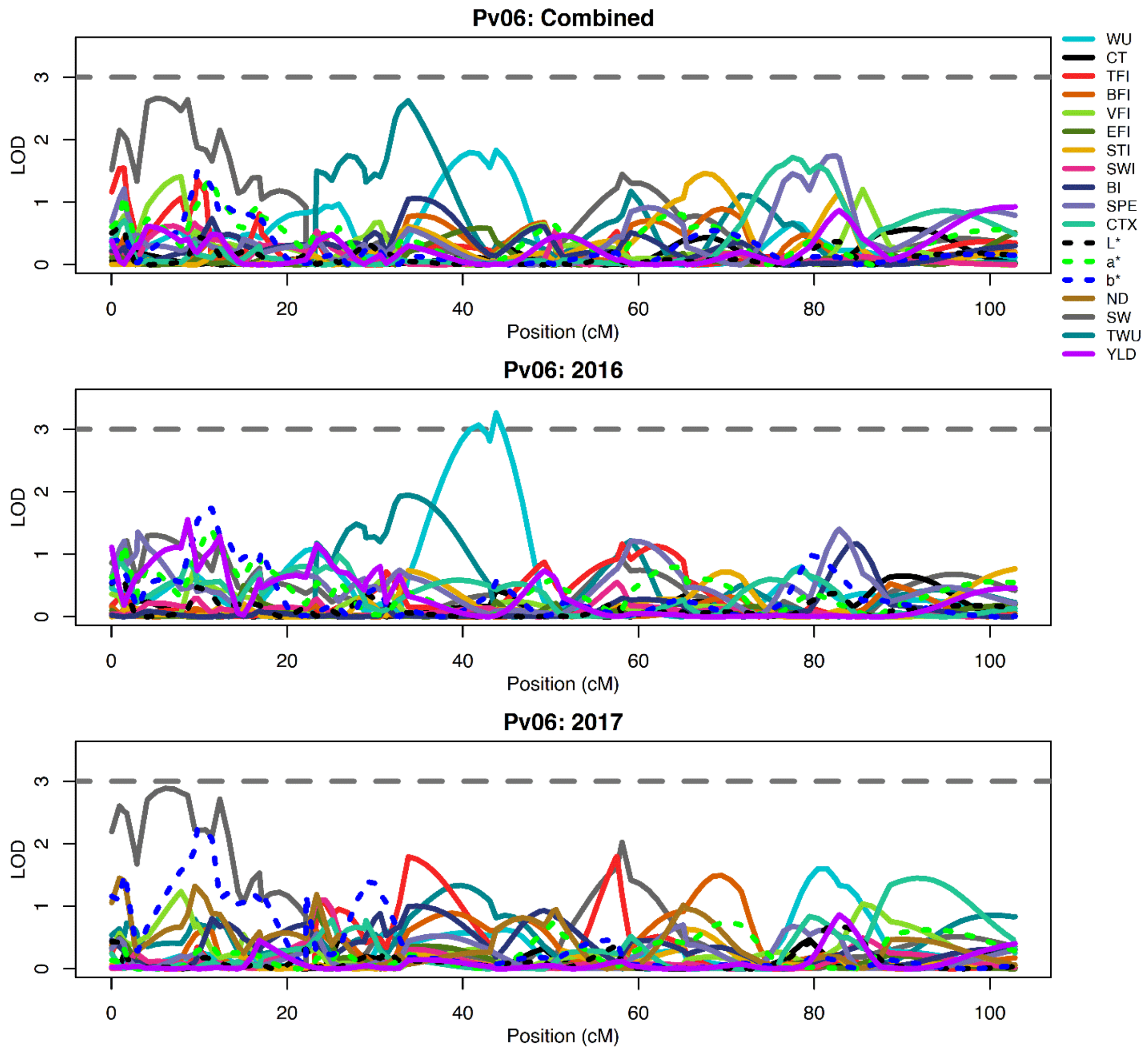


Figure S3.9 Line graphs of LOD by Pv07 position for both years combined, 2016, and 2017 for soak water uptake (WU), cooking time (CT), total flavor intensity (TFI), beany intensity (BFI), vegetative intensity (VFI), earthy intensity (EFI), starchy intensity (STI), sweet intensity (SWI), bitter intensity (BI), seed coat perception (SPE), cotyledon texture (CTX), L*, a*, b*, seed coat postharvest non-darkening (ND), seed weight (SW), total water uptake (TWU), and seed yield (YLD). The gray dashed line approximates the LOD threshold ($\alpha = 0.05$) for all traits.

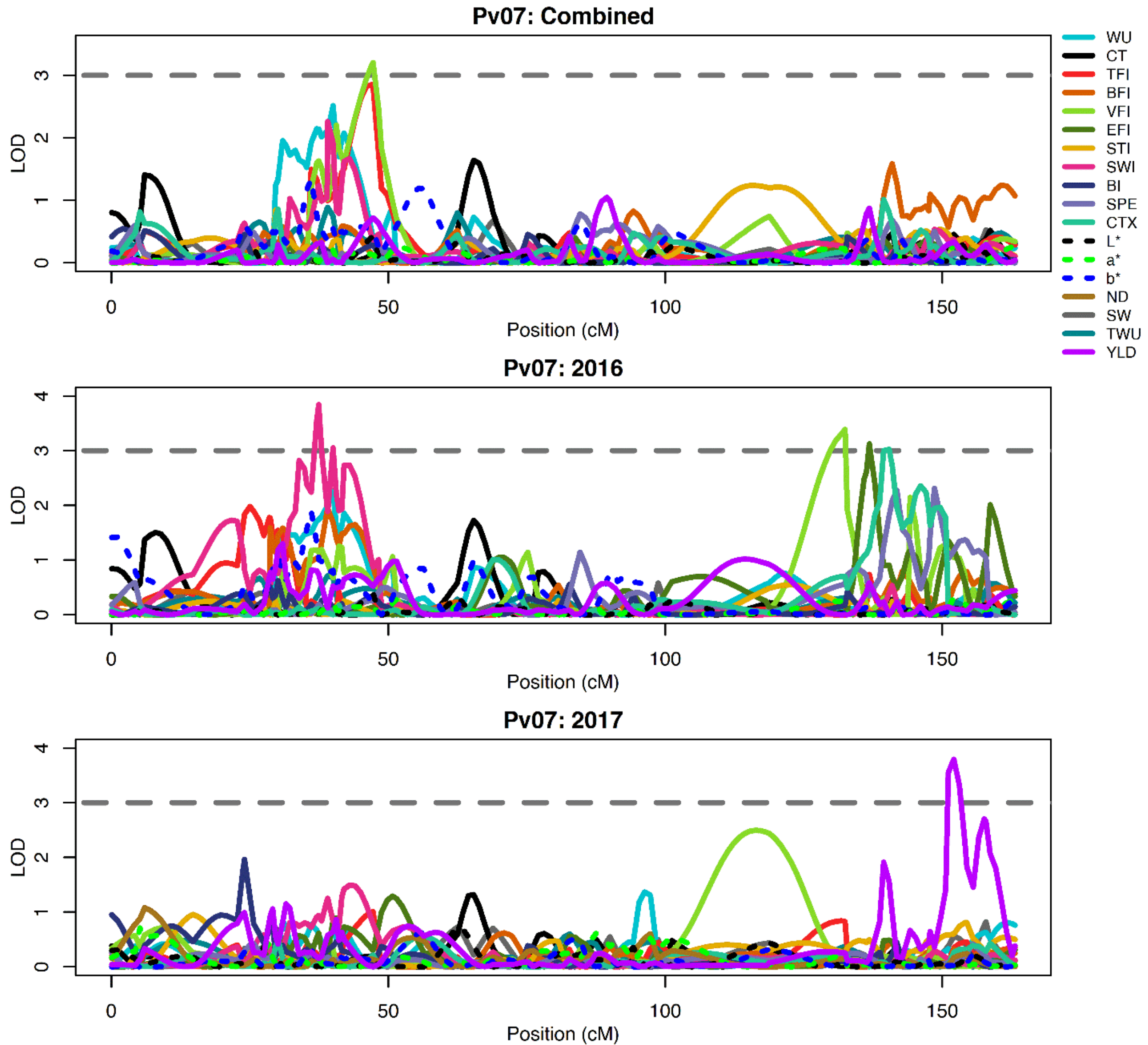


Figure S3.10 Line graphs of LOD by Pv08 position for both years combined, 2016, and 2017 for soak water uptake (WU), cooking time (CT), total flavor intensity (TFI), beany intensity (BFI), vegetative intensity (VFI), earthy intensity (EFI), starchy intensity (STI), sweet intensity (SWI), bitter intensity (BI), seed coat perception (SPE), cotyledon texture (CTX), L*, a*, b*, seed coat postharvest non-darkening (ND), seed weight (SW), total water uptake (TWU), and seed yield (YLD). The gray dashed line approximates the LOD threshold (α = 0.05) for all traits.

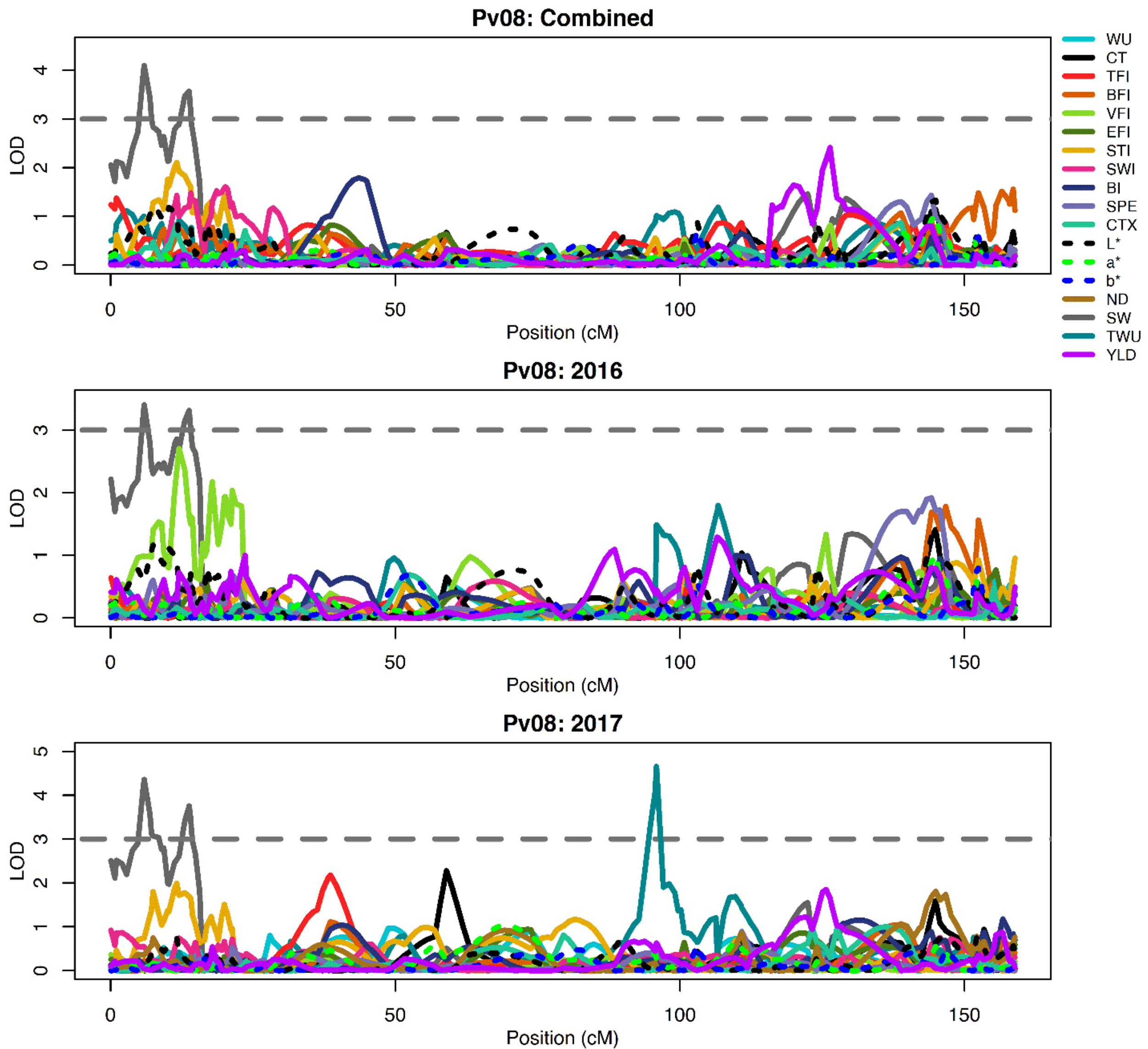


Figure S3.11 Line graphs of LOD by Pv09 position for both years combined, 2016, and 2017 for soak water uptake (WU), cooking time (CT), total flavor intensity (TFI), beany intensity (BFI), vegetative intensity (VFI), earthy intensity (EFI), starchy intensity (STI), sweet intensity (SWI), bitter intensity (BI), seed coat perception (SPE), cotyledon texture (CTX), L*, a*, b*, seed coat postharvest non-darkening (ND), seed weight (SW), total water uptake (TWU), and seed yield (YLD). The gray dashed line approximates the LOD threshold ($\alpha = 0.05$) for all traits.

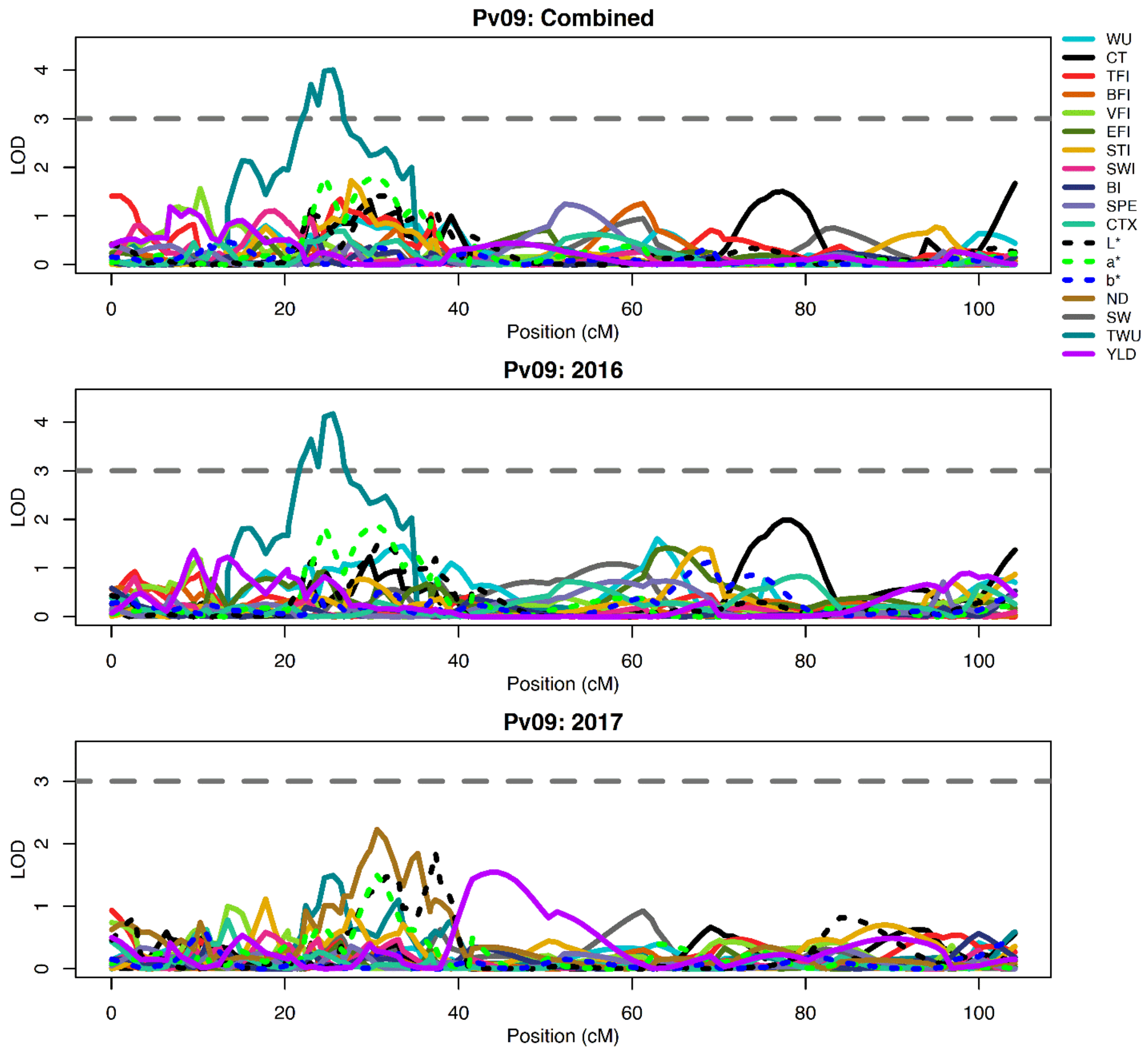


Figure S3.12 Line graphs of LOD by Pv10 position for both years combined, 2016, and 2017 for soak water uptake (WU), cooking time (CT), total flavor intensity (TFI), beany intensity (BFI), vegetative intensity (VFI), earthy intensity (EFI), starchy intensity (STI), sweet intensity (SWI), bitter intensity (BI), seed coat perception (SPE), cotyledon texture (CTX), L*, a*, b*, seed coat postharvest non-darkening (ND), seed weight (SW), total water uptake (TWU), and seed yield (YLD). The gray dashed line approximates the LOD threshold (α = 0.05) for all traits.

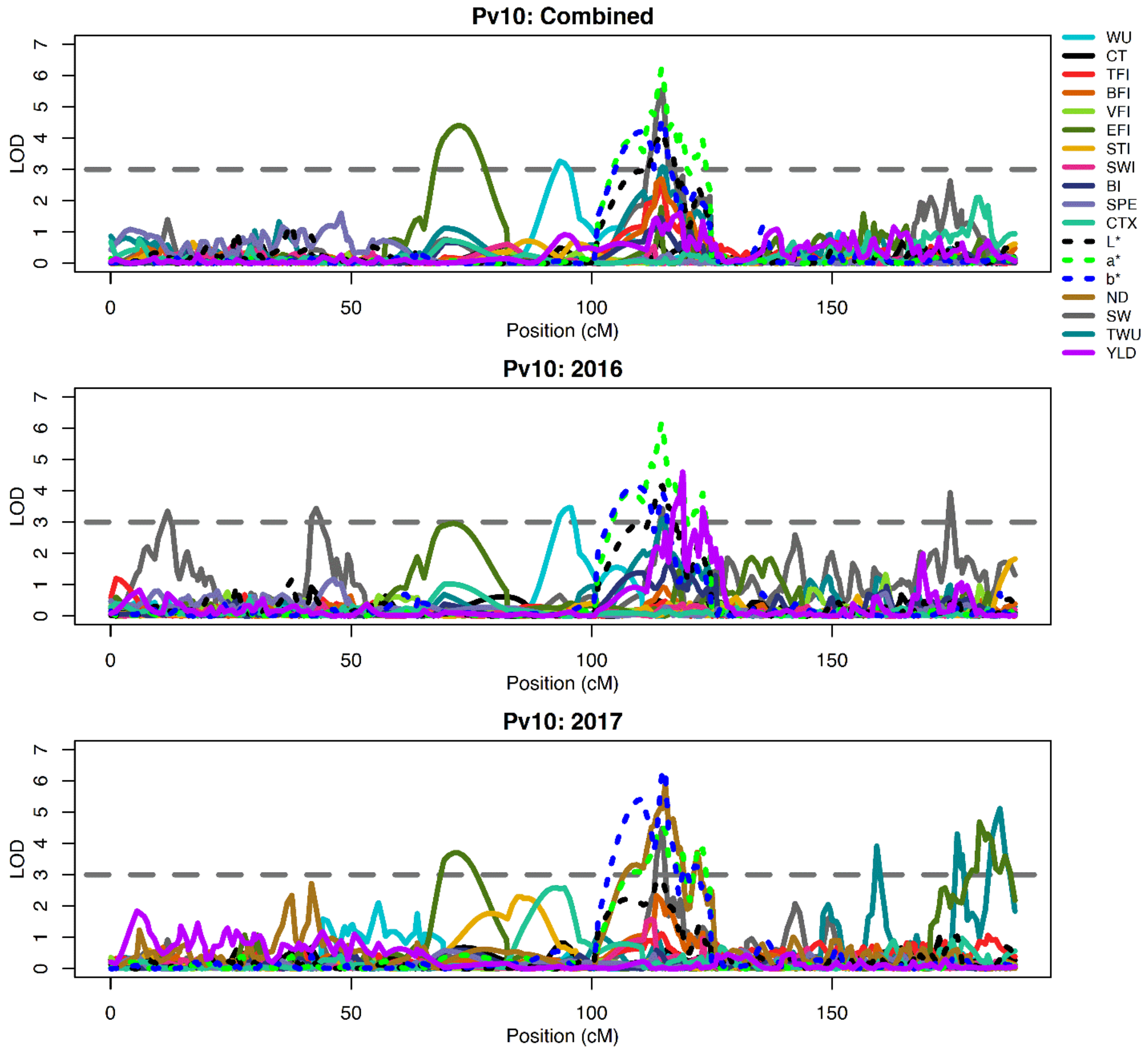
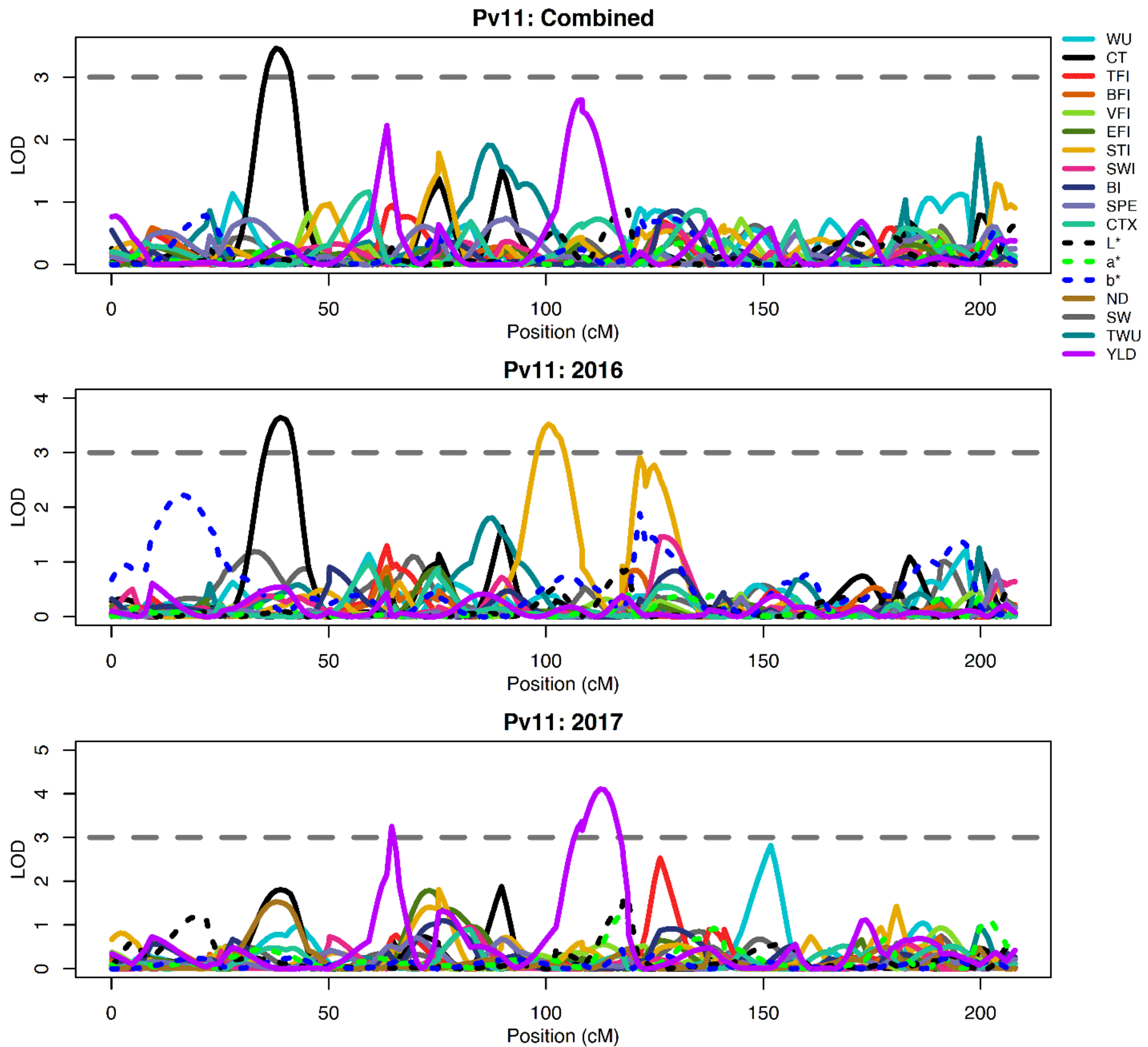


Figure S3.13 Line graphs of LOD by Pv11 position for both years combined, 2016, and 2017 for soak water uptake (WU), cooking time (CT), total flavor intensity (TFI), beany intensity (BFI), vegetative intensity (VFI), earthy intensity (EFI), starchy intensity (STI), sweet intensity (SWI), bitter intensity (BI), seed coat perception (SPE), cotyledon texture (CTX), L*, a*, b*, seed coat postharvest non-darkening (ND), seed weight (SW), total water uptake (TWU), and seed yield (YLD). The gray dashed line approximates the LOD threshold (α = 0.05) for all traits.



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CHAPTER 4:

REDUCED RETORT PROCESSING TIME IMPROVES CANNING QUALITY OF FAST-COOKING DRY BEANS (*Phaseolus vulgaris* L.)

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Reduced retort processing time improves canning quality of fast-cooking dry beans (*Phaseolus vulgaris* L.)

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ABSTRACT

While it is generally accepted that fast-cooking germplasm benefits consumers, benefits to the canning industry have not been established. Genotypes with good canning quality withstand the canning process while remaining intact with good appearance, but canning protocols used by breeders typically involve long processing times that may overcook some genotypes. The goal of this study was to identify whether cooking time influences canning quality in dry beans and whether reducing processing time could improve canning quality of fast-cooking genotypes. A set of 20 yellow bean genotypes including Ervilha, PI527538 and 18 derived recombinant inbred lines were selected for their varied cooking times. By comparing the genotypes processed across five retort times, differences in canning quality were identified. All genotypes performed better when processed for less time than the standard 45 min, but canning quality was highest at 10 min for fast- and medium-cooking genotypes and 15 min for slow-cooking genotypes. Cooking time was correlated positively with texture and intactness and negatively with washed-drained weights, indicating that slower cooking beans have higher canning quality. Color changed with retort processing such that longer times produced darker beans with more red and yellow. While fast-cooking beans exhibited lower canning quality at standard processing times, reduced retort

processing time allowed them to meet quality standards while still maintaining food safety. By accounting for cooking time as a component of canning quality, breeders can develop varieties that are convenient and cost efficient for preparation for both consumers and the canning industry.

INTRODUCTION

Dry beans are an affordable protein source with additional nutritional benefits, including soluble and insoluble dietary fiber, folate, and mineral content (Hornick and Weiss, 2011). In order to be edible, dry beans require hydrothermal processing to render inactive lectins and other anti-nutrients (Liener, 1979; Deshpande et al., 1984). Typically, dry beans are prepared by cooking in boiling water with or without prior soaking. The time required to cook beans can be significant, requiring hours of boiling to sufficiently soften the cotyledons. Genotype, age of the seeds, storage conditions, and moisture content all impact the ability of dry beans to take up water and cook in an acceptable amount of time (Hernandez-Unzon and Ortega-Delgado, 1989; Liu et al., 1995; Coelho et al., 2007; Cichy et al., 2015b). As a more convenient option, many consumers purchase canned beans. Canned beans are fully cooked and safe to eat without further processing, and they are more accessible to the average modern consumer with limited time for food preparation. However, some limitations to canned beans included their lack of prevalence in majority-world countries and their increased cost and negative health perceptions as compared to dry beans (Povey et al., 1998; Cichy et al., 2015b; Winham et al., 2019). While canned beans provide a partial solution to the inconvenience of long cooking times, fast-cooking beans are valuable to consumers who purchase dry beans. In addition, fast-cooking genotypes could benefit the canning industry by reducing the processing time required to prepare canned beans, resulting in energy savings (Deshpande et al., 1984).

The canning of beans is a hydrothermal process consisting of several steps that are modified depending on seed type: cleaning, soaking, blanching, filling, adding brine or sauce, sealing, and retort processing (Deshpande et al., 1984; Matella et al., 2013). Quality of canned bean products is evaluated on seed coat splitting, seed clumping, broth viscosity, extruded starch, or undesirable seed shape, color or size (Hosfield et al., 1995). Quality can be variable and is impacted by seed quality, canning protocol, and genotype (Hosfield and Uebersax, 1980; Hosfield et al., 1984; Hosfield, 1991; Ghasemlou et al., 2013; Matella et al., 2013). Retort temperature and duration are also important to prevent under- or over-cooking and to ensure safety for consumption.

Due to the impact of genotype on canning quality, some dry bean breeding programs incorporate canning quality as a selection criterion for germplasm development. A five-point scale is used by trained evaluators to indicate canning quality based on appearance, and it incorporates several factors including overall appearance, prevalence of splits, presence of clumps, viscosity of cooking broth, extent of extruded starch, and conformity of seed shape, color, and size to the relevant market class (Hosfield et al., 1995; Mendoza et al., 2017). A score of 3 for appearance is considered acceptable, although expectations vary depending on the market class. Appearance requires many trained evaluators and is difficult to rate accurately and consistently, but image analysis may be a suitable alternative for canning quality evaluation in the near future (Long et al., 2019). After being evaluated by trained panelists, samples are rinsed and weighed to determine water uptake during canning. Washed-drained weights indicate whether the samples were under- or over-hydrated following canning. Subsamples (100 g) of each washed-drained sample are evaluated for texture using peak force measurements recorded with a Kramer shear cell. Low peak force values indicate mushy, overcooked samples or those that could not withstand canning

without splitting. High peak force indicates firm, intact samples or those that are undercooked. Ideal peak force ranges from 50 to 75 kg depending on the market class (Hosfield and Uebersax, 1980). Appearance, washed-drained weights, and texture comprise the primary measurements for evaluating canning quality in a dry bean breeding program (Hosfield, 1991; Kelly and Cichy, 2012).

Dry bean breeding programs use a small-scale canning protocol that approximates industrial canning on limited sample sizes (Hosfield and Uebersax, 1980; Kelly and Cichy, 2012). Large numbers of genotypes and limited seed availability dictate that the canning protocol be standardized regarding retort time and temperature, preventing multiple processing methods from being applied to different samples. Breeding programs have generally relied on a 45 min retort time to evaluate canning quality. This practice may introduce bias against fast-cooking genotypes as they would be over-processed at 45 min and appear mushy with lower texture scores (Nordstrom and Sistrunk, 1977, 1979; Davis et al., 1980; Junek et al., 1980; Santoro et al., 2010). Cooking time measurements have not traditionally been part of the canning quality evaluation pipeline. However, if canning quality of fast-cooking lines is improved with reduced retort time, genotypes that are both fast-cooking and suitable for canning could be identified, benefitting the canning industry with reduced energy costs.

The goal of the study reported here was to characterize the relationship between cooking time and canning quality in dry beans. A set of 20 bean genotypes, including Ervilha (fast-cooking), PI527538 (slow-cooking), and 18 derived recombinant inbred lines (RILs) were processed in a stationary retort at 121 °F for 10, 15, 20, 30, and 45 min and evaluated for intactness, washed-drained weight, texture, and color. These parents and RILs were selected for their varied cooking times so that the relationship between cooking time and canning quality could be assessed

with minimal confounding genetic variation. The prevalence of splits was evaluated with other attributes of the appearance scale excluded to improve accuracy of the scores and target the trait with the largest impact on canning quality. This study explored whether canning quality of fast-cooking genotypes is improved in samples with reduced retort processing duration to determine whether cooking time should be considered as a component of canning quality.

MATERIALS AND METHODS

Germplasm

The germplasm relevant to this study consists of 18 yellow $F_{5:8}$ RILs and their parental lines Ervilha and PI527538 (Figure S4.1). Ervilha has a pale yellow Manteca seed type with a gray hilum. It was originally collected from a marketplace in Angola in 2010 and is part of the Andean Diversity Panel (ADP0512) (Cichy et al., 2015a). PI527538 is also part of the Andean Diversity Panel (ADP0468) and exhibits a greenish brown seed coat with hints of purple and a black hilum. It was collected in Burundi in 1985. Ervilha cooks faster than PI527538, and this relative difference in cooking time is stable across environments (Cichy et al., 2015b; Katuuramu et al., 2020). The RILs were developed by advancing F_2 seed via single seed descent to the F_5 generation and then bulking seeds from individual plants to form RILs. The RILs evaluated in this study are a subset from 242 lines selected for their varying cooking times (Bassett and Cichy, 2020). All RILs were evaluated for cooking time in 2017, and the nine fastest and nine slowest were selected for this study (Figure 4.1).

The genotypes were grown at the Montcalm Research Farm in MI in 2017. The soil type is Eutric Glossoboralfs (coarse-loamy, mixed) and Alfic Fragiorthods (coarse-loamy, mixed, frigid). Two row plots 4.75 m long with 0.5 m spacing between rows were arranged in a

randomized complete block design with two replications per genotype. Standard agronomic practices were followed to control biotic and abiotic stresses and ensure adequate growing conditions. Plants were hand-pulled at maturity and threshed with a plot Hege 140 plot harvester (Wintersteiger, Utah, USA). Seeds were cleaned by hand following harvest to remove field debris, off types, and damaged seed. All seeds were stored at room temperature and low humidity for 5 months following harvest.

Cooking Time Determination

Cooking times were determined using pre-soaked seeds with two replicates per genotype with automated Mattson cookers (Wang and Daun, 2005). Prior to cooking, 30 seeds per replicate were sorted into coin envelopes and equilibrated to 10-14% moisture in a humid cold room. Moisture content was checked using a moisture meter (Moisture Check Plus, Deere and Company, Moline, IL). After the desired moisture had been achieved, samples were weighed and soaked for 12 h in 250 ml distilled water in preparation for cooking. The seeds were then blotted dry and weighed to determine soaked weight. Twenty-five soaked seeds for each replicate were loaded onto 25-well Mattson cookers (Michigan State University Machine Shop, East Lansing, MI) with weighted (65 g) 2 mm diameter pins positioned in the center of each seed. Loaded Mattson cookers were placed into 4 L stainless steel beakers with 1.8 L of boiling distilled water. A low boil was maintained using a Cuisinart Countertop Burner (Cuisinart, Stamford, CT), and each replicate was cooked until 80% of the seeds were pierced completely. Cooking time was recorded, and samples were cooled for up to 10 min at room temperature and then weighed to determine cooked weight.

Canning Protocol

Each genotype was processed in duplicate across five retort times for a total of 10 samples per genotype. Prior to canning, seed moisture was increased to 14-17% moisture using a moisture

chamber. Once the seeds reached the desired moisture, 90 g dry weight of seeds per sample were placed in mesh bags and soaked for 12 h in 0.0028% $CaCl_2$ solution prior to canning. The soaked samples were placed into 300 x 407 tin cans, which were then filled with brine (1.5% sucrose, 1.25% NaCl, 0.03% $CaCl_2$). Filled cans were transported via a 5.6 m metal-tiled conveyor belt moving 2.15 cm/s through an exhaust box to facilitate water uptake and removal of air bubbles. The cans were heated to approximately 75 °C upon exiting the exhaust box, at which point they were sealed using a Dixie Double Seamer (Dixie Canner Co., Athens, GA). Cans were then placed in a Melco Steel Steam Sterilizer (Melco Steel Inc., Azusa, CA) and processed stationary at 121 °C for 10, 15, 20, 30, or 45 min. The come-up time to reach 121 °C in the retort was 15 min. All process times exceeded minimum safety requirements for the production of sterile canned bean products ($F_0 > 6$ min) (Matella et al., 2013) and destruction of anti-nutritional factors including lectins and protease inhibitors (Dhurandhar and Chang, 1990; Lajolo and Genovese, 2002; Nciri and Cho, 2018; Thompson, 2019). Following processing, cans were cooled to 40 °C via the addition of cold water to the retort. Once cooled, water was drained from the retort, and the cans were removed, dried, and left to equilibrate at room temperature for 1 week prior to opening.

Visual Evaluation

To prepare for visual evaluation, cans were opened and poured into paper food trays, with samples gently stirred and evenly distributed across each tray. Samples were randomly arranged to minimize bias. Trained reviewers then evaluated each sample using a five-point scale for intactness (1: 0-20% intact, 2: 21-40% intact, 3: 41-60% intact, 4: 61-80% intact, 5: 81-100% intact). Intactness is defined as an absence of splits. There were 14 total evaluators with a minimum of seven observations per sample due to absent evaluators during select can opening sessions.

Washed-drained Weight Determination and Image Analysis

Following visual evaluations, samples were rinsed to remove brine and randomly arranged in a large weigh boat to determined washed-drained weight. Samples were then imaged in the weigh boat for downstream image analysis (Figure S4.2). Images of canned samples were collected using the custom machine vision system described in Mendoza et al., 2017. The camera settings were as follows: manual exposure, auto focusing, lens aperture f = 5.6, shutter speed of 1/125, white balanced, ISO 100, and flash off.

A custom macro in ImageJ software developed for canned bean color analysis was used to determine CIELAB values for each sample (Bornowski, 2018). Each image was preprocessed to brighten samples and minimize reflections using a constant gamma correction and the noise reduction feature in the ImageJ software. To obtain CIELAB values, images were partitioned into L*, a*, and b* slices, and the mean value for each slice was recorded. L* measures black (0) to white (100); a* measure green (-) to red (+), and b* measures blue (-) to yellow (+).

Texture Analysis

After imaging each sample, texture was determined for two replicates of 100 g subsamples per can. The samples were evenly distributed in a 10 blade TA-91X Kramer shear cell attachment. Using a TA.XTPlus100 texture analyzer (Texture Technologies Corp., Hamilton, MA) with a 100 kg load cell, the samples were completely compressed for the 105 cm length of the Kramer shear cell at 20 mm/s. Peak force measurements were recorded in kilograms using Exponent version 6.1.4.0 (Stable Micro Systems, Godalming, UK).

Statistical Analysis

All analyses of variance (ANOVAs) in this study were conducted using the MIXED procedure in SAS version 9.4 of the SAS System for Windows (SAS Institute Inc. Cary, NC). For seed weight, water uptake, and cooking time phenotypes, the model included genotype as a fixed effect and replicate as a random effect. For intactness, washed-drained weight, texture, and CIELAB values, the model included genotype, retort time, genotype by retort time as fixed effects and replicate as a random effect. Intactness included evaluator and genotype by evaluator as random effects. Least squares estimates were reported in place of means for intactness to account for evaluator effects. Texture included subsample as a random effect. For ANOVAs within retort times, retort time and genotype by retort time were excluded from the fixed effects. Mean separation was determined using pdiff within the MIXED procedure and a Tukey multiple comparison adjustment. Pearson correlation coefficients were determined in R using the Cor function. Genotypes were separated into fast (18-20 min), medium (20-26 min), and slow (26-28 min) cooking groups determined via least squares differences to evaluate the relationship between cooking time and canning quality traits. Analyses for these groups were performed using PROC MIXED with the models including cooking group in place of genotype.

RESULTS

Cooking Time and Water Uptake

The genotypes selected for this study exhibited significant differences in seed weight, soak water uptake, and cooking time, although the parents did not differ in seed weight (Table 4.1). Significant correlations between seed weight and cooking time and between water uptake after soaking and cooking time were detected (Figure 4.2). Similar correlations have been identified in

a previous study (Cichy et al., 2015b). No significant differences in total water uptake were identified among genotypes (Table 4.1). The raw 100 seed weights of Ervilha and PI527538 were 52.84 and 47.99 g respectively. The seed weights of the 18 RILs ranged from 45.32 to 64.47 g. After the 12 h soak, water uptake of Ervilha was 114.1% and of PI527538 was 100.6%. The water uptake of the 18 RILs ranged from 98.4% to 116.4%. The cooking time for Ervilha and PI527538 were 18.8 and 26.3 min respectively. The cooking times of the RILs ranged from 18 to 28 min. The fast, medium, and slow groups were not significantly different for seed weight or total water uptake, but the fast group had a significantly higher water uptake as compared to the medium and slow groups (Figure 4.3). The fast, medium, and slow groups exhibited significant differences in their cooking times as intended when the groups were defined (Figure 4.3).

Canned Bean Intactness

Genotype, retort time, and genotype by retort time significantly affected intactness as rated by trained evaluators (Table 4.2). In addition, evaluator and genotype by evaluator were significant effects (data not shown). The least squares estimates for intactness were correlated positively with texture and negatively with retort time, washed-drained weight, L*, and b* (Figure 4.4). Cooking time was positively correlated with intactness ratings across all retort times (Figures 4.4-5, Table S4.1). The intactness ratings for Ervilha were 2.5, 2.8, 2.2, 2.3, and 2.8 and for PI527538 were 3.8, 3.4, 3.1, 3.2, and 3.0 for 10, 15, 20, 30, and 45 min processing times respectively (Table 4.3). For each retort time, the intactness ranges of the RILs are as presented in Table 4.3. Ervilha, PI527538 and the RILs showed a decrease in intactness as retort time increased, except for the 45 min retort time for Ervilha (Table 4.3). For all retort times except 45 min, Ervilha had a significantly lower intactness value than PI527538 (Table 4.3).

Cooking group and retort time for the fast, medium, and slow groups had a significant effect on intactness, but cooking group by retort time did not (Table 4.4). In addition, evaluator and cooking group by evaluator were significant effects (data not shown). The fast-cooking group had lower intactness scores than the slow-cooking group overall and across retort times (Table 4.5, Figure 4.6). In each group, a significant decline in intactness was observed as retort time increased (Figure 4.6, Table 4.6).

Washed-drained Weight

Genotype, retort time, and genotype by retort time significantly affected washed-drained weights (Table 4.2). The RILs increased in washed-drained weight as retort time increased (Table 4.3). For all retort times, Ervilha had a significantly higher washed-drained weight than PI527538 (Table 4.3). The washed-drained weights ranged from 271.1 to 280.7 g for Ervilha and 246.0 to 256.3 g for PI527538 across all retort times (Table 4.3). For each retort time, the washed-drained weight ranges of the RILs are as presented in Table 4.3. Washed-drained weight was correlated positively with retort time, L* and b* and negatively with intactness, texture, and a* (Figure 4.4). Cooking time was negatively correlated with washed-drained weight across all processing times (Figures 4.4-5, Table S4.1).

For the fast, medium, and slow groups, cooking group and retort time had a significant effect on washed-drained weight, but cooking group by retort time did not (Table 4.4). The fast group had a higher washed-drained weight as compared to the medium and slow groups for each retort time and overall (Table 4.5, Figure 4.6). In each group, a significant increase in washed-drained weights can be observed as retort time is increased (Figure 4.6, Table 4.6).

Texture Analysis

Genotype, retort time, and genotype by retort time significantly affected texture (Table 4.2). Ervilha, PI527538, and the RILs texture values decreased significantly as retort time increased (Table 4.3). For all retort times, Ervilha had a significantly lower peak force measurement than PI527538 (Table 4.3). The measurements ranged from 25.9 to 56.8 kg for Ervilha and 38.9 to 84.6 kg for PI527538 across all retort times (Table 4.3). For each retort time, the measurement ranges of the RILs are presented in Table 4.3. Texture was correlated positively with intactness and negatively with retort time, washed-drained weight, L*, and b* (Figure 4.4). Cooking time was positively correlated with texture across all processing times such that beans that take longer to cook in boiling water as determined with a Mattson cooker also have firmer texture when canned (Figures 4.4-5, Table S4.1).

Cooking group, retort time, and cooking group by retort time significantly affected texture for the fast, medium and slow groups (Table 4.4). The fast, medium, and slow groups had significantly different texture overall such that measurements increased from fast to medium to slow (Table 4.5, Figure 4.6). Within each retort time, the fast group had significantly softer texture than the medium and slow groups (Figure 4.6). The medium group had lower texture measurements than the slow group for the 10 min retort time, but otherwise was equivalent to the slow group (Figure 4.6). In each group, a significant decrease in firmness was observed as retort time increased (Figure 4.6, Table 4.6).

CIELAB Values

Genotype and retort time significantly affected L*, a*, and b*, but genotype by retort time was only significant for a* and b* (Table 4.2). For each retort time, the L*, a*, and b* ranges of the RILs are presented in Table 4.3. For Ervilha and PI527538 respectively, L* ranged from 62.4

to 66.7 and 44.3 to 48.8; a* ranged from 5.5 to 7.3 and 8.4 to 14.6, and b* ranged from 23.6 to 25.9 and 18.3 to 22.9 across all retort times. L*, a*, and b* values for Ervilha and PI527538 were significantly different such that Ervilha had higher L* and b* and lower a*. L* decreased and a* and b* increased as retort time increased for Ervilha, PI527538, and the RILs, although L* was not significantly different across retort times for Ervilha (Table 4.3).

L* was correlated positively with washed-drained weight and b* and negatively with retort time, cooking time, intactness, texture, and a*; a* was correlated positively with retort time and cooking time and negatively with washed-drained weight and L*; and b* was correlated positively with retort time, washed-drained weight, and L* and negatively with cooking time, intactness, and texture (Figure 4.4). Cooking time was correlated positively with a* and negatively with L* and b* across all retort times (Figures 4.4-5, Table S4.1). For the fast, medium and slow groups, cooking group and retort time significantly affected L*, a*, and b* and cooking group by retort time significantly affected a* and b* (Table 4.4). The fast, medium, and slow groups had significantly different CIELAB values such that the fast group had the highest L* and b* and lowest a* values and the slow group had the highest a* and lowest L* and b* values (Table 4.5, Figure 4.7). Within each retort time, the groups were distinct for L* and a* and the fast and slow groups were distinct for b* with the medium group falling in between the two other groups (Figure 4.7). In each group, a significant decrease in firmness was observed as retort time increased (Figure 4.6, Table 4.6). L* decreased and a* and b* increased as retort time increased for all groups (Table 4.6).

DISCUSSION

The genotypes in this study exhibited below-acceptable values for both intactness and

texture under normal retort processing conditions (45 min) (Tables 4.3, 4.6). However, intactness and texture improved and washed-drained weight decreased with reduced processing time, indicating that too long processing times negatively impact canning quality. While faster cooking genotypes displayed poorer canning quality overall, reducing retort time to as low as 10 min improved canning quality in faster cooking genotypes as indicated by decreased washed-drained weight and increased firmness and intactness approaching and in some cases exceeding quality standards. The 10 min retort time resulted in the best canning quality for the fast and medium cooking groups, but the slow group performed best with a 15 min retort time, as 10 min was insufficient to achieve texture within the ideal range of 50 – 75 kg. These results indicate that current small-scale canning protocols used for germplasm screening are biased toward slow cooking genotypes that can withstand longer processing times, preventing genotypes with acceptable canning quality at lower retort processing times from being identified. Energy costs from heat processing are a significant expense for canning companies (Featherstone, 2015a, 2015b), and retort time can be reduced substantially while maintaining safety of the canned product ($F_0 > 6$ min) (Matella et al., 2013). Previous research found that anti-nutritional factors including lectins and protease inhibitors can be deactivated by cooking for 10 min at 100 °C or pressure cooking for 7.5 min and that beans cooked to acceptable texture have minimal residual anti-nutrient activity (Dhurandhar and Chang, 1990; Lajolo and Genovese, 2002; Nciri and Cho, 2018; Thompson, 2019). Evaluating canning quality under shorter retort times would bias selection toward fast-cooking genotypes, which could allow the advancement of germplasm that is more convenient to prepare for consumers and requires less energy to process for canning companies.

While reduced retort processing time appears to improve canning quality in fast-cooking genotypes, it also affects the color of the canned product such that longer retort processing times

lead to darker beans exhibiting more red and yellow color. Fast-cooking time was associated with lighter (+L*) canned products exhibiting more green (-a*) and yellow (+b*) color, and this is also seen in the raw seed prior to processing (Figures S4.3-4). While darker color has previously been associated with longer cooking times (Cichy et al., 2015b), this correlation was also expected considering PI527538 is both darker and slower-cooking than Ervilha. If changes in color due to differences in retort time impact consumer preference, then color should be considered when evaluating canning quality in fast-cooking genotypes. For black beans, color is an important trait for consumer acceptance, and breeding programs use a separate scale for color to indicate degree of blackness (Cichy et al., 2014; Mendoza et al., 2017). CIELAB color measurements were recently found to be strongly correlated with the color scores from trained panelists in black beans indicating that CIELAB values are sufficient for evaluating color (Bornowski, 2018).

In order for new dry bean varieties to be successful, they must meet industry standards for canning quality (Kelly and Cichy, 2012). Canned products address the consumer need for convenience and as such are a common method of dry bean consumption in the USA. For these reasons, canning quality has been prioritized in breeding programs to the detriment of cooking time. While cooking time varies across all market classes, some market classes including yellow beans are more consistently fast-cooking, which makes them well-suited for the development of traits related to convenience. Currently, yellow beans have a low, but increasing prevalence in the USA, and it will be critical that they meet industry standards for canning quality to be successful as a market class. By prioritizing fast cooking time as an aspect of canning quality in future yellow bean varieties, they will be marketable to consumers prioritizing convenience and canning companies prioritizing low energy costs. Other market classes could also benefit from improved cooking times, but seed mixing at grain elevators could impact homogeneity of canned products if

the cooking times are not similar across genotypes. It could be worthwhile to isolate genotypes by cooking time to allow canning companies and consumers to benefit from fast-cooking times where possible, as well as allow growers to have a wider selection of varieties to choose from that meet canning quality standards when processed appropriately for their cooking time.

CONCLUSIONS

This study identified that fast-cooking genotypes benefit from shorter retort processing times, allowing for evaluation of their optimal canning quality. By reducing retort processing time, the bias toward slow-cooking beans can be mitigated, allowing future variety releases to maintain both fast-cooking time and high canning quality. This would benefit the canning industry, which may value the reduced time and energy required to prepare fast-cooking beans, as well as growers, who would have more options when selecting varieties to grow while still accounting for canning quality standards. The improved understanding gained from this research will allow dry bean breeders to better meet the needs of both consumers and the canning industry through the development of varieties that are convenient and cost efficient to prepare both in the kitchen and in the can.

ACKNOWLEDGEMENTS

This work was supported in part by funding from the U.S. Department of Agriculture, National Institute of Food and Agriculture (2017 67013 26212) and the Agricultural Research Service Projects 5050-21430-01000D (K.C.). We also thank Dr. Mohammad Siddiq and Dr. Jeff Swada for technical assistance with the retort and the Michigan State University dry bean breeding program for assistance with canning and evaluation.

APPENDICES

APPENDIX A:

CHAPTER 4 TABLES AND FIGURES

Table 4.1 Average values of seed weight, soak water uptake, cooking time, and total water uptake for Ervilha, PI527538, and RILs.

Trait	Parents		RILs		
	Ervilha	PI527538	Mean	Range	*P* value
Seed weight (g/100 seeds)	52.71[a] ± 2.3	49.72[a] ± 0.9	54.06	45.3 - 64.5	<0.0001
Soak water uptake (%)	114.05[a] ± 4.8	100.62[b] ±3.1	103.25	98.4 - 116.4	0.0017
Cooking time (min)	18.84[b] ± 0.3	26.25[a] ± 1.1	23.10	18.0 - 27.7	<0.0001
Total water uptake (%)	148.89[a] ± 11.7	159.19[a] ± 15.6	132.57	120.4 - 141.4	NS

P-values indicate the significance of the genotype effect determined via ANOVA. Mean separation between parents is indicated by superscript letter.

Table 4.2 ANOVA results indicating the significance of the fixed effects genotype, retort time, and genotype by retort time for intactness, washed-drained weight, texture, and CIELAB color.

Trait	Genotype	Retort Time	Genotype x Retort Time
Intactness	<0.0001	<0.0001	<0.0001
Washed-Drained Wt.	<0.0001	<0.0001	0.0331
Texture	<0.0001	<0.0001	<0.0001
L*	<0.0001	<0.0001	NS
a*	<0.0001	<0.0001	<0.0001
b*	<0.0001	<0.0001	<0.0001

Table 4.3 Means and ranges of intactness (1–5 scale), washed-drained weight (W-D Wt.) (g), texture measurements (kg), and CIELAB values for Ervilha, PI527538, and the RILs at the five retort processing times.

		Parents			RILs		
		Ervilha	PI527538	*P* value	Mean	Range	*P* value
	10 min	2.5^{a} ± 0.1	3.8^{a} ± 0.2	<0.0001	2.9^{a} ± 0.1	1.3 - 3.6	<0.0001
	15 min	2.8^{a} ± 0.2	3.4^{ab} ± 0.2	0.0071	2.7^{b} ± 0.1	1.2 - 3.4	<0.0001
Intactness[†]	20 min	2.2^{a} ± 0.3	3.1^{b} ± 0.3	0.0031	2.5^{bc} ± 0.1	1.1 - 3.5	<0.0001
	30 min	2.3^{a} ± 0.2	3.2^{b} ± 0.2	0.0002	2.5^{c} ± 0.1	1.2 - 3.3	<0.0001
	45 min	2.8^{a} ± 0.3	3.0^{b} ± 0.2	0.4635	2.4^{c} ± 0.2	0.8 - 3.6	<0.0001
	10 min	271.1^{a} ± 2.8	246.0^{a} ± 6.4	<0.0001	260.0^{c} ± 1.4	239.6 - 278.6	<0.0001
	15 min	280.0^{a} ± 1.7	247.8^{a} ± 1.5	<0.0001	260.2^{bc} ± 1.2	246.3 - 281.7	<0.0001
W-D Wt.	20 min	273.2^{a} ± 8.5	252.2^{a} ± 0.2	<0.0001	263.5^{ab} ± 1.3	252.0 - 281.6	0.0007
	30 min	280.0^{a} ± 4.0	255.4^{a} ± 2.1	<0.0001	264.9^{a} ± 1.0	253.3 - 283.9	<0.0001
	45 min	280.7^{a} ± 3.1	256.3^{a} ± 0.6	<0.0001	266.4^{a} ± 1.2	244.4 - 283.8	0.0033
	10 min	56.8^{a} ± 1.0	84.6^{a} ± 1.6	<0.0001	69.0^{a} ± 1.5	44.5 - 96.4	<0.0001
	15 min	40.9^{b} ± 1.2	68.7^{b} ± 1.1	<0.0001	58.3^{b} ± 1.1	37.4 - 76.4	<0.0001
Texture	20 min	32.9^{c} ± 1.0	51.9^{c} ± 0.6	<0.0001	43.8^{c} ± 0.9	28.5 - 59.1	<0.0001
	30 min	35.8^{c} ± 0.2	55.4^{c} ± 0.3	<0.0001	44.3^{c} ± 0.8	31.3 - 56.8	<0.0001
	45 min	25.9^{d} ± 1.3	38.9^{d} ± 1.4	<0.0001	32.1^{d} ± 0.5	21.3 - 41.7	<0.0001
	10 min	66.7^{a} ± 0.7	48.8^{a} ± 0.6	<0.0001	57.8^{a} ± 1.1	48.0 - 68.7	<0.0001
	15 min	66.2^{a} ± 0.4	48.0^{a} ± 0.2	<0.0001	57.2^{a} ± 1.2	47.4 - 68	<0.0001
L*	20 min	64.7^{a} ± 0.5	47.6^{ab} ± 0.3	<0.0001	56.6^{a} ± 1.1	46.8 - 67.3	<0.0001
	30 min	65.3^{a} ± 0.5	47.4^{ab} ± 0.8	<0.0001	55.4^{a} ± 1.2	44.8 - 66.7	<0.0001
	45 min	62.4^{a} ± 1.3	44.3^{b} ± 0.5	<0.0001	52.8^{b} ± 1.2	42.8 - 65.0	<0.0001
	10 min	5.5^{b} ± 0.1	8.4^{d} ± 0.0	<0.0001	6.8^{d} ± 0.2	5.0 - 8.8	<0.0001
	15 min	5.3^{b} ± 0.1	9.1^{cd} ± 0.2	<0.0001	7.3^{d} ± 0.2	4.7 - 9.9	<0.0001
a*	20 min	6.0^{b} ± 0.0	9.8^{c} ± 0.2	<0.0001	8.0^{c} ± 0.3	5.3 - 10.3	<0.0001
	30 min	6.3^{ab} ± 0.1	11.5^{b} ± 0.1	<0.0001	9.2^{b} ± 0.3	5.9 - 12.6	<0.0001
	45 min	7.3^{a} ± 0.4	14.6^{a} ± 0.0	<0.0001	9.9^{a} ± 0.4	6.2 - 13.0	<0.0001
	10 min	23.6^{b} ± 0.3	18.3^{c} ± 0.0	<0.0001	20.2^{d} ± 0.3	16.6 - 24.4	<0.0001
	15 min	23.9^{b} ± 0.0	19.4^{bc} ± 0.4	<0.0001	21.0^{d} ± 0.3	18.1 - 24.4	<0.0001
b*	20 min	24.1^{b} ± 0.3	20.5^{b} ± 0.0	<0.0001	22.2^{c} ± 0.2	20.1 - 26.2	<0.0001
	30 min	24.9^{ab} ± 0.3	22.3^{a} ± 0.4	<0.0001	23.0^{b} ± 0.2	20.4 -26.8	<0.0001
	45 min	25.9^{a} ± 0.4	22.9^{a} ± 0.1	<0.0001	23.7^{a} ± 0.2	21.1 - 26.6	<0.0001

[†] Means for Intactness are least squares estimates.

P-values indicate the least squares differences between the parents and the significance of the genotype effect determined via ANOVA. Mean separation across retort times is indicated by superscript letter.

Table 4.4 ANOVA results indicating the significance of the fixed effects cooking group, retort time, and cooking group by retort time for intactness, washed-drained weight, texture, and CIELAB color.

Trait	Cooking Group	Retort Time	Cooking Group x Retort Time
Intactness	<0.0001	<0.0001	NS
Washed-Drained Wt.	<0.0001	<0.0001	NS
Texture	<0.0001	<0.0001	<0.0001
L*	<0.0001	<0.0001	NS
a*	<0.0001	<0.0001	<0.0001
b*	<0.0001	<0.0001	0.0138

Table 4.5 Means and ranges of intactness (1–5 scale), washed-drained weight, texture measurements, and CIELAB values for the fast-, medium-, and slow-cooking groups across all retort times.

Trait	Fast		Medium		Slow	
	Mean	Range	Mean	Range	Mean	Range
Intactness[†]	$2.4^{b} \pm 0.1$	1.6 - 3.5	$2.5^{b} \pm 0.1$	0.8 - 3.6	$2.9^{a} \pm 0.1$	1.9 - 3.8
W-D Wt.	$269.6^{a} \pm 1.0$	253.6 - 283.9	$259.8^{b} \pm 0.8$	242.4 - 277.6	$258.0^{b} \pm 0.9$	239.6 - 270.5
Texture	$40.5^{c} \pm 1.0$	21.3 - 73.9	$51.7^{b} \pm 1.2$	25.5 - 96.4	$55.6^{a} \pm 1.5$	31.0 - 90.5
L*	$65.0^{a} \pm 0.3$	58.3 - 68.7	$54.0^{b} \pm 0.7$	42.8 - 67.9	$49.6^{c} \pm 0.4$	43.8 - 57.3
a*	$6.1^{c} \pm 0.1$	4.7 - 8.7	$8.5^{b} \pm 0.2$	5.0 - 12.5	$9.9^{a} \pm 0.2$	6.8 - 13.0
b*	$23.4^{a} \pm 0.2$	20.5 - 26.4	$21.7^{b} \pm 0.3$	16.6 - 26.8	$21.0^{c} \pm 0.2$	18.2 - 24.2

[†] Means for Intactness are least squares estimates.
Mean separation across cooking groups is indicated by superscript letter.

Table 4.6 Means and ranges of intactness (1–5 scale), washed-drained weight, texture measurements, and CIELAB values for the fast-, medium-, and slow-cooking groups at the five retort times.

		Fast		Medium		Slow	
		Mean	Range	Mean	Range	Mean	Range
Intactness[†]	10 min	$2.7^{a} \pm 0.2$	1.9 - 3.5	$2.8^{a} \pm 0.2$	1.3 - 3.6	$3.3^{a} \pm 0.2$	2.5 - 3.8
	15 min	$2.6^{a} \pm 0.2$	1.9 - 3.3	$2.6^{ab} \pm 0.3$	1.2 - 3.4	$2.9^{b} \pm 0.2$	1.9 - 3.4
	20 min	$2.4^{ab} \pm 0.2$	1.8 - 3.0	$2.3^{bc} \pm 0.3$	1.1 - 3.2	$2.8^{b} \pm 0.2$	2.4 - 3.5
	30 min	$2.2^{b} \pm 0.2$	1.6 - 3.2	$2.4^{abc} \pm 0.3$	1.2 - 3.2	$2.8^{b} \pm 0.2$	2.1 - 3.3
	45 min	$2.3^{ab} \pm 0.2$	1.8 - 2.8	$2.1^{c} \pm 0.3$	0.8 - 3.6	$2.7^{b} \pm 0.2$	2.2 - 3.2
W-D Wt.	10 min	$265.4^{a} \pm 2.1$	253.6 - 278.6	$253.0^{c} \pm 1.4$	242.4 - 262.5	$250.5^{b} \pm 1.8$	239.6 - 262.5
	15 min	$266.3^{a} \pm 2.2$	256.8 - 281.7	$257.8^{bc} \pm 1.7$	246.3 - 273.7	$257.3^{a} \pm 1.4$	246.3 - 263.6
	20 min	$271.9^{a} \pm 2.3$	257.0 - 281.6	$261.0^{a} \pm 1.5$	254.2 - 277.1	$258.4^{a} \pm 1.4$	252.0 - 265.7
	30 min	$271.8^{a} \pm 1.7$	263.7 - 283.9	$262.1^{a} \pm 1.1$	253.9 - 271.0	$261.7^{a} \pm 2.0$	253.3 - 270.5
	45 min	$272.4^{a} \pm 2.0$	258.6 - 283.8	$265.1^{a} \pm 1.5$	255.2 - 277.6	$262.2^{a} \pm 2.1$	244.4 - 270.5
Texture	10 min	$55.5^{a} \pm 1.5$	44.6 - 73.9	$70.3^{a} \pm 2.4$	49.4 - 96.4	$80.9^{a} \pm 1.0$	70.0 - 90.5
	15 min	$48.0^{b} \pm 1.5$	37.4 - 64.8	$61.9^{b} \pm 1.7$	41.9 - 76.4	$63.9^{b} \pm 1.0$	55.0 - 71.3
	20 min	$35.3^{c} \pm 1.1$	28.5 - 46.7	$46.6^{c} \pm 1.2$	31.2 - 59.1	$48.6^{c} \pm 0.6$	44.0 - 54.2
	30 min	$36.9^{c} \pm 0.7$	31.3 - 44.0	$46.2^{c} \pm 1.0$	35.5 - 56.8	$49.2^{c} \pm 0.9$	42.9 - 55.7
	45 min	$27.0^{d} \pm 0.5$	21.3 - 30.9	$33.4^{d} \pm 0.7$	25.5 - 41.7	$35.4^{d} \pm 0.6$	31.0 - 41.4
L*	10 min	$66.6^{a} \pm 0.5$	63.3 - 68.7	$55.8^{a} \pm 1.4$	48.7 - 67.9	$51.5^{a} \pm 0.8$	48.0 - 56.7
	15 min	$66.2^{a} \pm 0.5$	62.5 - 68.0	$55.2^{a} \pm 1.4$	47.8 - 67.3	$50.9^{a} \pm 1.0$	47.4 - 57.3
	20 min	$65.2^{a} \pm 0.3$	63.1 - 66.5	$55.0^{a} \pm 1.4$	47.2 - 67.3	$49.9^{a} \pm 0.8$	46.8 - 54.6
	30 min	$64.7^{ab} \pm 0.5$	61.0 - 66.3	$53.5^{a} \pm 1.6$	45.9 - 66.7	$50.1^{ab} \pm 1.2$	46.3 - 61.2
	45 min	$62.4^{b} \pm 0.6$	58.3 - 64.8	$50.5^{a} \pm 1.5$	42.8 - 65.0	$46.4^{b} \pm 0.6$	43.8 - 51.6
a*	10 min	$5.3^{c} \pm 0.1$	5.0 - 5.7	$6.9^{c} \pm 0.2$	5.2 - 8.7	$8.2^{d} \pm 0.2$	6.8 - 8.8
	15 min	$5.5^{bc} \pm 0.1$	4.7 - 6.2	$7.6^{c} \pm 0.3$	5.0 - 9.3	$8.6^{d} \pm 0.2$	7.3 - 9.9
	20 min	$6.0^{b} \pm 0.1$	5.6 - 6.6	$8.2^{bc} \pm 0.3$	5.3 - 10.2	$9.6^{c} \pm 0.2$	8.5 - 10.3
	30 min	$6.7^{a} \pm 0.2$	6.2 - 7.9	$9.6^{ab} \pm 0.4$	5.9 - 11.8	$10.8^{b} \pm 0.3$	7.7 - 11.7
	45 min	$7.3^{a} \pm 0.2$	6.2 - 8.7	$10.3^{a} \pm 0.4$	6.3 - 12.5	$12.0^{a} \pm 0.3$	9.8 - 13.0
b*	10 min	$22.5^{c} \pm 0.3$	20.5 - 24.0	$19.6^{c} \pm 0.6$	16.6 - 24.4	$18.9^{d} \pm 0.2$	18.2 - 20.2
	15 min	$22.7^{bc} \pm 0.3$	20.7 - 24.4	$20.7^{bc} \pm 0.4$	18.1 - 24.4	$19.9^{c} \pm 0.2$	18.9 - 21.3
	20 min	$23.2^{bc} \pm 0.3$	21.6 - 25.0	$22.1^{ab} \pm 0.4$	20.1 - 26.2	$21.2^{b} \pm 0.2$	20.4 - 23.0
	30 min	$23.9^{ab} \pm 0.4$	22.0 - 26.0	$22.8^{a} \pm 0.5$	20.4 - 26.8	$22.6^{a} \pm 0.4$	21.5 - 26.4
	45 min	$24.9^{a} \pm 0.2$	24.0 - 26.4	$23.4^{a} \pm 0.4$	21.1 - 26.6	$23.1^{a} \pm 0.2$	22.1 - 24.2

[†] Means for Intactness are least squares estimates.
Mean separation across retort times is indicated by superscript letter.

Figure 4.1 Histogram of the cooking times of Ervilha, PI527538, and the 242 RILs, determined using the Mattson cooker method following a 12 h soak. Seeds were grown at the Montcalm Research Farm in Michigan, USA in 2016. The nine fastest (in blue) and slowest cooking lines (in red) were selected for this study.

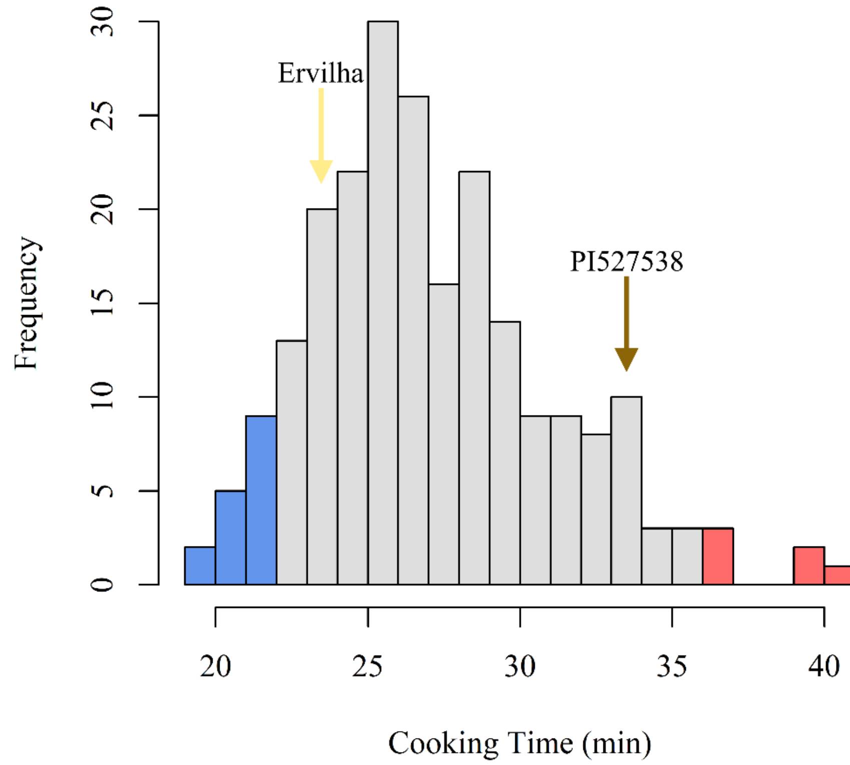


Figure 4.2 Pearson correlation matrix of seed weight, soak water uptake, cooking time, and total water uptake. Correlation coefficients are indicated in the lower left and represented by colored, directional ellipses in the upper right. *P < 0.05, **P < 0.01, ***P < 0.001.

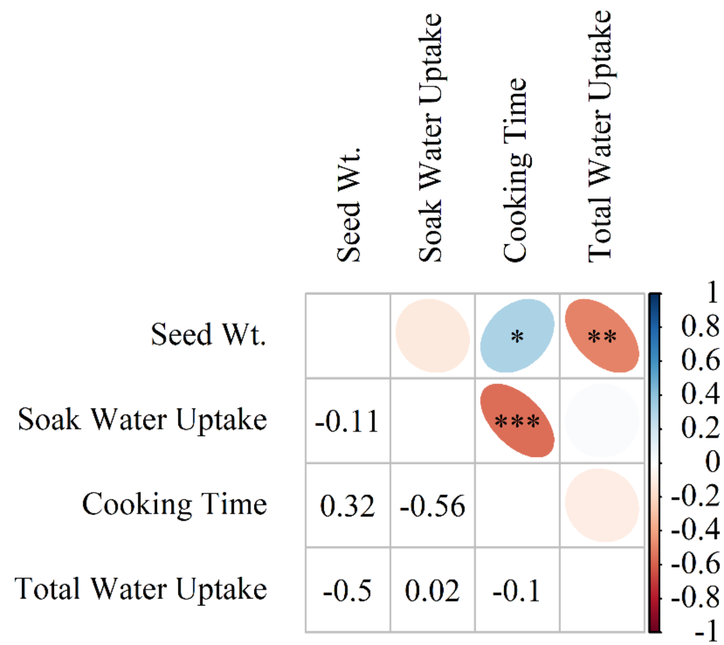


Figure 4.3 Boxplots of seed weights, soak water uptake, cooking times, and total water uptake for Ervilha, PI527538, and selected RILs separated into fast-, medium-, and slow-cooking groups. Lines indicate Ervilha (yellow) and PI527538 (brown). Mean separation is indicated by letters above each boxplot.

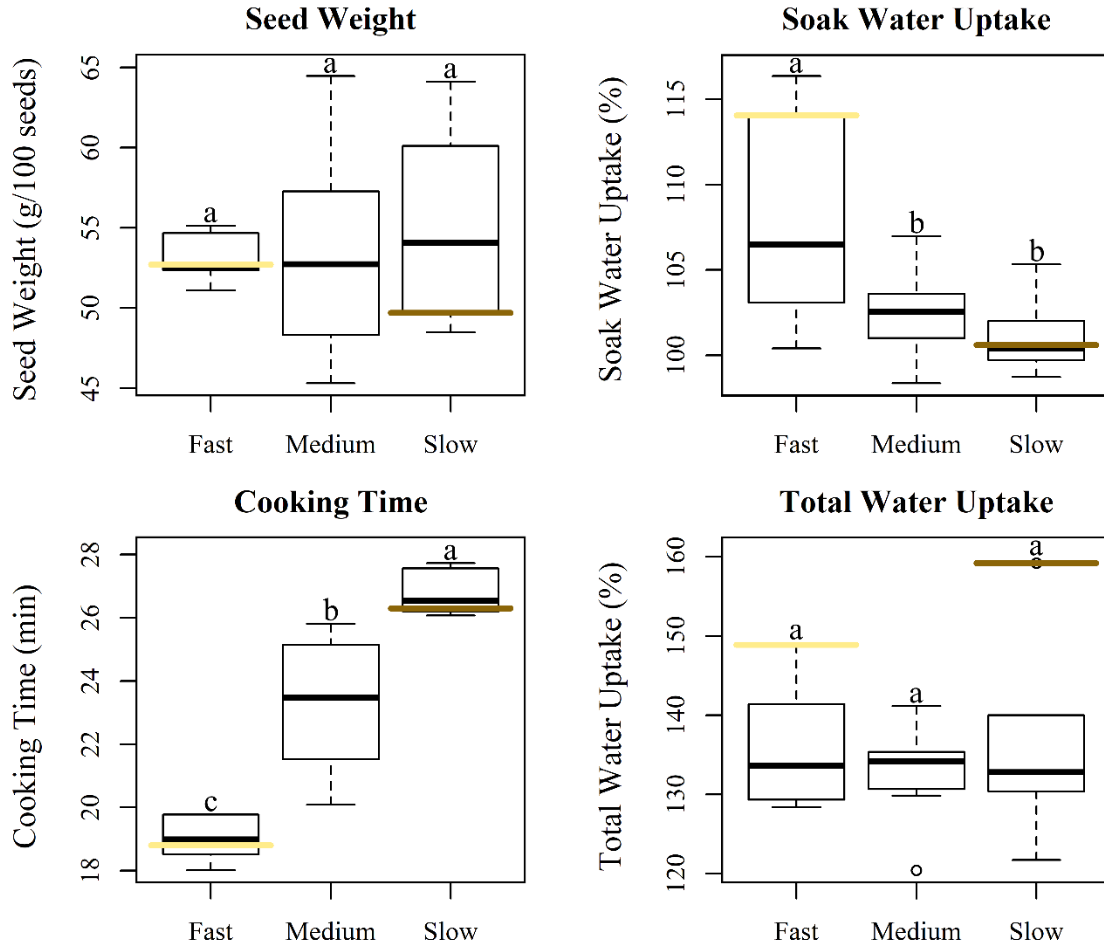


Figure 4.4 Pearson correlation matrix of retort time, cooking time, washed-drained weight, texture, intactness, and CIELAB color values across all genotypes and retort times. Correlation coefficients are indicated in the lower left and represented by colored, directional ellipses in the upper right. *P < 0.05, **P < 0.01, ***P < 0.001.

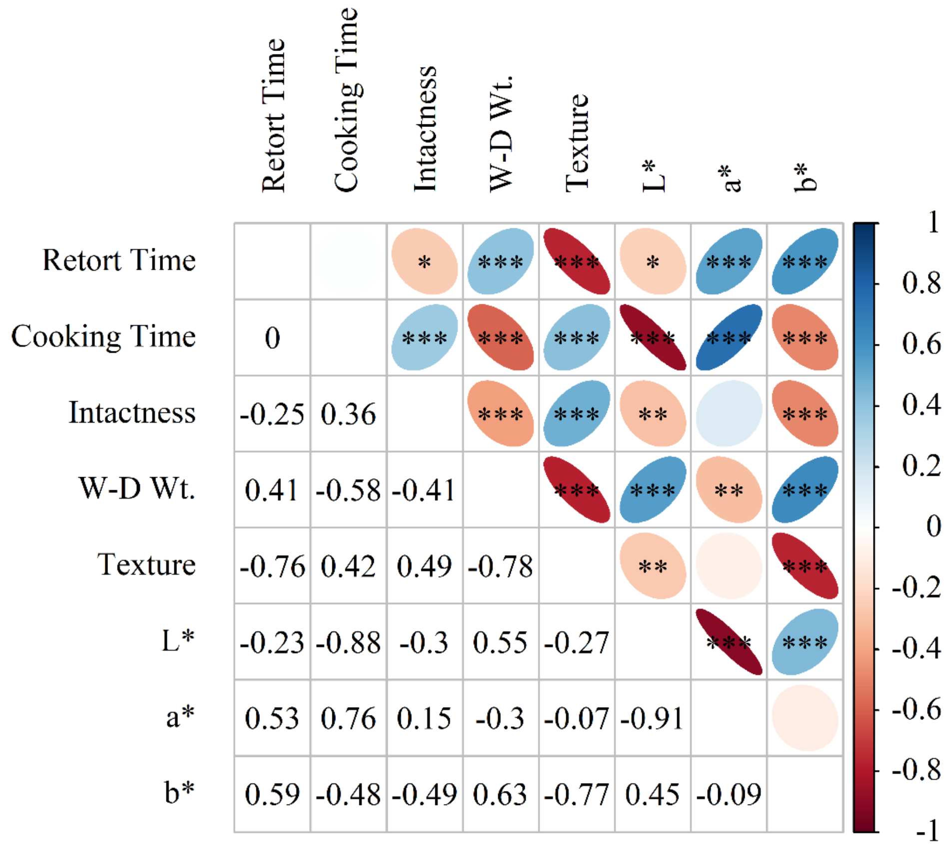


Figure 4.5 Scatterplots showing the relationship between cooking time and washed-drained weight, texture, intactness, and CIELAB color values separated by retort time. The five retort time series are indicated by colors and symbols as specified.

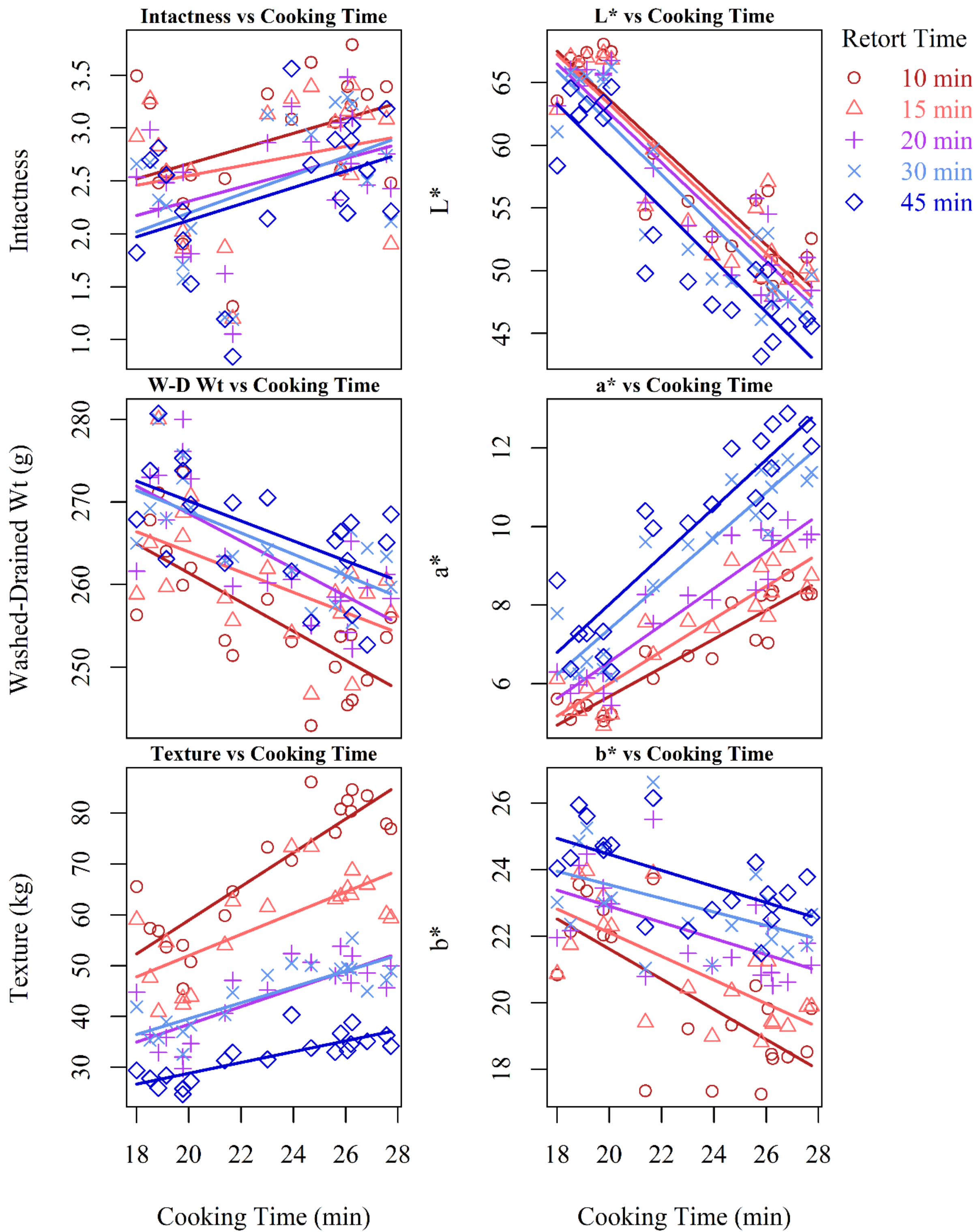


Figure 4.6 Boxplots of washed-drained weights, texture and intactness values across all retort times for Ervilha, PI527538, and selected RILs separated into fast-, medium-, and slow-cooking groups. Lines indicate Ervilha (yellow) and PI527538 (brown). Mean separation within each retort time is indicated by letters above each boxplot.

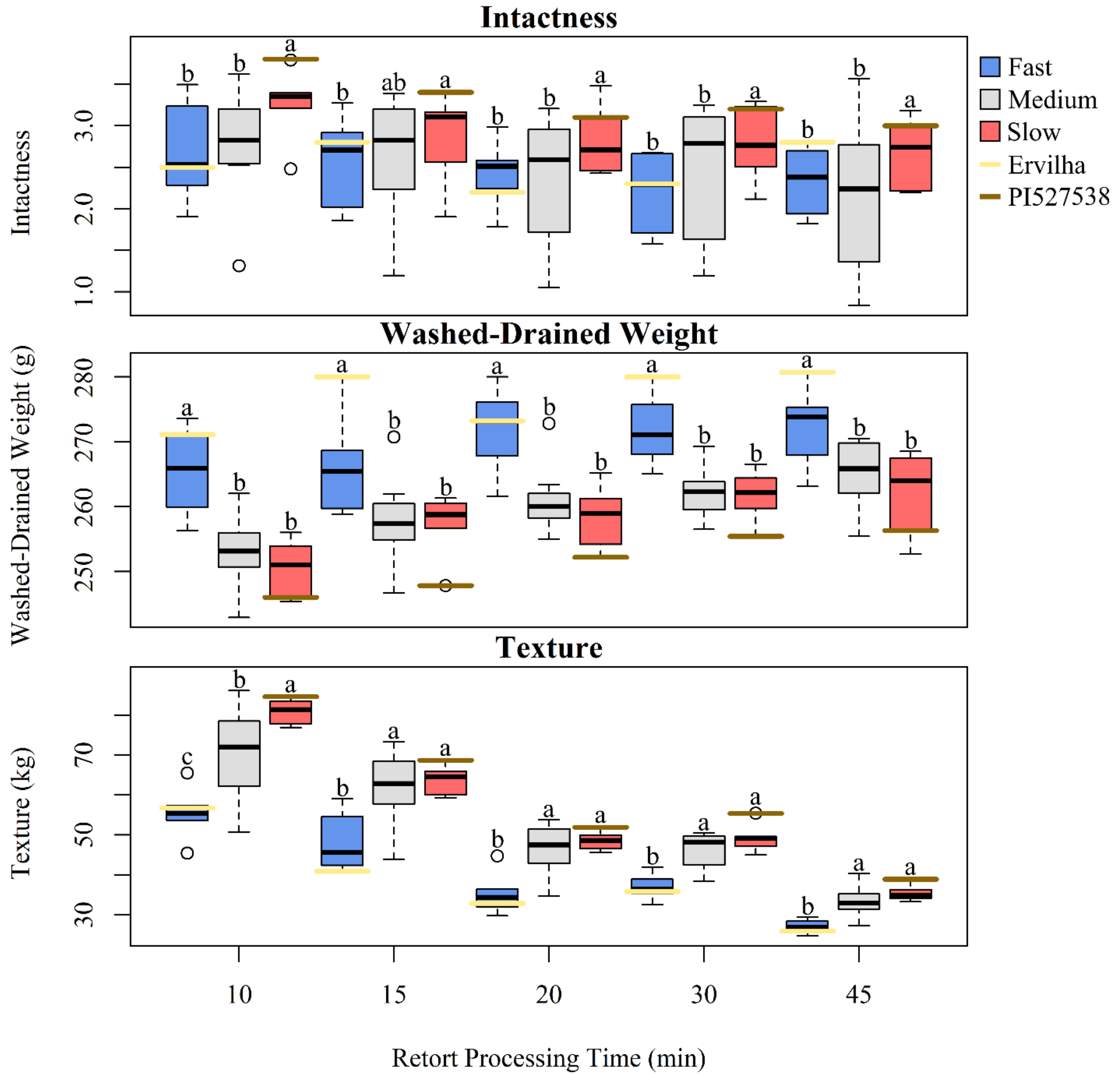
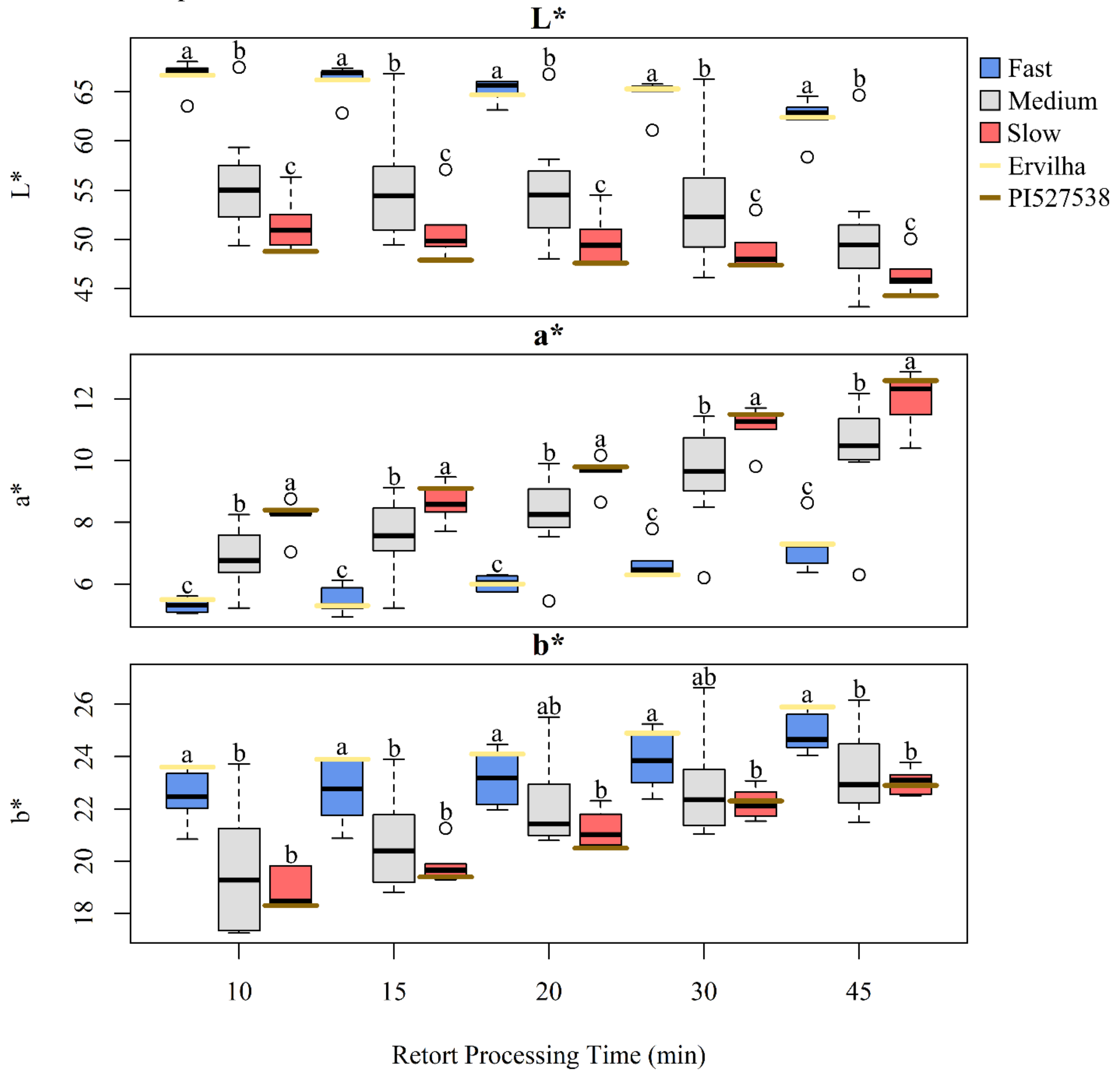


Figure 4.7 Boxplots of CIELAB color values across all retort times for Ervilha, PI527538, and selected RILs separated into fast-, medium-, and slow-cooking groups. Lines indicate Ervilha (yellow) and PI527538 (brown). Mean separation within each retort time is indicated by letters above each boxplot.



APPENDIX B:

CHAPTER 4 SUPPLEMENTAL TABLES AND FIGURES

Table S4.1 Pearson correlation coefficients and *P*-values for correlations between cooking time and washed-drained weight, texture, intactness, and CIELAB color values at the five retort times.

Trait		10 min	15 min	20 min	30 min	45 min
Intactness	r	0.39	0.25	0.39	0.46	0.39
	P-value	NS	NS	NS	0.0408	NS
Washed-Drained Wt	r	-0.71	-0.55	-0.72	-0.69	-0.59
	P-value	0.0004	0.0114	0.0003	0.0007	0.0064
Texture	r	0.87	0.71	0.78	0.83	0.81
	P-value	<0.0001	0.0005	<0.0001	<0.0001	<0.0001
L*	r	-0.90	-0.90	-0.92	-0.91	-0.90
	P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
a*	r	0.93	0.90	0.93	0.93	0.91
	P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
b*	r	-0.70	-0.70	-0.58	-0.47	-0.62
	P-value	0.0006	0.0007	0.0071	0.0378	0.0033

Figure S4.1 Images of the raw seed of Ervilha, PI527538, and the RILs selected for this study separated into fast-, medium-, and slow-cooking groups.

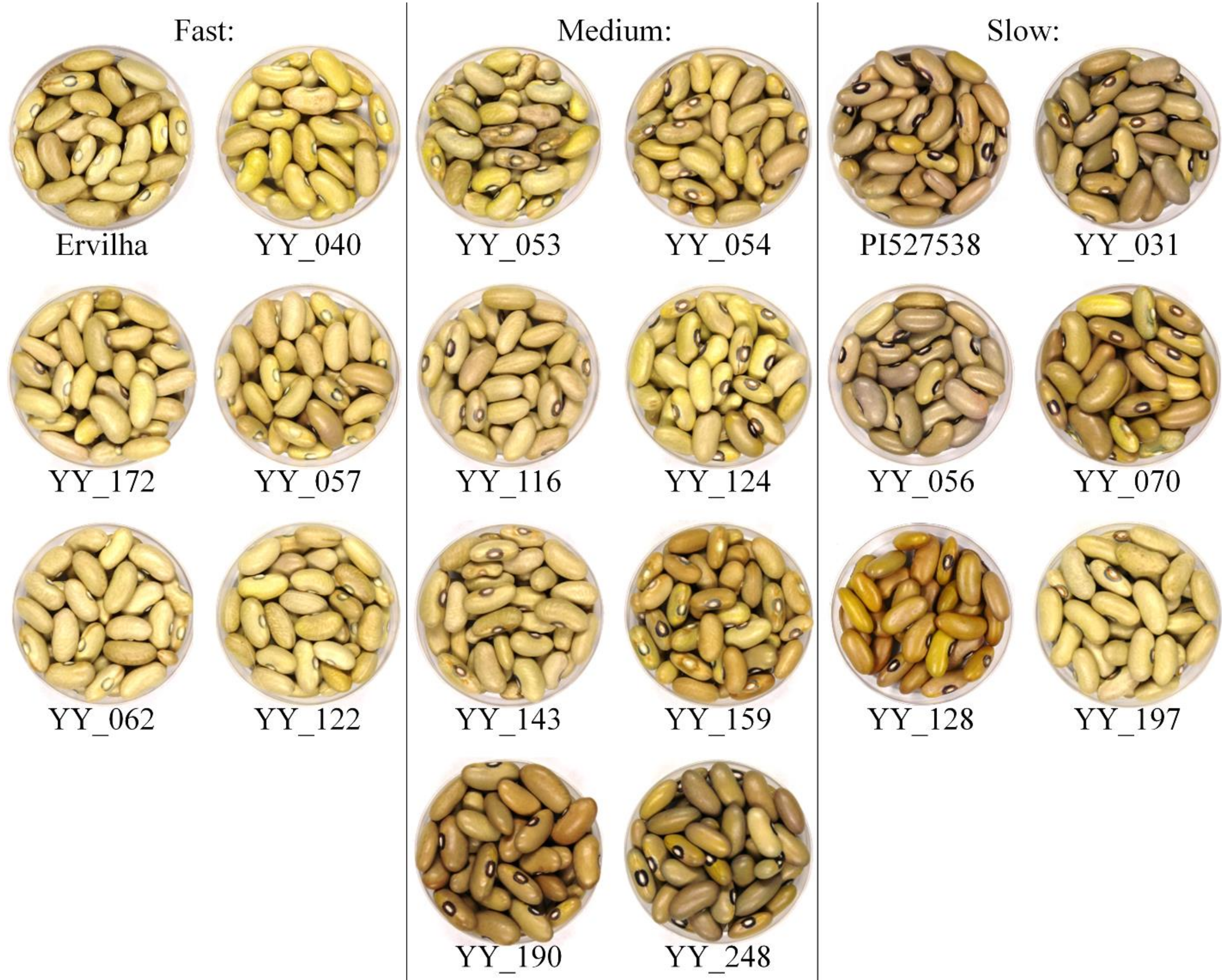


Figure S4.2 Images of the washed-drained canned samples for Ervilha and PI527538 after retort processing for 10, 15, 20, 30, and 45 minutes.

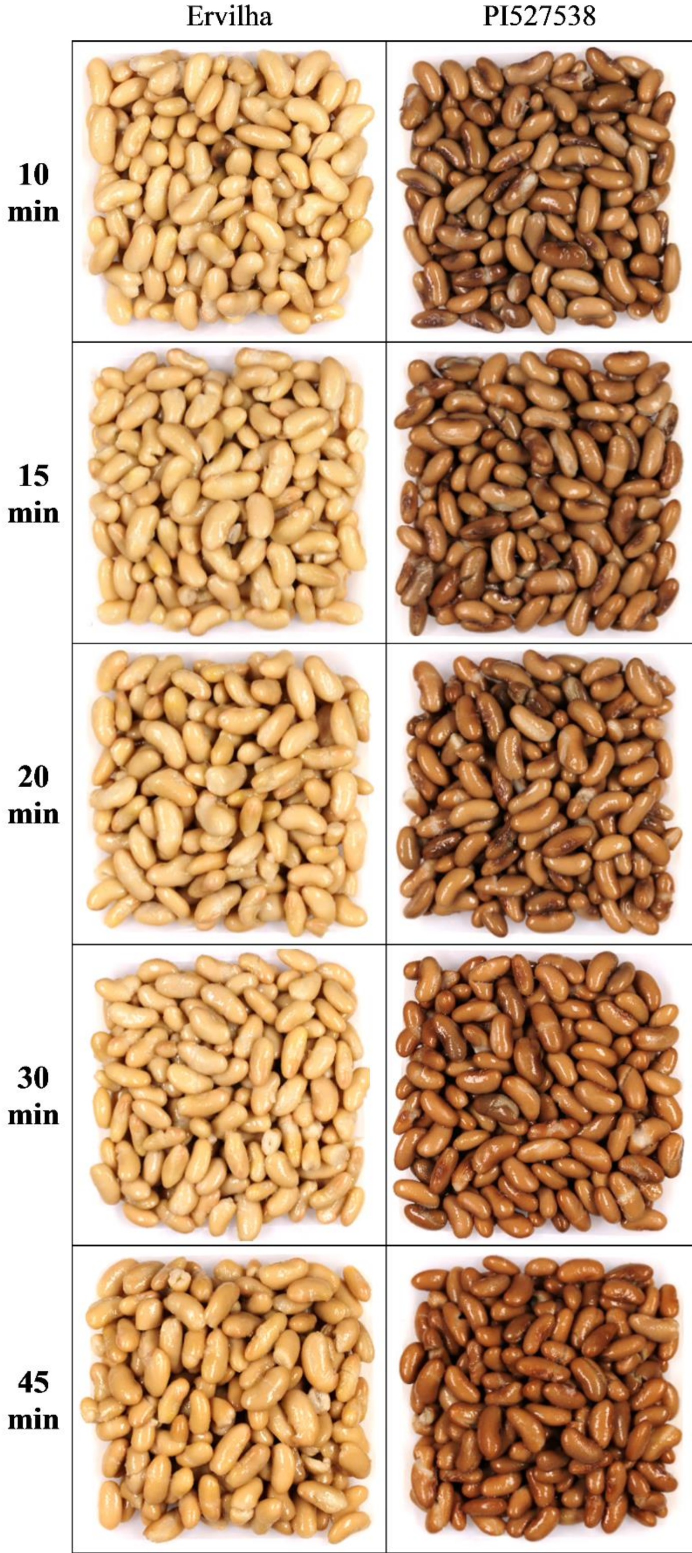


Figure S4.3 Boxplots of the CIELAB values for the raw seed of Ervilha, PI527538, and the RILs selected for this study separated into fast, medium, and slow cooking groups. Lines indicate Ervilha (yellow) and PI527538 (brown). Mean separation is indicated by the letters above each boxplot.

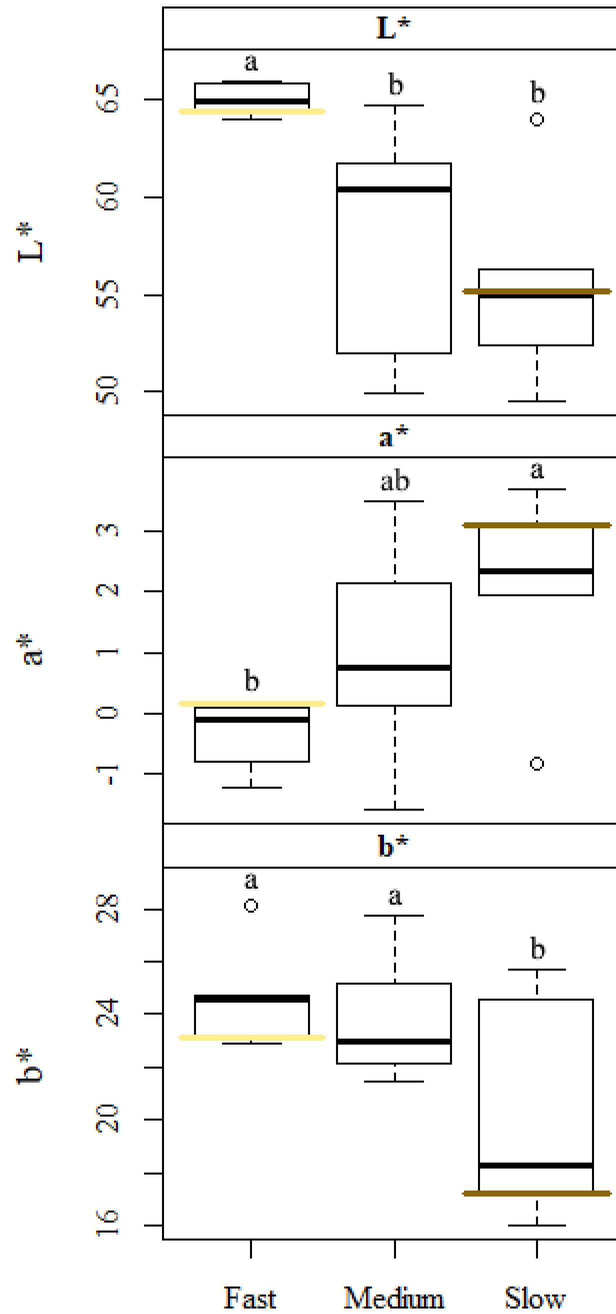
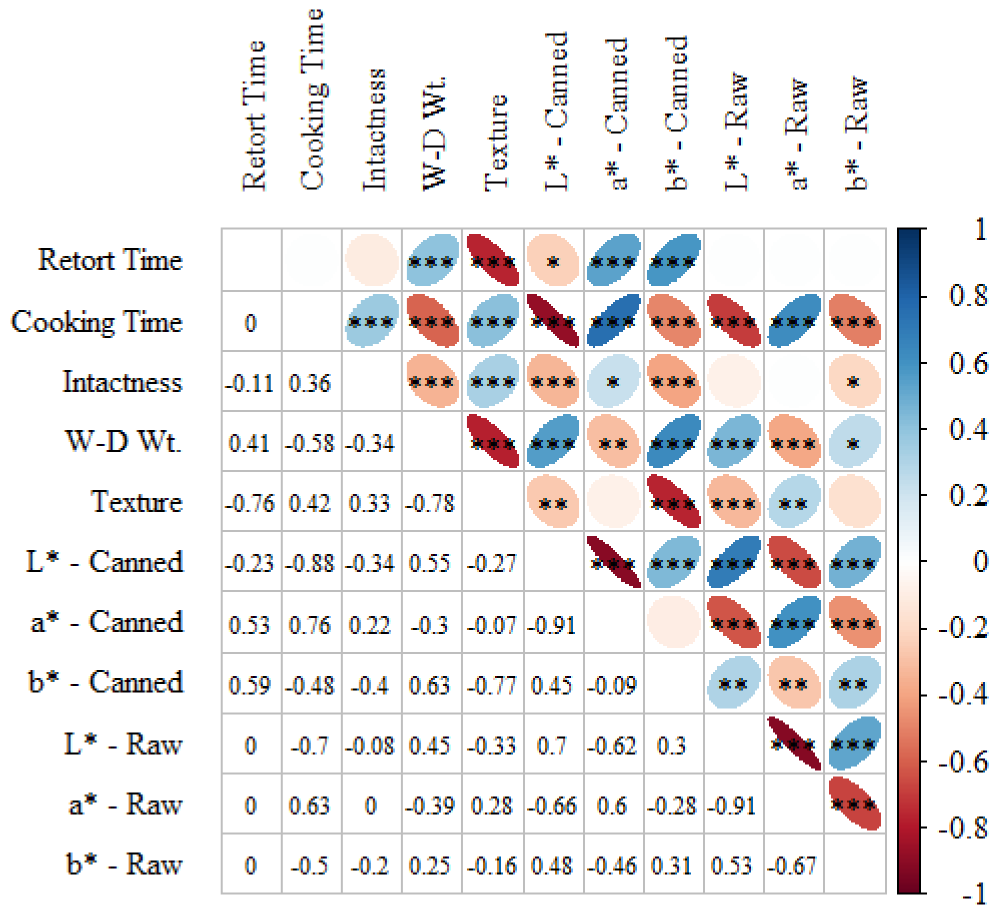


Figure S4.4 Pearson correlation matrix of retort time, cooking time, washed-drained weight, texture, intactness, and CIELAB color values (canned and raw) across all genotypes and retort times. Correlation coefficients are indicated in the lower left and represented by colored, directional ellipses in the upper right. *P*-values are indicated by asterisks, where *, **, and *** represent <0.05, <0.01, and <0.001 respectively.



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SUMMARY AND CONCLUSIONS

Cooking time, flavor, and texture are important consumer-valued traits that contribute to consumer purchasing decisions. Incorporating these traits into breeding programs will expand appeal of beans to consumers that are deterred by the long cooking times and undesirable flavor and texture present in dry beans as well as contribute to success of bean products in new markets. These studies explored genetic variability and the mechanism of cooking time as it relates to the seed coat and cell wall, identified genomic loci relevant for cooking time and sensory attributes using quantitative genetics approaches, and determined the relevance of cooking time to the canning industry.

Chapter 1 evaluated cooking time, pre-soaking time, physical traits, and cell wall and seed coat compositional traits across four seed types of dry beans. The relationships among cooking time and these attributes suggest that cooking time of unsoaked and pre-soaked beans are controlled by different mechanisms. Cooking time of pre-soaked beans was associated with seed weight, cotyledon/seed coat percent, cotyledon cell wall thickness, insoluble cell wall isolate, and total and insoluble whole seed dietary fiber. Previous studies have associated these traits with cell separation, water uptake, and water transport during cooking. Cooking time of unsoaked beans was associated with thicknesses of seed coat layers. These traits also affect water uptake and transport, but at an earlier stage in the hydration process. Both seed coat and cotyledon cell wall traits have been previously associated with cooking time, but only in the context of hardshell and the hard-to-cook phenomenon. This work revealed that genetic variability for these traits contributes to genetic variability for cooking time outside the context of textural defects. Understanding the factors associated with genetic variability for cooking time in unsoaked and

pre-soaked beans can help direct progress in breeding fast-cooking beans as well as reveal potential consequences of faster-cooking germplasm, including trade-offs like reduced fiber or seed coat integrity.

Chapter 2 lays a foundation for incorporating sensory attributes into dry bean breeding programs and contributes to the limited genetic resources available for breeding fast cooking beans. Broad ranges of sensory attribute intensities and cooking times were observed both across and within seed types, revealing a lack of uniformity within seed type, but also a wealth of genetic variability for sensory quality and cooking time. This genetic variability can be harnessed to improve cooking time in new varieties and target specific sensory profiles to be defined according to consumer preference for each seed type. Limited correlations were observed among sensory attributes and cooking time, indicating that they can combine in multiple ways with limited effort required to break undesirable linkages. The modified QDA approach used to screen materials and the significant genetic SNPs identified for flavor, texture, and cooking time could allow breeders to improve agronomic traits without sacrificing desirable sensory quality and cooking time. The set of genotypes exhibiting extreme sensory attribute intensities identified during this study can be used for panel training as well as future work exploring sensory attributes and consumer preference. Improving flavor, texture, and cooking time in dry beans can ensure they are appreciated as a delicious and tasteful component of a healthful diet in all the versatile ways consumers choose to eat them.

Chapter 3 further adds to the currently limited pool of resources available for dry bean breeders to target fast cooking time, flavor, and texture in their breeding programs. This chapter also highlights the potential for yellow beans, particularly the Manteca seed type, to deliver desirable traits to consumers in an easily identifiable package. The QTL identified can be used to

develop molecular markers for the incorporation of fast cooking time and desired sensory attribute intensities into new bean varieties. Yellow beans may appeal to USA consumers, who are seeking bean products with improved culinary characteristics and unique appearance. With the recent increased interest in plant-based proteins, now is an opportune time to address consumer preference in dry beans to remain competitive with other pulses, and yellow beans might be an ideal vehicle to a fast-cooking, flavorful, and flourishing future of dry beans.

Chapter 4 identified the relationship between cooking time and canning quality. Fast-cooking genotypes were found to benefit from shorter retort processing times, allowing for evaluation of their optimal canning quality. Current processing times used to evaluate germplasm are biased toward slow-cooking genotypes, as they overcook fast-cooking genotypes, causing reduced canning quality. By reducing retort processing time, the bias toward slow-cooking beans can be mitigated, allowing future variety releases to maintain both fast-cooking time and high canning quality. Reduced processing times could benefit the canning industry, which may value the reduced time and energy required to prepare fast-cooking beans, as energy is a major expanse for canning facilities. This may also benefit growers, as they would have more options when selecting varieties to grow while still accounting for canning quality standards. Understanding the relationship between cooking time and canning quality will allow dry bean breeders to better meet the needs of both consumers and the canning industry through the development of varieties that are convenient and cost efficient to prepare both in the kitchen and in the can.

Ultimately, this work aims to support breeders and researchers in their goals to increase bean consumption and ensure this nutritious crop is accessible to a growing global population. The genetic resources provided by this work will be useful to breeders as they develop new varieties with a focus on cooking time and sensory quality.