ELUCIDATING THE GENETIC CONTROL AND MECHANISMS OF COOKING TIME DIFFERENCES IN DRY BEAN

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ABSTRACT

ELUCIDATING THE GENETIC CONTROL AND MECHANISMS OF COOKING TIME DIFFERENCES IN DRY BEAN

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Dry bean (*Phaseolus vulgaris* L.) is a nutritious crop that is high in protein, fiber, vitamins and minerals and provides a large portion of dietary protein in the human diet. Beans require significant time to cook making them inconvenient for consumers potentially reducing the consumption rate. Considerable genetic variability exists for cooking time in *P. vulgaris*, but the underlying genetic causes of cooking time differences are largely unknown.

To elucidate the genetic control of cooking time in dry bean, two Andean bean genotypes (TZ-27 and TZ-37) from Tanzania were identified with contrasting cooking times but similar seed color and plant growth habit. The average cooking time was 72.3 min and 33.1 min for TZ-27 and TZ-37, respectively, across nine growing locations globally. Additionally, TZ-37 had a 100 dry seed weight 1.2 to 1.5 times greater than TZ-27 in all test environments. The concentration of Ca and Mn were 1.7 and 1.3 times higher in TZ-27 than in TZ-37 respectively, while TZ-37 had 1.1 times the level of phytate than TZ-27.

TZ-27 and TZ-37 were used to develop a biparental recombinant inbred line population (RIL) of 161 genotypes. The RIL population was evaluated for cooking time, hydration capacity, seed coat percentage, protein concentration, and other agronomic traits in Arusha and Morogoro in Tanzania in 2016 and 2017. Morogoro typically has higher temperatures, lower altitude, and lower soil fertility soil than Arusha. Significant variation was found for cooking time within the RIL population with a range of 21.4 min to 134.6 min across both years and locations. An environmental influence on cooking time was observed with the average cooking

time in Morogoro being 15 min longer than Arusha. Five lines were identified that cooked faster than TZ-37 showing transgressive segregation for cooking time. Seed weight, hydration capacity, and protein concentration were inversely correlated with cooking time, while seed coat percentage was directly correlated.

A quantitative trait loci (QTL) analysis was conducted using 2,427 single nucleotide polymorphism (SNP) markers discovered through genotyping by sequencing. Ten QTL were discovered for cooking time. A previously reported QTL, CT1.1, was found in Arusha in 2016 and 2017. CT3.2 was found in Arusha and Morogoro in 2017 and explained 20.5% of variation for the trait. CT6.1 was identified in both locations in 2017 and co-localized with multiple traits including hydration capacity, protein concentration, and 100 seed weight. CT10.1 was identified in Morogoro in both years. These 4 QTL, collectively, resulted in a reduction of cooking time by 16 minutes. Genes of interest within the cooking time QTL included one for polygalacturonase and another for the inhibitor of pectin methylesterase, which have previously been shown to affect cooking time. The QTL described serve as potential targets for improvement of cooking time in commercial dry bean genotypes and could be used to increase the efficiency in a breeding program by selecting for genotypes with multiple QTL for fast cooking. Developing faster cooking varieties would eliminate a major barrier for consumption. Faster cooking beans would be especially helpful in developing countries by reducing fuel burned as well as time required to gather firewood, which allows more time for other tasks.

To my husband

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LITERATURE REVIEW

INTRODUCTION

Dry beans (*Phaseolus vulgaris*) are an inexpensive and nutritious food containing large amounts of fiber, vitamins, and minerals (Drewnowski and Rehm, 2013). Beans are also rich in protein providing up to 35% of the worldwide protein intake (Broughton et al., 2003) earning the nickname "poor man's meat" in some areas (Uebersax,2006; Chianu et al., 2010). Domestication of this crop began 7,000 years ago (Gepts et al., 1986) after the species had split into two gene pools, Middle American and Andean (Gepts, 1998). From their original centers of domestication, beans were transported around the world by explorers of the new world, such as Columbus and Pizarro (Papa et al., 2006, Angioi et al., 2010). Once in Europe, beans were further disseminated by different peoples to Africa and Asia. Founder effect, preference, and performance resulted in different areas having different concentrations of the two gene pools (Papa et al., 2006, Angioi et al., 2010). Andean varieties are favored in sub-Saharan Africa and Europe, while Middle American varieties are mainly grown in China, Brazil, and Central Africa. (Angioi et al., 2010, Bellucci et al., 2014).

BEANS AS A NUTRITIOUS CROP

Dry bean, are a highly nutritious food crop. Benefits provided by dry beans include high fiber, high protein, and an abundance of vitamins and minerals. Dietary fiber levels reported in the literature range from 10.5 to 32 g per 100 g serving (Havemeir et al., 2017, Tosh and Yada, 2010). Work by Wang et al. (2010) revealed that dry beans, on average, contained 24 g 100 g^{-1} dietary fiber when cooked. In contrast, vegetables including carrots, potatoes, lettuce, spinach, and tomato, have a much lower amount of fiber with a range of 0.3 to 0.7 g 100 g^{-1} (Hanif et

al., 2006). Furthermore, fiber in dry bean was at least 1.2 times greater than that found in other pulses (Tosh and Yada, 2010).

Similarly, protein is present in large amounts in beans, and seeds have, on average, 26% protein (Guzmán-Maldonado, 2000; Singh et al., 2006). Because of the high protein content of beans, they are currently in both the vegetable and protein groups according to current dietary guidelines (U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2015). The high nutrient content of beans also makes them an ideal crop for consumption in developing countries where meat can be too costly to comprise an average meal. Out of the countries where beans contribute more than 10% of per capita total protein intake, 14 of the top 20 countries are in Africa (Akibode and Maredia, 2011). In Burundi specifically, pulses provide 55% of the total protein per capita (Akibode and Maredia, 2011). Comparing the presence of different B vitamins also suggests that beans tend to have higher concentrations compared to most other crops. Vitamins B_1 and B_2 were present at levels of 2 mg g^{-1} and 1.2 mg g^{-1} respectively in dry beans (Granito, 2002). In comparison, vitamins B_1 and B_2 in vegetables including lettuce, Swiss chard, and red mustard were present in amounts 5 to 15 times less (Santos, 2012). Minerals such as Fe and K are also present in large amounts in beans. Current dietary guidelines identify pulses as an excellent source of Fe (U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2015). Iron in beans is variable with a range of 1.6 to 9.2 mg per 100 g (Wiesinger et al., 2017, Havemeier et al., 2017, Asare-Marfo et al., 2017). Iron is especially important for adolescents, which is why some researchers are attempting to develop high Fe bioavailable lines (Wiesinger et al., 2017, Asare-Marfo et al., 2017). Dry beans contain large amounts of K with a range of 325 to 1630 mg per

100 grams (Havemeier et al., 2017, Messina, 2014, Abt et al., 2016). In contrast, banana, a fruit most would say is high in K has, 357 mg per 100 g (Abt et al., 2016).

HEALTH BENEFITS PROVIDED BY BEANS

Fiber and protein have been shown to increase satiety (Li et al., 2014), which is one reason bean consumption has been linked to a decrease in obesity as well as other nutritional benefits (Li et al., 2014, Slavin, 2005). Additional studies showed that beans or pulses in general helped decrease hunger and/or increase fullness following consumption (Clark and Duncan, 2017). Winham and Hutchins (2007) compared people who included baked beans in their diet to those who followed a diet including canned carrots instead. The people in the bean group showed lower LDL cholesterol levels, an indicator of heart health (Winham and Hutchins, 2007). Beans are also considered a low glycemic index (GI) food (any food that releases glucose slowly and steadily) and has been used in glycemic index studies. For example, Bell and Sears (2010) found that when low GI foods comprised one's diet, cardiovascular disease risks decreased. Other indicators of heart health such as lower blood pressure have been linked to pulse consumption (Viguiliouk et al., 2017, Jayalath et al., 2014). Another study focused on mortality by researching the diets of cohorts of people in different countries compared to their mortality. Daily consumption of vegetables, fruits and nuts, meat, legumes, and other foods were assessed in five different countries and only legume consumption was a statistically significant predictor of survival (Darmadi-Blackberry et al., 2004). Specifically, the researchers from this study concluded that for every 20 g increase in legume consumption per day, there was an 8% decrease in death risk (Darmadi-Blackberry et al, 2004). There are many similar studies available as this relationship has been well studied between bean consumption and health. Despite these findings, beans are not consumed in the US to the same degree as in

other parts of the world such as Latin America and Africa, (Siddiq et al., 2012). For example, as of 2013, Mexico, Brazil, and Guatemala consumed 10.4, 16.1, and 12.1 kg per capita respectively. In addition, Tanzania, Rwanda, and Uganda consume 13.0, 34.1, and 22.6 kg per capita respectively. However, the US consumes 2.8 kg of beans per capita (FAOSTAT Database, 2017). Comparing beans to potato, a crop that is quite ubiquitous in the US, reveals a startling trend. In the previously mentioned countries, potatoes are consumed about 3 times more than beans, at most, but in the US, potatoes are consumed 18 times more than beans (FAOSTAT Database, 2017). All of these studies thus far beg the question, if beans are both inexpensive and healthy, why aren't they consumed more?

INTRODUCTION TO COOKING TIME IN BEANS

Despite health benefits and distribution around the world, beans require a long time to cook. Cooking time is one of the leading factors effecting consumption of beans in many parts of the world (Chege et al., 2016; Leterme and Muuoz, 2002). This observation has driven research in cooking time of legumes for over 150 years. For example, development of the Mattson cooker which is a pivotal instrument used in cooking time measurement was first used in 1946 (Mattson, 1946). This instrument has changed little over the years except streamlining the process by decreasing the number of seeds measured from 100 to 25 and using sensors that record cooking time so that the apparatus does not require constant observation. Prior to development of the Mattson cooker, cooking times were recorded by measuring the softness of beans (measuring the pressure required to cut, smash, or pierce the seed) or by crushing beans using the finger press method (Snyder, 1936). At the time, it was noted that using the finger press method "will be open to criticism, because it is not entirely objective" (Snyder, 1936).

Cooking time is an ongoing area of research as it is an important consumer trait. The success of a variety relates not only to agronomic characteristics important to the grower, but also end-use quality traits important to the consumer. Despite the value consumers place on meal preparation time (Chege et al., 2016; Leterme and Muuoz, 2002, Winham, 2017), research into cooking time has mostly focused on external factors such as storage conditions or different soaking treatments prior to cooking. Only recently, has research into the genetics underlying cooking time differences been explored (Jacinto-Hernandez, 2003, Garcia et al., 2012, Cichy et al., 2015). Subsequently, studying the genetic cause of cooking time variation and other traits related to cooking time should yield valuable insights that could help in developing fast cooking varieties of dry bean.

HOW TO MEASURE COOKING TIME

Cooking time in legumes has been researched for over 150 years as it is an important consumer trait. Many methods have been explored to assess cooking time in legumes. One of the oldest methods involve crushing seed between the thumb and forefinger and has been used in experiments dating back to 1872 (Wood, 2016). This method is still used today, and some work has shown that this method can approach more objective methods (Kinyanjui et al., 2015). Human panels have also been used as a way to measure cooking time (Wood, 2016). Yeung et al. (2009) found that a single trained evaluator could assess doneness in beans based on mouthfeel. It was unclear based on the described methods, how the evaluator was trained. Another study used a semi-trained panel of 20 students to assess texture, taste, aroma, color, and overall acceptability in pigeon pea (Ghadge et al., 2008). Again, how the students were trained was not explained, but results from that study showed no significant differences among the criteria studied, which could mean the panel needed additional training or the preparations

of pigeon pea were similar (Ghadge et al., 2008). Another method includes using a Mattson Cooker, which uses weighted pins that push through the bean as it softens from cooking (Wood, 2016, Wang et al., 2005). The weighted pins and consistency of the weight is important as heavier pins could lead to beans being reported as faster cooking than if lighter pins were used. Each method has drawbacks as well as advantages, but each method is an attempt to obtain data regarding how long beans or other pulses require to cook.

ENVIRONMENTAL INFLUENCES ON COOKING TIME

Growing Conditions: Growing conditions and environment may also influence cooking time. The effect of soil type on cooking time was examined by Iliadis (2003). Differences between the 14 studied soil types included organic matter, K, and Ca, and all were grown in the same location to minimize environmental differences such as temperature and rainfall (Iliadis, 2003). Interesting to note was that while soil type did influence cooking time, it had little effect on the order of cooking time in the lines tested such that lines that cooked fastest in one soil type tended to be the fastest cooking in every tested soil type (Iliadis, 2003). The authors ultimately concluded that "plant breeding to create new varieties with short cooking time has greater merit than efforts to identify soil types, which can produce lentils with a short cooking time" (Iliadis, 2003). Exploring soil fertility in detail on *Pisum sativum*, Mattson et al. (1950) found that if K (a monovalent cation) was added to soil, seeds cooked in 40 minutes, which was faster than if the plants were fertilized with Mg or Ca, which are divalent cations. Environmental conditions also have been shown to effect cooking time in dry beans (Erskine et al., 1985, Proctor and Watts, 1987, Singh et al., 1990, and Coşkuner and Karababa, 2003). For example, three varieties of dry bean were grown at various locations in Uganda and evaluated for cooking time (Balamaze et al., 2008). Similarly to findings by Iliadis (2003), some varieties showed very

little cooking time variation between locations while others had a greater variation (Balamaze et al., 2008). The variety K131 grown in Lira required nearly two hours of cooking after a four-hour soak in acetate buffer (pH 4), whereas the same variety grown in Naari needed much less time to cook (45 minutes) after the same soaking treatment (Balamaze et al., 2008). Other varieties in their study varied by 10 minutes or less across the two locations.

Post-Harvest Handling and Storage: When testing the effect of storage temperature on cook time, Coelho et al. (2007) found that storing beans at a warmer temperature (29 ºC) post-harvest for 135 days lengthened cook time by 2 hours in the Andean yellow bean variety Peruano and nearly 30 minutes in the landrace Paraiso compared to seed that was freshly harvested. The relationship between cooking time and storage temperature was also shown by Maurer et al. (2004) where storage at 29 ºC for 3.5 months more than doubled the cooking time of red beans and black beans. Similarly, Aguilera and Ballivan (1987) found that storage at increasing temperatures from 8.5 ºC to 40 ºC increased hardness values overall and at a faster rate. Additionally, it was observed that seed with lower moisture content (around 8.5 to 10.4%) required less force to pierce beans than the same seed with a higher moisture content (12.4 to 13.4%) (Aguilera and Ballivan, 1987). It should be noted that the study did not directly measure cooking time, but rather, the authors measured seed hardness. Evidence that cold storage could affect cook time has been discovered. Beans stored at 29 ºC and 65% humidity followed by storage at 2 ºC and 79% humidity had faster cook times than beans only stored at 29 ºC and 65% humidity (Maurer et al., 2004). These results were similar to those previously obtained by Hentges et al. (1990), who found that storage at 29 ºC and 65% humidity for 6 months added at least 4 hours to cowpea and dry bean varieties, but then moving that same seed to storage at 6.5 ºC and 71% humidity for another 6 months shortened cooking time to 2 hours

or less. This suggests that the hard to cook defect resulting from adverse storage can be reversed to some degree. Briefly, the hard to cook defect is observed when legumes require a longer cooking time to achieve a proper tenderness, and it should be noted that hard, in this instance, does not necessarily mean hard to cook seed are harder than easy to cook seed at the raw or soaked stage of cooking preparation (Liu and Bourne, 1995). It is typically associated with prolonged storage. The hard to cook defect could be prevented from developing if recently harvested seed was thermally processed prior to storage. Thermal treatment (heated with steam or in a retort) as low as 2 to 10 minutes resulted in hardness values similar to consistent storage at 4 °C even after the treated seeds were stored at 25 °C and 70% relative humidity for 9 months (Molina, 1976). In contrast, untreated seed stored in the same conditions consistently had the highest hardness values (Molina, 1976).

INTRODUCTION OF HARD TO COOK TRAIT AND MECHANISMS

Adverse storage conditions cause molecular changes within the seed coat and cotyledons of a bean seed. Various hypotheses regarding these changes have been explored including the interaction of phytase and phytate, tannins migrating to the middle lamella, and lignification of the middle lamella. The most widely held hypothesis is the phytate/phytase hypothesis, which explains longer cooking times resulting from high temperature and high humidity conditions are caused by a reduction of phytate as the temperature increases. Phytase breaks down phytate reducing its ability to chelate the metals Ca, Zn, and Mg (Coelho et al., 2007, Njoroge et al., 2016, Njoroge et al., 2014). As a result, free ions (especially Ca) can form cross links in the middle lamella with pectin (Fig. 1.01) leading to reduced cell separation and increased cooking times (Coelho et al., 2007, Njoroge et al., 2016, Njoroge et al., 2014). Galiotou-Panayotou et al. (2008) similarly found a relationship between Ca and phytate by using increasing Ca

solutions in cooking water and measuring phytate in the seed. Seed hardness (the force required to pierce or crush a seed) in common bean and lentils increased as Ca concentration increased and phytate concentration decreased (Galiotou-Panayotou et al., 2008).

The concentration of phytate in the seed has been correlated to cooking time (Hentges et al., 1991; Del Valle et al., 1995; Coelho et al., 2007). The relationship found in those studies was an inverse correlation such that increasing amounts of phytate resulted in shorter cooking times.

Another hypothesis that has been proposed to explain the hard to cook defect in dry beans involves tannins, a component of the seed coat. Tannins are thought to effect cooking time by migrating from the seed coat to the cotyledons during the soaking and cooking processes resulting in the formation of cross links within the middle lamella (Coelho et al., 2007). This strengthens the middle lamella making it difficult for cell separation to occur leading to increased cooking times. This mechanism was suggested by Elia et al. (1997) where a high correlation of 0.8 was found between tannin content and cooking time. This result was obtained by using 15 Andean varieties and one Middle American variety as parents to generate a population that was tested for cooking time after growing in Morogoro, Tanzania in 1993 and 1994 (Elia et al., 1997). Other researchers have shown tannins do not affect cooking time (Akinyele et al., 1986).

Cooking time has also been hypothesized to be affected by lignification. Martín-Cabrejas et al. (1997) found a moderate correlation of 0.5 indicating that as the percentage of lignin increased due to prolonged storage (5 years) in adverse conditions, cooking time lengthened.

Lignifiaction was also linked to cooking of faba bean (Nasar-Abbas et al., 2008). Cooking quality (bean hardness) was measured rather than cooking time, but as the storage temperature increased from 5 °C to 50 °C bean hardness, measured after a 2 h cook, increased from 3.3 N to

15.2 N of force to puncture the seed, and percentage of lignin increased 3 fold over the same range of temperatures (Nasar-Abbas et al., 2008). The process of lignification involves polyphenols migrating to the middle lamella where enzymes such as peroxidase can use the polyphenol substrate to form lignin (Coelho, 2007). Other studies have found similar relationships between lignin content and cooking time (Hincks and Stanley, 1987; Balamaze et al., 2008). However, additional research has led to the conclusion that lignin does not influence cooking time (Rozo et al., 1990; Mujica et al., 2011). All three hypotheses suggest the same end result, which is the middle lamella is insolubilized to some degree leading to more energy required to separate cells in the cotyledons to cook the bean. It is likely that each of these play a role in the hard to cook phenomenon of beans and the genetic differences for cooking time.

ENZYMATIC CONTROL OF HARD TO COOK

Phytase has been discussed previously in this review as an enzyme that could affect cooking time, but other enzymes and their relationship to cooking time have also been studied. Pectin methylesterase (PME) is thought to affect cooking time through hydrolysis of methyl groups from pectin (Mafuleka et al., 1990). This enzyme requires at least 15 minutes at 50 °C to deactivate. As methyl groups are removed from $R\text{-}COOCH_3$, more $R\text{-}COO^-$ sites are available for Ca and other divalent cations to bind, which can lead to crosslinking of pectin meaning that an increase in PME activity could lead to longer cooking times. Martínez-Manrique (2011) found that the longest cooking bean genotype studied had the greatest PME activity. Other research has revealed similar results (Garcia et al., 1998). However, other studies have not been able to link PME activity to cooking time (Mafuleka et al., 1990, Njoroge et al., 2014, Njoroge et al., 2016). In the study done by Mafuleka et al. (1990), two landraces were assessed for PME activity in cotyledon samples with seed coats removed. A small sample size

combined with altering seed structure prior to experimentation could have affected PME activity in one or both landraces tested. Studies that look at enzymatic activity typically have small samples sizes because one or more steps are low throughput. For example, enzyme extraction typically requires large volumes of solution and time-consuming steps limiting the number of samples that can be processed at a time (Martínez-Manrique et al., 2011). Polygalacturonase further breaks down pectin following the action of PME (Vu et al., 2004). Polygalacturonase is activated during the soaking process and breaks down homogalacturonan (a component of pectin) by cleaving the bonds holding galacturonic acid molecules together, which further weakens pectin overall. Though little research has been conducted in legumes, Martínez-Manrique et al. (2011) discovered that polygalacturonase becomes more active during the soaking process (compared to raw seed) and results in a shortening of cooking time. In the three genotypes that were tested, the two genotypes with the highest polygalacturonase activity (9.6 and 7.2 nmol of galacturonic acid min^{-1} $protein^{-1}$) also cooked the fastest after soaking, but they both had similar levels of activity when raw (4.1 and 4.0 nmol of GA min^{-1} $protein^{-1}$) (Martínez-Manrique et al., 2011). Additional bean genotypes should be tested to provide more evidence of the relationship between polygalacturonase and cooking time.

Peroxidase has also been linked to cooking time by its role in lignification. Peroxidase produces H_2O_2, which is one of many reactive oxygen species. Hydrogen peroxide then acts as an oxidant joining monomers of lignin together (Lewis and Yamamoto, 1990). By repeating the process, lignin polymers are formed (Lewis and Yamamoto, 1990). Research in this area has also produced mixed results with some researchers finding evidence for a link between longer cooking time and greater peroxidase activity, while others found evidence against a causal relationship such as how inactivating peroxidase did not allow for seed softening (Liu

and Bourne, 1995). Though there is not a clear consensus on which enzymes, if any, affect cooking time, it is still an important area of research into cooking time as there is agreement that in most cases, soaking shortens cooking time making it possible for some enzymatic process to occur that would soften beans while soaking. Also, because different studies use different varieties, contrasting results could be caused by underlying genetic differences between varieties.

HARD TO COOK PHYSIOLOGICAL CHANGES

The hard-to-cook defect has been reported in beans as changes that occur to the bean that prolong cooking time, usually in reference to storage. Changes leading to the hard to cook defect including lignification and phytate-Ca binding have been explored in the review above. A change in protein composition has been noted in hard to cook lines with proteins of size 30 and 31 kDa present in larger amounts in hard to cook lines compared to those that are easy to cook (Parmar et al., 2017). Additional work would need to be done to discover the specific proteins present in that size range. Differences in starch have been reported where larger quantities of large starch granules are present in lines requiring longer cooking times compared to those that cook quickly (Parmar et al., 2017). Similarly, micrographs of starch granules appeared larger as they were clumped together with pieces of the cell wall when soaked in $CaCl_2$ (inducing the hard to cook defect), compared to the same variety soaked in distilled water (Njoroge et al., 2016). The clumped granules and cell wall fragments indicated that a $CaCl_2$ soak resulted in reduced cell separation. In addition, Shomer et al. (1990) found smaller starch granules, a lack of cell adhesion, and separation of primary and secondary cell walls in easy to cook seed when compared to hard to cook seed (Fig. 1.02).

COOKING TIME AS RELATED TO COOKING METHOD

Soaking treatments can dramatically shorten or lengthen cooking time based on the solutes added to the soaking solution. Bivalent cations such as Ca and Mg can form cross-links with pectin in the middle lamella resulting in insoluble pectates that reduce cell separation and lengthen cooking time (Fig. 1.01) (Caffall and Mohnen, 2009; Njoroge et al., 2016; Mattson, 1946; and Kinyanjui et al., 2015). In contrast, soaking in a solution containing monovalent cations such as Na and K can significantly shorten cooking time (Mattson, 1946; Kinyanjui et al., 2015; Njoroge et al., 2016). To illustrate this point, Njoroge et al. (2016) soaked Canadian Wonder beans in distilled water, Na_2CO_3, and $CaCl_2$ solutions. The 95% cooking time in distilled water was 97 min, while use of NaCl reduced cooking time down to 23 min. On the other hand, the authors did not obtain a cooking time for beans soaked in $CaCl_2$ because they ended the experiment after 300 minutes of cooking. This trend is also observed in other legumes. In yam bean and cowpea, soaking with NaCl shortened cooking times by 20 and 10 min respectively, while soaking in $CaCl_2$ lengthened cooking time by 60 min in yam bean and 100 min in cowpea (Onwuka and Okala, 2003).

Acidic and basic solutions can also affect cooking time. Starting from a neutral pH, as the solution becomes more acidic, cooking time increases until very low pH (under 4) at which point cooking time begins to decrease again (Mattson, 1946; Kinyanjui et al., 2015). As the solution becomes basic beyond a pH of 7, cooking time decreases (Mattson, 1946; Kinyanjui et al., 2015). Different hypotheses have been developed to explain this property of cooking time including that alkaline pH precipitate Ca and Mg, break down proteins and pectin, and favor beta elimination of pectin (Mattson, 1946; Potter and Hotchkiss, 1973; and Sila et al., 2006).

At more acidic pH, it is thought that the Ca pectin complex is broken down more readily (Mattson, 1946).

SEED COMPOSITIONAL VARIABILITY AND COOKING TIME

Seed Size

A relationship between seed size and cooking time is frequently reported in the literature. Specifically, seed weight is used as seed size, and results often show that smaller and lighter seed cook faster than larger, heavier seed (Cichy et al., 2015, Black et al., 1998, Bressani et al., 1988). The reasoning behind this trend is logical in that water and heat must travel a smaller distance to reach the center of the seed if the seed is small (Wood, 2016). But not all research into this area has noted the same trend. Four kidney bean cultivars were measured for a variety of traits including seed size, but no correlation for any measurement of seed size was found with cooking time (Wani et al., 2014).

Seed Coat Percentage

Cooking time could be influenced by the thickness of the seed coat by impeding movement of water into the seed as well as inhibiting the movement of heat into the seed. Seed coat thickness is directly measured via microscopy or indirectly measured using seed coat percentage, which is the mass of the seed coat as a percentage of the total mass of the seed. Giani and Okwechime (1993) found no relationship between seed coat percentage and cooking time as did Pirhayati et al. (2011). In pea, a thin seed coat was directly related to hydration capacity as well as cooking time (Wang et al., 2003), and this trend was also observed in chickpea (Avola and Patanè, 2010). In addition, a correlation of 0.97 was found in beans such that seed with a thicker seed coat required longer cooking times (Santos et al., 2016).

Protein Concentration

The relationship between protein concentration and cooking time is of great interest because a link has been proposed in the literature that longer cooking times means greater leaching of nutrients and anti-nutrients. (Mubarak, 2005). Because of the abundance of proteins in beans, it is important to see if a link exists between proteins and cooking time. In mung beans, a high correlation between protein concentration and cooking time was observed such that as cooking time decreased, protein concentration increased (Afzal et al., 2003). In dry bean, a study across 4 market classes and 12 lines revealed a moderate inverse correlation between retention of protein after cooking and cooking time (-0.5) (Wiesinger, 2016). In the same study, an inverse correlation of -0.5 was found between protein concentration and cooking time (Wiesinger, 2016). Shimelis and Rakshit (2005) also found that varieties that cooked faster tended to have a higher protein concentration as well. No significant relationship between protein concentration and cooking time was discovered in another study of cowpea (Giami and Okwechime, 1993) or dry bean (Elia et al., 1997). A study of multiple legumes showed that chickpea, faba bean, and white kidney beans all lost similar amounts of protein even though each required different cooking times to render them palatable (Güzel and Sayar, 2012).

Starch Content

Resistant starch (a component of dietary fiber) has been explored in beans as legumes contain high amounts of fiber. The role of fiber in cooking time is important as components of the cell wall including pectin have been implicated in effecting cooking time and are part of dietary fiber. A specific component of dietary fiber, soluble dietary fiber, was shown to influence cooking time (Hooper et al., 2016). Elapsed cooking time and analysis of dietary fiber in eight genotypes, comprising 4 market classes, was measured (Hooper et al., 2016). Soluble dietary

fiber was present at a greater percentage in the fast cooking lines and lower in the slow cooking lines (Hooper et al., 2016). Shimelis and Rakshit (2005) did not find a relationship between cooking time and total fiber content, but in this study, total fiber was not further parsed into soluble and insoluble fiber meaning differences could exist if those lines were analyzed for soluble fiber.

GENETIC CONTROL OF COOKING TIME

Limited research has been conducted to investigate underlying genetic variation in relation to cooking time differences in dry bean genotypes. Two studies by Elia et al. (1997, 2002) found nearly identical narrow sense heritability values for cooking time, which in both studies were around 0.9 suggesting a very strong genetic component to cooking time. In addition to heritability analyses, some research has been done on marker analyses related to cooking time. One such study used random amplified polymorphic DNA markers (RAPDs). Jacinto-Hernandez et al. (2003) found a single RAPD associated with shorter cooking time in dry bean they named UNAM 16. A more recent study used microsatellites to discover QTL related to cooking time. Few loci were found in this study similar to the work by Jacinto-Hernandez et al. (2003, Garcia et al., 2012). QTL were found on chromosome 1 and 9 though most were only discovered for one generation or one location of the study (Garcia et al., 2012). CT1.1, a promising QTL discovered on chromosome Pv01 was found in two successive generations and explained up to 21% of the variation in cooking time (Garcia et al., 2012). A GWAS study that examined over 200 accessions found multiple SNPs on chromosomes Pv02, Pv03, and Pv06 associated with cooking time (Cichy et al., 2015). SNPs on chromosome Pv06 explained 8.7% of variation for cooking time (Cichy et al., 2015). These two studies, taken together, reveal that chromosomes 1, 2, 3, 6, and 9 should be explored further for effects on cooking time.

COOKING BEANS AROUND THE WORLD

In developing countries, especially in East Africa, legumes are a dietary staple (Bouchenak and Lamri-Senhadjii, 2013; Taiwo et al., 1998, Uebersax, 2006). In addition, countries in Africa primarily use firewood as a fuel source for cooking (Hoffman et al., 2015; Munalula and Meincken, 2009; Adkins et al., 2012, Uebersax, 2006). Pollutants released into the air, time spent gathering wood, and incidence of disease are all effected by the length of time that meals take to cook.

In East Africa, women are mainly responsible for cooking as well as gathering firewood. Each week, on average, 6 hours are spent traveling 12.9 km to collect firewood (Adkins et al., 2012). Harvesting and burning of firewood on a weekly basis not only increases pollutants such as CO, CO_2, and particulates, but also results in local deforestation that typically would have a mitigating effect on pollution (Edwards et al., 2004). According to the WHO, 2 million tons of biomass are burned each day (Rehfuess and World Health Organization, 2006). As women and children are often responsible for cooking in these countries (spending an average of 25.6 h per week on this task alone), they tend to have a higher exposure to these pollutants and, thus, are more likely to have health issues (Wang and Daun, 2005, Saksena et al., 1992, Smith et al., 2000). Diseases from indoor cooking range from direct effects such as inflamed airways to indirect effects such as exacerbating a pre-existing illness like tuberculosis, which lead to over 1.5 million deaths per year from indoor smoke related causes (World Health Organization, 2006).

Production of faster cooking beans will decrease time spent cooking as well as time spent gathering firewood. Time gained could then be devoted to additional childcare or income-generating activities (Uebersax, 2006). In addition, less wood would need to be burned

lessening damage to local and global air quality as well as decreasing health risks to women and children who cook. It has been estimated 1.3 kg of wood per cooking session would be conserved if cooking time in beans could be reduced by 15 minutes, and that would equate to 150,000 metric tonnes of wood being conserved for Rwanda alone annually (Shellie-Dessert and Hosfield, 1990).

CONCLUSION

Research in cooking time of dry bean has sought to identify what mechanisms can result in beans being easy or hard to cook. Most work to date has focused on storage conditions or soaking treatments. What exactly causes the hard to cook mechanism is still not fully understood as conflicting results can be found in the literature. These conflicts could be due, in part to the *P. vulgaris* variety, which already includes two genepools, 7 races, and numerous market classes. Differences could also be due to environmental differences such as heat, rainfall, or soil composition, and many other factors could be at play as well. Despite this, some agreement is found in phenomena such as how soaking in distilled water or NaCl reduces cooking time, while soaking in $CaCl_2$ lengthens it. Agreement can also be found in the ultimate reason cooking time is lengthened in dry bean, which is that pectin is affected in some fashion leading to decreased cell separation. The methods leading to a change of pectin differ by research group, but the three main methods proposed are phytase/pectate, tannins, and lignification. Some researchers have tried to minimize some of these factors by examining cooking time differences within one market class, which was what was done in the current study. By minimizing differences in the population being examined, the likelihood of existing differences being related to cooking time increases.

Genetic studies have shown promise in beginning to identify underlying genetic causes to cooking time. High heritabilities around 0.9, and a minimal number of QTL discovered to date suggest that a better understanding of what areas of the genome control cooking time is achievable.

Though much work has been accomplished in understanding cooking time in dry bean in the last 100+ years, the next big leap in this area will be when a better understanding of the genetic cause of cooking time differences is achieved. To accomplish that objective was a goal of the current research.

APPENDIX

APPENDIX

Chapter 1 Figures

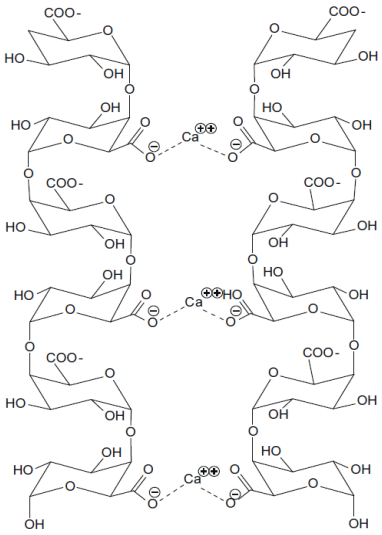

Figure 1.01. The egg box model of Ca cross linking in pectic polysaccharides (Caffall and Mohnen, 2009)**.**

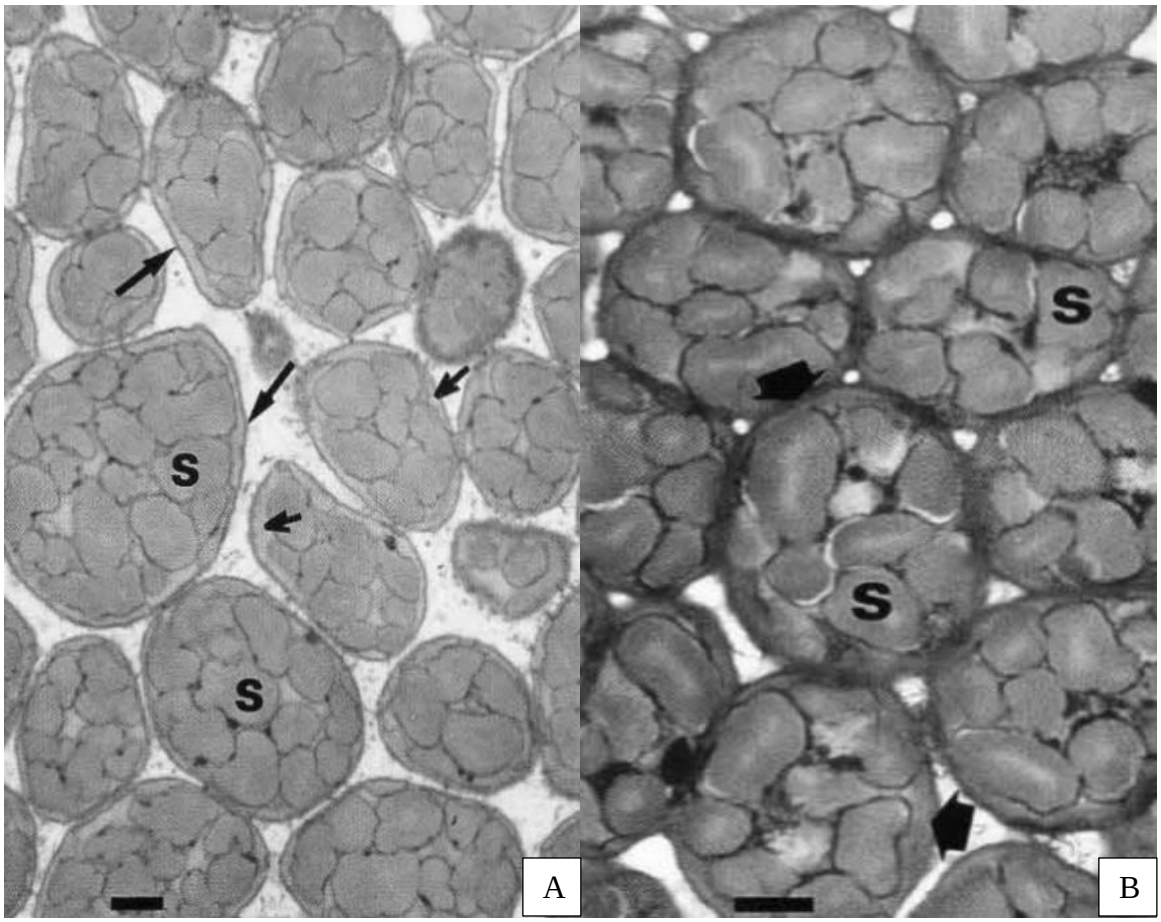

Figure 1.02. Light micrographs of bean cotyledon cross sections of easy to cook (A) **and hard to cook** (B) **bean.** S = starch. Pointed arrows indicate primary cell walls and blunt arrows indicate secondary cell walls (Shomer et al., 1990).

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CHAPTER 2

ASSESSMENT OF PHENOTYPIC STABILITY AND UNDERSTANDING THE MECHANISMS BEHIND THE COOKING TIME TRAIT IN *PHASEOLUS VULGARIS*.

ABSTRACT

Dry beans (*Phaseolus vulgaris*) are an inexpensive and nutritious food containing high amounts of protein, fiber, vitamins, and minerals. However, beans require long cooking times to render them palatable. An understanding of the genetics involved in cooking time would be beneficial as this trait is important to consumer quality. Two Andean bean genotypes of Tanzanian origin, 'Incomparable' (TZ-27) and 'WG616488' (TZ-37) were evaluated for cooking time in nine different environments and under three soaking treatments. TZ-37 cooked in 33.14 min following a 12 h soaking in DI water prior to cooking whereas TZ-27 cooked in 72.29 min under the same conditions. The exception is that when these two genotypes were cooked without a pre-soaking step (unsoaked) they had similar cooking times of 103.28 and 109.28 min respectively. Nutrient content, enzyme activity, hydration capacity, cellular structures, and different cooking methods were explored to understand the possible mechanism behind differences in soaked bean cooking time. Hydration capacity and cooking without a prior soaking step were not significant between TZ-27 and TZ-37. However, phytate was significantly lower in TZ-27 (12.5 mg g^{-1}) than in TZ-37 (13.7 mg g^{-1}), and the percentage of Ca was double that of TZ-37. Protein was also 7% higher in TZ-37 than TZ-27, but pectin methylesterase activity was lower in TZ-37. These findings suggest that TZ-27 has a diminished capacity to chelate Ca due to its reduced phytate levels, and the greater activity of pectin methylesterase leaves more binding sites within pectin for Ca cross-linking to occur. These results can explain the increased cooking time seen in TZ-27 compared to TZ-37.

INTRODUCTION

Dry bean (*Phaseolus vulgaris*) is one of the most nutritious and inexpensive crops available (Drewnowski and Rehm, 2013). Dry bean offers more fiber and protein than many other commonly consumed crops (Wang et al., 2010, Guzmán-Maldonado et al., 2000, Granito et al., 2002), and beans are the main source of protein in many parts of the world including developing countries such as areas in Africa, as well as South and Central America (Hanif et al., 2006, Bouchenak and Lamri-Senhadji, 2013, and Akibode and Maredia, 2011). Preparing beans for consumption requires a long cooking time and this is one of the main barriers to increased bean consumption (Chege et al., 2016; Leterme and Muuoz, 2002, Winham, 2017). There is considerable variation for cooking time in dry beans ranging from ~20 min. to over 1.5 h for freshly harvested beans under optimal growing and storage conditions. (Cichy et al., 2015). Despite this observation, little is known about the genetic basis for these differences in cooking time. To date, the research focus on cooking time differences in beans has been on environmental conditions during the growing season or storage conditions post-harvest (Jackson and Varriano-Marston, 1981; Alfredo, 1993; and Coelho et al., 2007).

In some developing countries where beans are consumed, such as those in Africa, firewood is the main fuel used for cooking (Adkins and Modi, 2012). A longer cooking bean thus requires more time harvesting firewood in addition to more pollutants released as a result of burning wood that can lead to negative health impacts (Saksena et al., 1992, Smith et al., 2000, Adkins and Modi, 2012). A faster cooking bean could lead to increased health benefits as faster cooking lines tend to have higher nutrient retention of protein, iron, Zn, K, etc. (Wiesinger et al., 2016).

Cooking time is influenced by the genotype, environment and storage conditions. Most of what is known about this trait is how post-harvest bean storage influences cooking time. Storage at high temperatures (29 °C and above) and high humidity, can lengthen cooking time dramatically, and this is referred to as hard-to-cook (Maurer et al., 2004, and Coelho et al., 2007). Multiple hypotheses have been used to explain the hard-to-cook mechanism including phytate/phytase, tannins, and lignin. Phytase breaks down phytate (a metal chelator), which reduces the ability of phytate to bind with Ca, Zn, and Mg (Coelho et al., 2007, Njoroge et al., 2016, Njoroge et al., 2014). These free ions can form cross links in the middle lamella with pectin, reducing cell separation resulting in increased cooking times (Coelho et al., 2007, Njoroge et al., 2016, Njoroge et al., 2014). Tannins were also proposed as leading to hard-to-cook beans. During soaking and cooking, it was thought that tannins migrate from the seed coat to the cotyledons where cross links are formed in the middle lamella resulting in decreased cell separation and increased cooking times (Coelho et al., 2007, Elia et al., 1997). Lignification, like the previous hypotheses, also involves the middle lamella. This hypothesis explains that the hard-to-cook mechanism is the result of polyphenols being converted to lignin in the middle lamella by peroxidase, which results in decreased cell separation (Martín-Cabrejas et al., 1997, Nasar-Abbas et al., 2008). Though each hypothesis involves a different cause, the result is the same, which is insolubilization of the middle lamella leading to decreased cell separation during the cooking process, which increases cooking time. Since there is very little information on the mechanisms associated with genetic variability in cooking time, the research findings on hard to cook may also help provide clues to possible mechanisms.

Two lines were chosen as parental lines to use to develop a RIL population because of their similarity for many traits including seed coat color, growth habit, and location of collection. They differ, however, in cooking time with TZ-27 (Incomparable), on average, requiring twice as long to cook as TZ-37 (W6 16488). This study seeks to determine the phenotypic stability of TZ-27 and TZ-37 across different environments and determine the underlying mechanisms that cause variability in cooking time between these dry bean genotypes.

MATERIALS AND METHODS

Plant Material

Two bean genotypes were studied from the US Plant Germplasm Collection. 'W6 16488' is a brown bean from Lushoto, which is in the Tanga region of Tanzania and was collected in 1994. As part of the *P. vulgaris* Andean Diversity Panel (Cichy et al., 2015), W6 16488 is also known as TZ-37 and ADP-0037. 'Incomparable' is a brown bean from Tanzania, collected in 1943. As part of the Andean Diversity Panel (Cichy et al., 2015), Incomparable is also known as TZ-27 and ADP-0027. TZ-27 and TZ-37 were grown at the following locations: Arusha (2014), Morogoro (2014), and Mbeya (2014) in Tanzania, Cedara (2014) and Potchefstroom (2014) in South Africa, Hawassa, Ethiopia (2015), and Entrican, Michigan (2013), Isabela, Puerto Rico (2014), and Pullman, Washington (2014) in the US (Table 2.01). Average temperatures during the growing season ranged from 14 to 30 °C and average rainfall ranged from 0.1 to 518.1 mm (Table 2.01). Harvested seed was then sent to Michigan State University and stored in a controlled atmosphere cabinet (Storage Control Systems Inc., Sparta, MI) until the moisture content was between 10 and 12%. Within six months of harvest, cooking time was determined.

Seed Characteristics

One hundred seed weight of dry seed was determined for TZ-27 and TZ-37 prior to preparation for cooking. After soaking in distilled water for 12 hours, 100 seed weight for soaked seed was determined.

Hydration Capacity

Before soaking, dry seed was weighed. The same seed was weighed after 12 hours of soaking. Hydration capacity was calculated by subtracting the weight of soaked seed from the weight of dry seed, which was then divided by the weight of the dry seed and multiplied by 100 to determine the grams of water taken up by 100 g of seed. This method is adapted from AACC method 56-35.01 though seed was soaked for 12 h instead of 16 h (AACC International, 2012). For the time course experiment, seed from the 2016 season at Montcalm Research Center (Entrican, Michigan) was weighed before soaking as well as after 6, 12, 18, or 24 hours of soaking.

Cooking Time

TZ-27 and TZ-37 were grown in multiple locations (Table 2.01). The seed was harvested, and the humidity of the seed was equilibrated by storing open packets of seed in a controlled atmosphere storage cabinet to bring seed to a humidity of 10 – 12% before cooking. For unsoaked seed, a sample of 25 dry seeds were cooked in distilled water with a Mattson bean cooker (also known as a pin drop cooker) (Customized Machining 246 and Hydraulics Co., 247 Winnipeg, Canada). The elapsed cooking time was recorded as the time required for 80% of the pins to pierce the seeds. This technique has been utilized by others to test cooking times (Paredes-López et al., 1988; Alfredo, 1993; Wang and Daun, 2005). For soaked seed, the same protocol was performed with the following changes: seed was soaked in distilled water for 12

hours at room temperature prior to placement in the Mattson cooker. For seed soaked and cooked in hard water, the same protocol was performed for soaked seed except seed was soaked and cooked in tap water ($CaCO_3$ of 390 ppm) from Michigan State University. When performing the time course cooking experiment, seed was soaked for 0, 6, (8, 10), 12, 18, and 24 hours before cooking.

Nutrient Content

Seed from the 2012 and 2013 growing seasons at the Montcalm Research Center were used in the nutrient analyses. Total N concentration was determined in 1 g of lyophilized powder from cooked samples of TZ-27 and TZ-37 by the Dumas combustion method at A & L Great Lakes Laboratories (Fort Wayne, IN, USA). The resulting N content was converted to crude protein percentage by multiplying by 6.25.

Phytic acid phosphorus (PA-P) was measured in 50 mg of lyophilized powder from cooked samples using a modified colorimetric assay (adapted from Gao et al., 2007). To calculate phytate, PA-P (g/g) was multiplied by 3.6, which was derived from the molar mass of phytic acid (660 g mol^{-1}) divided by the product of the molecular weights of phosphorus (P, 31 g mol^{-1}) and the molar ratio of phosphorus:phytic acid (6 mol mol^{-1}) as explained in Brooks et al. (2001).

Mineral analyses were adapted from Wiesinger et al. (2016) and conducted by first treating 500 mg of lyophilized powder from cooked samples with ultrapure nitric acid for 16 h at room temperature. Samples were then placed in a digestion block (Martin Machine, Ivesdale, IL, USA) for 4 h and incubated at 125 °C with refluxing. Samples were then cooled for 5 min before 2 mL of H_2O_2 was added and incubation at 125 °C for an additional hour. This step was repeated a second time before the digestion block temperature was raised to 200 °C and

maintained until each sample was completely dry. Digested samples were resuspended in 2% ultrapure nitric acid and incubated overnight prior to analysis using inductively coupled plasma-optical emission spectroscopy (ICP-OES) (CIROS ICP model FCE12, Spectro, Kleve, Germany) with daily calibration of certified standards. To ensure batch-to-batch accuracy, all samples were digested and measured alongside tomato leaf standards purchased from the National Institute of Standards and Technology (SRM 1573A; Gaithersburg, MD, USA).

Electrolyte Leakage

Seed from the 2016 growing season at the Montcalm Research Center was used to evaluate electrolyte leakage. Seed was soaked and cooked as previously described. For high temperature conditions, seed was stored in an incubator chamber at 34-35 °C and a humidity of 70.5-71.5% for 10 days. For low temperature conditions, temperature was held constant near 4 °C and 79% humidity for 10 days. Electrical conductivity (EC) of each soaking solution before and after soaking for 12 h was measured using the SevenCompact S230 Conductivity meter (Mettler-Toledo AG, Schwerzenbach, Switzerland) to check the effect of soaking solution on seed leachate with relation to cooking time.

Microscopy of Cellular Structures

Seed from the Entrican, MI 2012 growing season were evaluated at the MSU Scanning Electron Microscopy Facility following the methods explained by Nakalema (2015). Freeze-drying was performed on both seed soaked in distilled water for 12 h and unsoaked seed. Freeze-dried seeds were fixed in platinum prior to slicing. A cross-sectional cut was made across the cotyledons near the narrowest part of the bean. Scanning electron microscopy was performed with a JEOL JSM-7500F (cold field emission electron emitter).

Enzyme Extraction

Three month-old seed from Arusha, Tanzania in 2017 that had been stored at 4 °C was used for enzyme extraction and enzyme activity assays. The enzyme extraction was adapted from the method of Martínez-Manrique et al. (2011). One gram of cotyledon flour was homogenized in 15 mL of 0.05 M sodium acetate of pH 5.5 containing 1.0 M NaCl. The solution was mixed for 1.5 h at 4 °C before centrifugation at 5000 x g for 10 min at 4 °C. The pellet was removed, and the supernatant was dialyzed against 2 L of 0.05 M sodium acetate buffer of pH 5.5 at 4 °C using Spectra/Por pre-treated dialysis tubing 8 kD (SpectrumLabs). The sodium acetate buffer was replaced, and dialysis was allowed to run overnight. The dialyzed extract was then centrifuged at 10,000 x g for 10 min at 4 °C. The supernatant was the working enzyme solution used in the enzymatic assay.

Pectin Methylesterase Activity

Activity of pectin methylesterase was measured using the spectrophotometric method adapted from Cameron et al. (1992). One modification to this method was that bromothymol blue was used in the colorimetric assay at a final concentration of 0.00250% (w/v) instead of 0.00125%. This change allowed for better optical density. Citrus esterified pectin (Sigma) was used as the substrate for pectin methylesterase to act upon. Stock solutions of potassium phosphate, bromothymol blue, and pectin were made as described by Cameron et al. (1992). The standard curve was produced through dilution of concentrated galacturonic acid with distilled water to produce solutions of 0, 5, 35, 50, 65, 80, 150, and 250 nmoles per mL in the final reaction volume. In triplicate, 10 µL of enzyme solution was placed in 3 wells of a 96 well plate. This was done for all 16 samples for a total of 48 wells. One hundred ninety µL of the reaction

cocktail was added to each well for a total of 200 µL. The reaction cocktail was composed of 750 µL of 0.5% w/v pectin, 100 µL bromothymol blue, and 100 µL distilled water. The previous volumes produced enough reaction cocktail for 5 reactions per 1 sample to ensure that enough reaction cocktail would be available for all reactions. All reaction cocktail solutions and enzyme preparations were adjusted to pH 7.5 before beginning the assay. The galacturonic acid standards were not pH adjusted. The reaction of methanol released by the breakdown of pectin by pectin methylesterase resulted in a color change in bromothymol blue that was measured at an absorbance of 620 nm by a Biotek Synergy HT plate reader (Biotek Instruments, Winooski, VT) each minute for 30 minutes. Enzyme activity of pectin methylesterase (PME) of TZ-27 and TZ-37 was determined by subtracting activity at 30 minutes from activity at 0 minutes and dividing by thirty to obtain activity per minute. Enzyme activity was then divided by mg protein resulting in nmoles of methanol released per minute per mg of protein.

RESULTS

Seed Characteristics

Seed weights were compared prior to and after soaking and cooking. TZ-27 weighed, on average, 39.6 and 83.4 g when raw and soaked, respectively, while TZ-37 weighed 51.4 and 111.8 g when raw and soaked (Table 2.02). Parental lines grown in Hawassa, Ethiopia had the smallest dry and soaked weight, while those grown in Cedara, South Africa resulted in the greatest weight for both lines.

Cooking time in each line following soaking treatment

Significant cooking time differences between TZ-27 and TZ-37 were observed following a 12-hour soak in deionized water (Fig. 2.01, Table 2.08). Additionally, location effected cooking time ($p < 0.0001$), and there was a genotype by location effect ($p < 0.0001$) (Table 2.08). Locations spanned 75.8 degrees latitude, 158.2 degrees longitude, and 1,764.8 meters of altitude (Table 2.01). These growing sites encompassed three agro-ecological zones (tropical, sub-tropical, and temperate) (Table 2.01). TZ-37 cooked faster than TZ-27 at every location tested when the lines were soaked prior to cooking (Fig. 2.01, Table 2.03). This is an important finding because it suggests cooking time as a stable trait across environments. Specifically, TZ-37 cooked at least 1.7 times faster than TZ-27 at most tested locations, and TZ-37 was observed to cook up to 3.4 times faster than TZ-27 when grown at the Cedara location. The difference observed at the Cedara location was because TZ-27 required the most time to cook. The fastest cooking times of 19.1 and 39.3 minutes for TZ-37 and TZ-27 respectively were observed for beans grown in Hawassa, Ethiopia. Because growth in Hawassa results in faster cooking lines, it could be of interest to grow potential fast cooking varieties there to observe how short of a cooking time can be achieved. Other lines from the Andean Diversity Panel grown in Hawassa in the same field trial were also observed to cook faster than other locations (data not shown). Similarly, TZ-27 and TZ-37 required longer cooking times (97.8 and 57.2 min respectively) after being grown in Morogoro, which was also observed in other lines of the Andean Diversity Panel in the same field trial (data not shown).

Cooking time in each line without prior soaking treatment

When unsoaked, beans took between 90 to 140 min to cook, which was 2.6 to 1.4 times longer than the same genotypes at the same locations when soaked. Cooking time differences between TZ-27 and TZ-37 were much smaller when seed was not soaked prior to cooking (Fig. 2.02, Table 2.04). A significant difference was only observed at the Morogoro location with TZ-37 cooking 1.3 times faster than TZ-27 (Fig. 2.02, Table 2.04) Significant differences were not observed at any other location.

Hydration Capacity of TZ-27 and TZ-37

Hydration capacity was significantly different based on location ($p=0.0006$), but significant differences between lines were only observed in Pullman, WA. The remaining 8 locations showed no significant differences for hydration capacity between TZ-27 and TZ-37 (Table 2.03). The range of hydration capacities for TZ-27 was 99.0 to 115.5 g water per 100 g seed, and for TZ-37, it was 100.5 to 119.0 g water per 100 g seed (Table 2.03).

Cooking Time and Hydration Capacity over Time

To determine the soaking time required to observe significant cooking time differences between TZ-27 and TZ-37, both lines were cooked after soaking in distilled water for different amounts of time (Fig. 2.03). No significant differences in cooking time between lines grown in Entrican, MI were observed at 0 and 6 hours of soaking prior to cooking. However, significant differences were observed at 12 hours of soaking ($p=0.0079$), as well as after 18 hours ($p = 0.02$) and 24 hours ($p = 0.04$). Regardless of genotype, no significant differences were observed when comparing a 12 h soak to either the 18 or 24 h soak. Genotypes from Arusha showed significant differences at 6 h through 24 h of soaking. Both lines from Arusha were not

significant when comparing a 6 h soak to 8 h or more. In contrast, no significant differences were observed between TZ-27 and TZ-37 for hydration capacity. Hydration capacity, like cooking time, stabilized at 12 h through 24 h (Fig. 3). A 12 h soak was used for all further cooking experiments where seed was soaked prior to cooking.

Cooking Time in Hard Water

Calcium has been linked to longer cooking times, and it is often cited that the binding of pectin and Ca form an insoluble product making cell separation during the cooking process difficult (Coelho et al., 2007, Njoroge et al., 2016, Njoroge et al., 2014, Galiotou-Panayotou et al., 2008 Caffall and Mohnen, 2009, Mattson, 1946, and Kinyanjui et al., 2015). The effect of Ca on cooking time was tested by using tap water from Michigan State University. MSU tap water was used as it has a high degree of hardness ($CaCO_3$ of 390 ppm) compared to other areas of North America ($CaCO_3$ range of 1.4 to 135.5 ppm) (Morr et al., 2006; Infrastructure Planning and Facilities, 2016). As with distilled water, there was a significant difference between genotypes when they were soaked in hard water prior to cooking ($p = 0.0327$). Compared to soaking and cooking in distilled water, cooking times were at least 5.4 times longer when soaked and cooked in hard water, but TZ-37 cooked at least 1.4 times faster than TZ-27 when soaked prior to cooking (Table 2.06). Location, again, had an effect on cooking time ($p<0.0001$). Cooking times were longer when unsoaked prior to cooking in hard water with the fastest cooking time being 462.6 min (Table 2.06). When unsoaked, no difference was observed between TZ-27 and TZ-37 (Table 2.06) though location had an effect on cooking time ($p = 0.0001$).

Hydration Capacity in Hard Water

Hydration capacity was lower when soaked in hard water compared to distilled water (Table 2.05, Table 2.07). Also, soaking in hard water resulted in hydration capacities that were significant by genotype ($p=0.0098$) with TZ-37 taking up more water, but location and genotype by location were not significantly different.

Nutrient Content

Nutrient contents of TZ-27 and TZ-37 were measured because nutrients including Ca, phytate, Mg, Na, and K have been shown to effect cooking time. In bean, soaking in a Na solution shortened cooking time, while cooking time was lengthened when soaked in a Ca solution (Njoroge et al., 2016), which was also observed in pea (Mattson, 1946). Increased Ca in the soaking solution also led to increased hardness, which can result in longer cooking times (Galiotou-Panayotou et al., 2008). Monovalent cations like K and Na resulted in shorter cooking times in pea when used as a soaking solution (Mattson, 1946). Phytate is a bioactive compound that can chelate minerals including Ca and increasing concentrations of phytate in the seed have been shown to lead to faster cooking in bean (Wiesinger et al., 2016, Coelho, 2007) and decreased hardness (Galiotou-Panayotou et al., 2008). Cooked seed was chosen to measure because it represented the maximum amount available for digestion. Phytate was significantly higher in TZ-37, while Ca and Mn (divalent cations) were higher in TZ-27 (Table 2.09). The lowest Ca observed (0.8 mg g^{-1}) was in TZ-37 in Morogoro in 2016, while the highest Ca concentration (2.5 mg g^{-1}) was found in Arusha and Morogoro in 2016 and 2017 respectively (Table 2.09). In addition, the concentration of Ca was at least 1.7 times higher in TZ-27 than TZ-37 regardless of environment, and Mn was at least 1.3 times higher in TZ-27

than TZ-37 (Table 2.09). Phytate was higher in TZ-37 by 1.1 times in both years it was measured for lines grown in Entrican, Michigan (Table 2.09).

Microscopy of cell structures

Scanning electron microscopy on 12 soaked seed revealed differences between TZ-27 and TZ-37. Organized cells with starch granules mostly bound within a protein matrix were evident in the micrograph of TZ-27 (Fig. 2.04). Individual cells were more loosely organized in TZ-37 with starch granules that were more visible and less bound by the protein matrix (Fig. 2.04).

Electrolyte Leakage

Electrolyte leakage has been linked to cooking time as well as viability (Boros and Wawer, 2005, Powell, 1986, Leão et al., 2012). Boros and Wawer (2005) showed significant variation in genotypes across 21 cultivars for conductivity (a measure of electrolyte leakage) as well as cooking time. Whether stored in adverse conditions or not, conductivity was not significantly different between TZ-27 and TZ-37 (Table 2.05). The same result was observed regardless of soaking treatment, though conductivity was significantly increased when comparing distilled water to hard water (Table 2.05).

Enzyme Activity

Enzyme activity has been connected to cooking time through the breakdown of cell wall components leading to cell separation during the cooking process (Mafuleka et al., 1991, Martínez-Manrique, 2011, Garcia et al., 1998). Pectin methylesterase (PME) activity can lead to longer cooking times, as PME removes methyl groups, which leave exposed carboxyl groups that can bind with divalent cations. This binding leads to pectin becoming crosslinked, which

strengthens the cell wall and results in decreased cell separation and prolonged cooking time. PME activity in TZ-27 was similar whether raw or soaked in both locations. However, PME activity decreased in TZ-37 when soaked (Table 2.07). Because greater PME activity leads to more free sites for Ca cross-linking and longer cooking times, the decrease in activity in soaked TZ-37 is a possible cause behind shorter cooking time in TZ-37 when seed is soaked.

DISCUSSION

Seed Characteristics

An inverse correlation (r = -0.4) was observed for dry weight and cooking time and for soaked weight and cooking time (r = -0.4) (Table S2.02), which suggests that larger seeds cooked faster than smaller seeds. TZ-37 was also significantly greater in seed mass regardless of environment compared to TZ-27, and TZ-37 always cooked faster than TZ-27 when soaked prior to cooking. These results contrast with other research that has found larger seeds result in longer cooking times (Cichy et al., 2015, Black et al., 1998, Bressani et al, 1988). These contrasting results could be due to the small number of genotypes tested (n = 2) in the current study. TZ-37 weighed between 1.2 and 1.5 times as much as TZ-27 across environments whether dry or soaked, suggesting a strong genetic component to seed weight. Cooking time related genes that are in tight linkage with seed weight related genes could result in larger seeds that are fast cooking if that linkage between small seed weight and fast cooking was broken. Since TZ-37 is fast cooking with large seed size, these traits might be inherited together by offspring making this an ideal candidate to move two sought after traits into a population simultaneously.

Cooking time

Research on cooking time, thus far, has focused on environmental influences. Less is known about the mechanisms associated with genetic differences in cooking time. If a substantial environmental component determined cooking time in dry beans, it could lead to difficulty in identifying regions of the genome that affect cooking time. It would also be difficult to apply genetic findings to beans grown in different locations than the ones tested here. Thus, it was important to phenotype lines grown in many different environments for cooking time to determine the effect of the environment. An important trend was noted despite the environment having an effect on cooking time, which was that TZ-37 cooked 2.2 times faster than TZ-27 on average regardless of location. In other words, a difference in location can make TZ-27 require a longer or shorter cooking time, but it never cooks faster than TZ-37.

Research has shown that storage at high heat and humidity can lengthen cooking time as these conditions trigger the breakdown of phytate by phytase (Coelho et al., 2007, Njoroge et al., 2016, Njoroge et al., 2014). For both TZ-27 and TZ-37, environments that had high humidity and temperature such as Cedara, South Africa and Morogoro, Tanzania, took longer to cook. Though cooking time for both genotypes changed based on environment, TZ-37 always cooked faster than TZ-27 when soaked prior to cooking. This trend was observed irrespective of location or soaking treatment. Also, though soaking and cooking in hard water greatly lengthened cooking time to over 3.5 h for the fastest cooking sample, TZ-37 cooked 1.5 times faster than TZ-27, on average. These results point to a possible genetic cause that underlies cooking time. The few studies that have focused on this area agree that there is a genetic component to cooking time. For example, Garcia et al. (2012) found a QTL for cooking time on Pv01, and Cichy et al. (2015) found SNPs on Pv02, Pv03, and Pv06. An understanding of

what causes these two lines to cook at different rates will be studied further by creating a mapping population where TZ-27 and TZ-37 are parental lines.

Hydration Capacity

Because soaking significantly affected cooking time between the two lines, hydration capacity was also measured to determine if differences existed for water uptake. In addition, research has suggested that water absorption could be a useful predictor of cooking time (Elia, 1997). However, research into TZ-27 and TZ-37 indicate that hydration capacity does not influence cooking time. These results conflicted with work by Elia et al. (1997) and Cichy et al. (2015) where an inverse correlation was discovered between hydration capacity and cooking time providing evidence that beans that take up more water cook faster. These conflicting findings could be the result of two possibilities. The populations in the other studies had differences in hydration capacity and cooking time suggesting a link between the two traits that was correlation rather than causation. Another possibility is that the small number of genotypes used in this current study do not have differences in hydration capacity, but that does not mean that hydration capacity would not influence cooking time when in different genetic backgrounds. With hydration capacity excluded as a cause of cooking time differences and soaking causing cooking time differences between these two lines, a possible enzymatic cause becomes a more plausible explanation for cooking time differences in dry bean.

Nutrient Content

Calcium and Mg are divalent cations that can cause insolubilization of the middle lamella lengthening cooking time (Caffall and Mohnen, 2009; Njoroge et al., 2016; Mattson, 1946; and Kinyanjui et al., 2015, Coelho, 2007). In contrast, Na and K are monovalent cations that have

been shown to shorten cooking time (Mattson, 1946; Kinyanjui et al., 2015). Phytate is a bioactive compound that chelates Ca and other divalent cations such that those minerals cannot be absorbed by the body (Cheryan and Rackis, 1980). On the other hand, because of its chelation function, phytate reduces the number of divalent cations that would otherwise bind with pectin and lengthen cooking time. Since TZ-27 has 1.1 times less phytate and twice the Ca than TZ-37, it's possible that more free Ca ions are not being chelated by phytate leading to crosslinking with pectin and lengthening cooking time in TZ-27. Though the role of Mn in cooking time is unclear, it can act as a divalent cation and the greater amount of Mn in TZ-27 could also bind with pectin as Ca or Mg would.

Microscopy

After 12 hours of soaking, differences were visible between TZ-27 and TZ-37 at the cellular level. Specifically, adhesion between the cells as well as adhesion of starch granules within the protein matrix was less pronounced in TZ-37 than TZ-27. This result was similarly found in cowpea where control seed was softer and had starch granules that were less embedded within a protein matrix compared to aged seed and seed soaked in $CaCl_2$ (Liu et al., 1993). While the uptake of water was not related to cooking time in these lines, there is some process occurring during the soaking period that results in the different appearances of the cells that is likely also effecting cooking time to differing degrees in TZ-27 and TZ-37.

Enzyme Activity

A possible cause for differences in cooking time could be differences in enzymatic activity. TZ-27 had significantly higher PME activity when the lines were grown in Morogoro. PME activity lengthens cooking time by exposing areas on pectin where crosslinking between pectin

molecules can occur when a divalent cation is present (Jolie et al., 2010). In a study of enzyme activity and cooking time, the slowest cooking line in the study had more than double the PME activity when not soaked and almost 3.5 times greater activity when soaked compared to the fastest cooking line (Martínez-Manrique et al., 2011). Interestingly, TZ-37 PME activity decreased after soaking while TZ-27 activity remained relatively constant. This result could be caused by a greater expression of pectin methylesterase inhibitor (PMEi) in TZ-37 than TZ-27. PMEi has been identified in several crops and reduces activity by binding non-covalently with PME (Giovane et al., 2004).

CONCLUSION

Cooking time in dry bean is a complex trait that has yet to be fully understood. A summary of trends found in TZ-27 and TZ-37 that effect cooking time is presented in Figure S2.01. Trends such as smaller seed or seed with a great hydration capacity leading to fast cooking times were not evident in TZ-27 or TZ-37. However, important data regarding cooking time and possible mechanisms were uncovered. TZ-37 cooks at least 1.7 times faster than TZ-27 regardless of location as long as seed is soaked prior to cooking. Cooking time was not different between the genotypes when they are cooked without soaking. Seed compositional differences included that TZ-27 contained two times more Ca and 1.1 times less phytate than TZ-37. These data suggest that some process occurs during soaking that results in TZ-37 cooking faster. The process could be PME being inhibited in TZ-37 so it cannot cleave methyl groups from pectin, which would keep Ca crosslinking from occurring in pectin resulting in shorter cooking times. A biparental RIL population will be developed using TZ-27 and TZ-37 as parental lines to elucidate the genetic cause underlying cooking time differences.

APPENDICES

APPENDIX A

CHAPTER 2 FIGURES AND TABLES

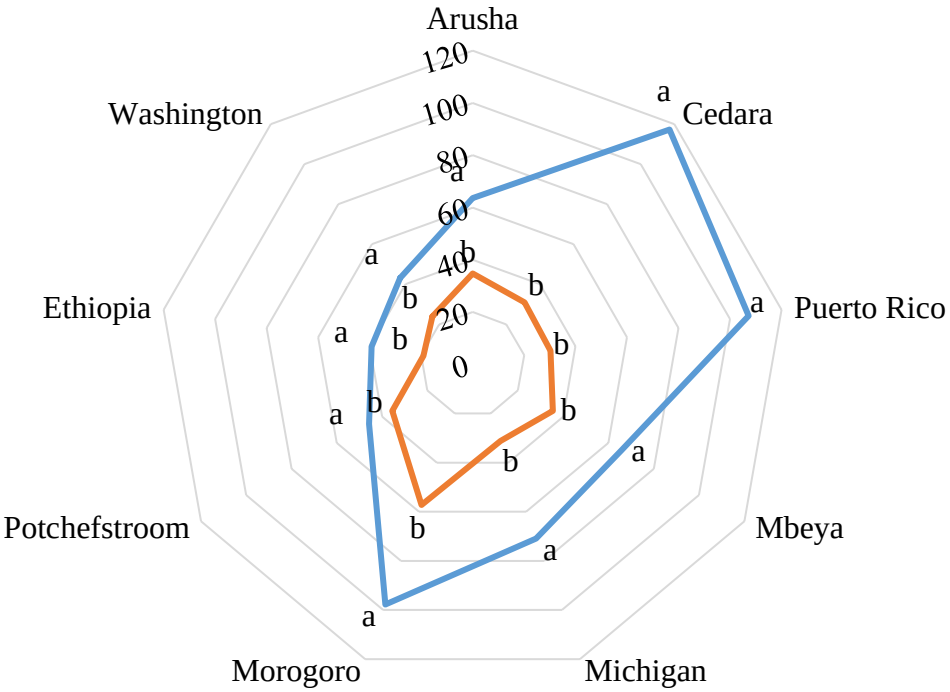

Figure 2.01. Spider plot of elapsed cooking time of beans grown at different locations (soaked). TZ-27 (blue) and TZ-37 (orange) were cooked after soaking in distilled water for 12 hours. The elapsed cooking time (in minutes) for 80% of the beans to cook was recorded. Results shown were represented as a mean of two to four replicates for each location. Each ring in the figure represented 20 minutes. The same letter for each location indicates no significant difference at $p < 0.05$.

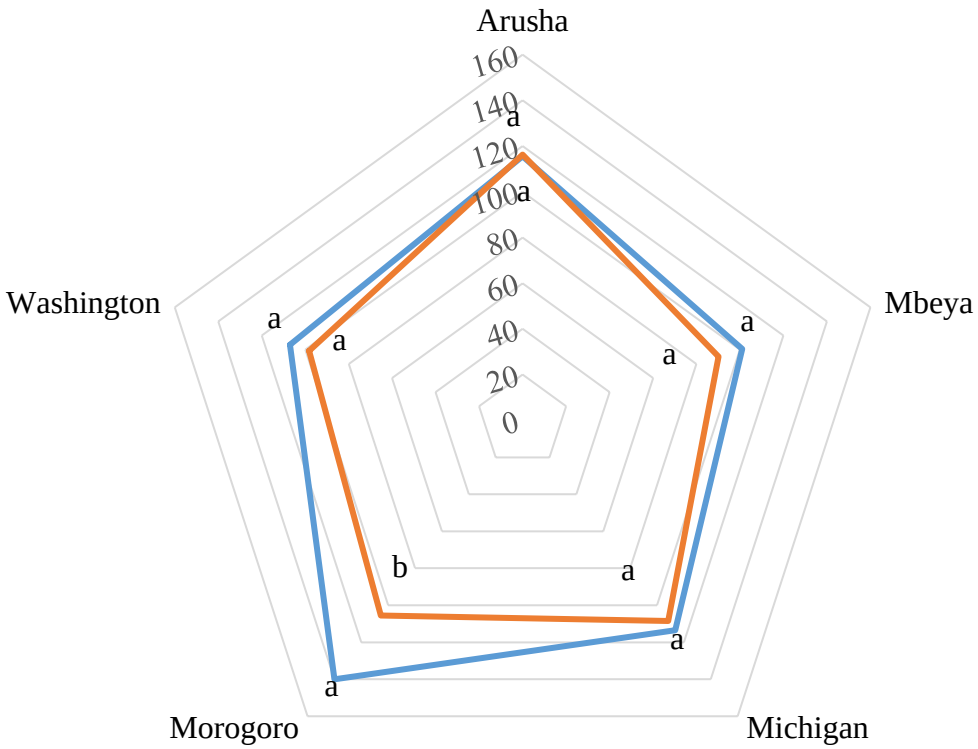

Figure 2.02. Spider plot of elapsed cook time of beans grown at different locations (unsoaked). TZ-27 (blue) and TZ-37 (orange) were cooked in distilled water without soaking beforehand. The elapsed cooking time (in minutes) for 80% of the beans to cook was recorded. Results shown were represented as a mean of two replicates for each location. Each ring in the figure represented 20 minutes. The same letter for each location indicates no significant difference at $p < 0.05$.

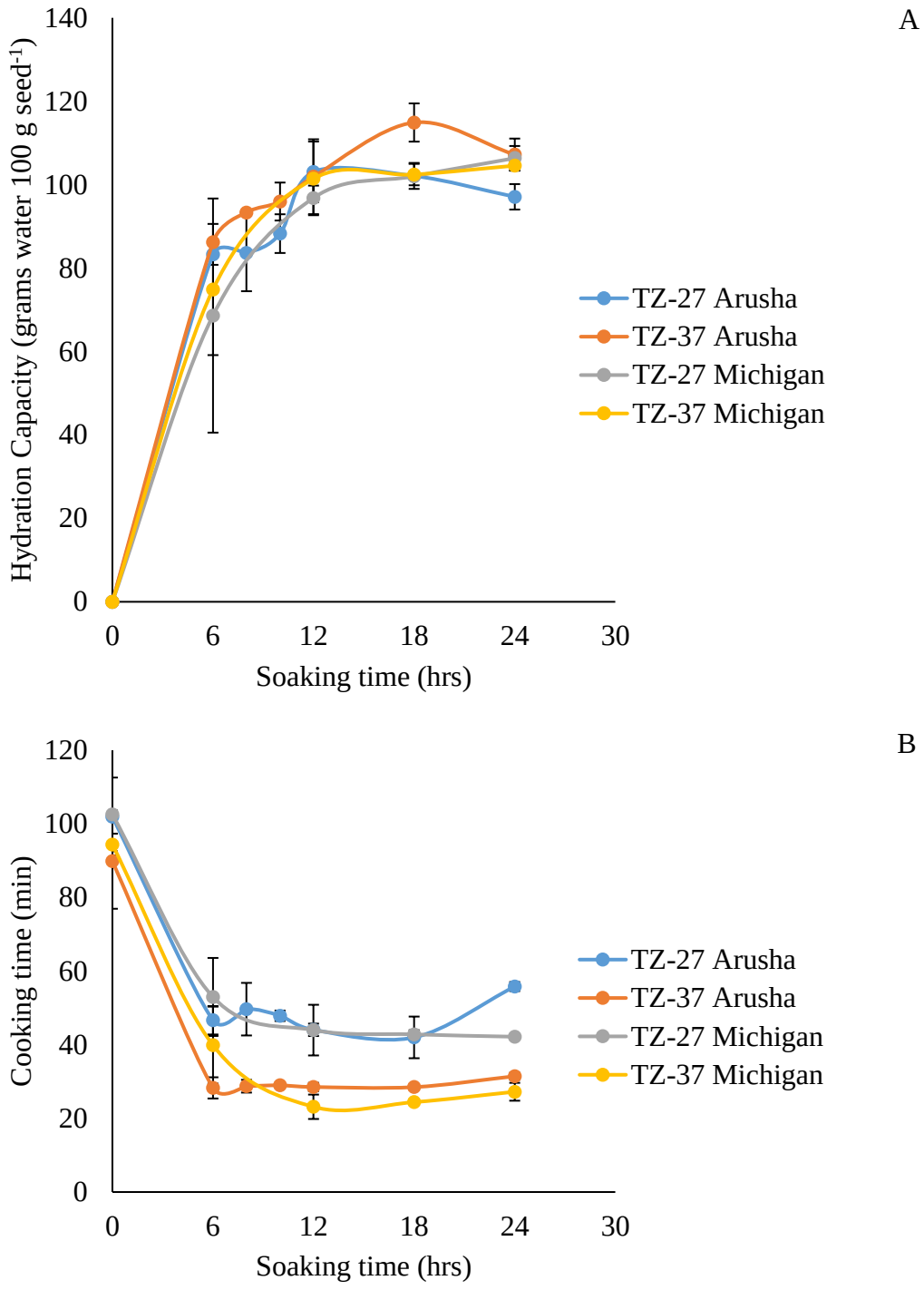

Figure 2.03. Cooking time and hydration capacity of beans over time. TZ-27 and TZ-37 were cooked after soaking in distilled water for 0, 6, 8, 10, 12, 18 and 24 hours. Hydration capacity for TZ-27 and TZ-37 was recorded after soaking in distilled water for 0, 6, 8, 10, 12, 18, and 24 hours (A). The elapsed cooking time for 80% of the beans to cook was reported (B). Results shown represented the mean of two replicates.

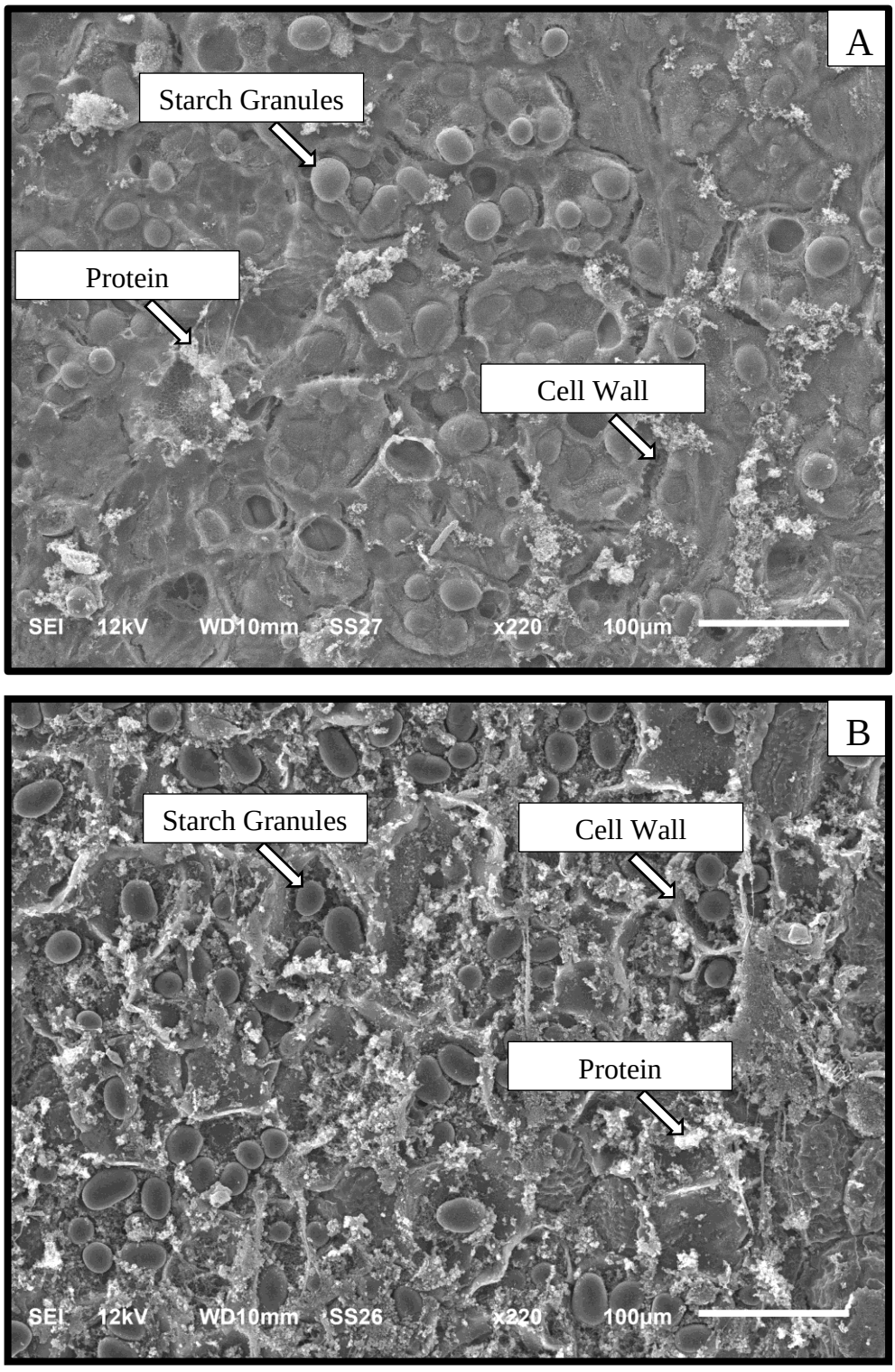

Figure 2.04. SEM images of bean samples soaked for 12 h in distilled water. Once samples were fixed and dehydrated, a cut was made perpendicular to the cotyledons near the narrowest part of the bean. A) TZ-27 B) TZ-37.

Country	Location	Latitude	Longitude	Altitude (m)	Average Temperature (°C)	Average Rainfall (mm)	Agro-Ecological Zone
Ethiopia (Hawassa)	Southern Agricultural Research Institution	7.1	38.5	1709.0	21 – 26	24.4 – 144	Tropics, warm
South Africa (Cedara)	Cedara College near Pietermaritzburg	-29.5	30.3	1047.3	16 – 25	12.3 – 138.3	Subtropical, warm/moderate cool
South Africa (Potchefstroom)	Potchefstroom Station	-26.7	27.1	1357.6	14 – 25	0.1 – 74.5	Subtropical, warm/moderate cool
Tanzania (Arusha)	Selian Agriculture Institute	-3.4	36.6	1412.2	20 – 25	5 – 158.3	Tropics, warm
Tanzania (Mbeya)	Uyole Research Station	-8.9	33.5	1777.2	17 – 20	5.5 – 318.7	Tropics, warm
Tanzania (Morogoro)	Sokoine University of Agriculture	-6.8	37.7	527.2	23 – 27	31.3 – 518.1	Tropics, warm
U.S.A. (Entrican, MI)	Montcalm Research Center	43.4	-85.2	286.2	19 – 25	25.6 – 93.2	Temperate, cool
U.S.A. (Isabela, PR)	Tropical Crops and Germplasm Research	18.5	-67.0	12.4	28 – 29	15.1 – 71.7	Tropics, warm
U.S.A. (Pullman, WA)	Othello Research Farm	46.3	-119.7	258.6	18 – 30	8.3 – 62.2	Temperate, cool

Table 2.01. Field locations for TZ-27 and TZ-37. Latitude and Longitude are in degrees, and average temperature and rainfall are presented as ranges of temperatures and rainfall during each growing season at each location.

Country or State	City	Raw 100 Seed Weight (g)		Soaked 100 Seed Weight (g)	
		TZ-27	TZ-37	TZ-27	TZ-37
South Africa	Cedara	51.6^{cde}	68.5^{a}	106.0^{de}	143.7^{a}
South Africa	Potchefstroom	37.7^{ij}	45.3^{efgh}	79.5^{ij}	97.1^{efgh}
Tanzania	Arusha	40.3^{hi}	52.8^{bcd}	89.5^{ghi}	122.9^{bc}
Tanzania	Mbeya	37.0^{ij}	45.6^{efg}	78.3^{ij}	98.3^{efgh}
Tanzania	Morogoro	34.5^{jk}	47.4^{ef}	74.3^{jk}	104.3^{def}
Ethiopia	Hawassa	29.4^{k}	41.0^{ghi}	63.7^{k}	92.6^{fgh}
Puerto Rico	Isabela	33.3^{jk}	49.3^{def}	71.4^{jk}	103.5^{def}
Michigan	Entrican	44.1^{fgh}	55.6^{bc}	87.8^{hi}	114.2^{cd}
Washington	Pullman	48.6^{def}	57.5^{b}	100.6^{efg}	126.5^{b}

Table 2.02. Weight characteristics for raw and soaked bean seeds from TZ-27 and TZ-37. Values represent the mean of 2 replicates. The same letter in a column or row within a weight category indicates no significant difference at $p < 0.05$.

Country	Location	Cooking Time (min)		Hydration Capacity	
		TZ-27	TZ-37	TZ-27	TZ-37
Ethiopia	Hawassa	39.3^{gh}	19.1^{k}	115.5^{abc}	127.0^{a}
Tanzania	Arusha	63.6^{ef}	34.8^{hi}	112.0^{bcdef}	119.0^{ab}
Tanzania	Mbeya	65.8^{de}	35.5^{hi}	113.4^{bcde}	116.1^{ab}
Tanzania	Morogoro	97.8^{c}	57.2^{f}	104.3^{efgh}	100.5^{gh}
South Africa	Cedara	117.2^{a}	30.9^{i}	105.6^{cdefgh}	109.8^{bcdefg}
South Africa	Potchefstroom	45.7^{g}	35.5^{hi}	111.0^{bcdef}	114.9^{bcd}
U.S.A.	Entrican, MI	71.0^{d}	31.2^{i}	99.0^{h}	105.3^{defgh}
U.S.A.	Isabela, PR	107.20^{b}	30.2^{ij}	114.6^{bcd}	109.9^{bcdefg}
U.S.A.	Pullman, WA	43.2^{g}	23.9^{jk}	102.7^{fgh}	113.9^{bcde}

Table 2.03. Cooking time and hydration capacity following a 12 h soak in distilled water for TZ-27 and TZ-37 in 9 locations. Each value is an average of two replicates, and columns or rows within a trait with the same letter indicate no significant difference at $p < 0.05$.

Country	Location	Cooking Time (min)	
		TZ-27	TZ-37
Tanzania	Arusha	115.5^{b}	116.3^{b}
Tanzania	Mbeya	101.0^{bc}	90.1^{c}
Tanzania	Morogoro	140.0^{a}	105.5^{bc}
U.S.A.	Entrican, MI	113.5^{b}	108.5^{bc}
U.S.A.	Pullman, WA	107.0^{bc}	98.2^{bc}

Table 2.04. Cooking time without presoaking treatment for TZ-27 and TZ-37 in 5 locations. Values represent the mean of two replicates, and columns or rows with the same letter indicate no significant difference at $p < 0.05$.

Soaking Solution	Low Temperature		High Temperature	
	Electrical Conductivity (µS cm^{-1})		Electrical Conductivity (µS cm^{-1})	
	TZ-27	TZ-37	TZ-27	TZ-37
Distilled Water	266.6^{c}	249.0^{c}	298.2^{bc}	355.6^{b}
Hard Water	1166.5^{a}	1183.0^{a}	1141.2^{a}	1185.8^{a}

Table 2.05. Electrical conductivity of soaking solution. Electrolyte leakage from TZ-27 and TZ-37 were measured in the soaking solution after each line had soaked for 12 hours in either distilled water or hard water. Values within a row or column with the same letter represent no significant difference at $p < 0.05$. Each value represents an average of 6 replicates.

Country	Location	Soaked		Unsoaked		Soaked	
		Cooking Time (min)		Cooking Time (min)		Hydration Capacity	
		TZ-27	TZ-37	TZ-27	TZ-37	TZ-27	TZ-37
Tanzania	**Arusha**	513.9^{d}	365.5^{g}	608.5^{bc}	576.8^{c}	79.5^{bc}	95.2^{a}
Tanzania	**Mbeya**	352.3^{g}	228.9^{h}	471.5^{e}	462.6^{e}	70.1^{d}	87.7^{ab}
Tanzania	**Morogoro**	616.2^{b}	418.3^{f}	697.7^{a}	680.8^{a}	74.1^{cd}	80.7^{bc}

Table 2.06. Hard water effects on cooking time and hydration capacity. TZ-27 and TZ-37 were cooked after soaking in hard water for 12 hours. The elapsed cooking time for 80% of the beans to cook was recorded. Results shown were represented as a mean of two replicates for each location. Each ring in the figure represented 100 minutes. All soaked results were significant but all unsoaked results were not significant using an alpha of 0.05.

	Raw				Soaked			
	Arusha		Morogoro		Arusha		Morogoro	
Enzyme Activity	TZ-27	TZ-37	TZ-27	TZ-37	TZ-27	TZ-37	TZ-27	TZ-37
Pectin Methylesterase (nmol of methanol min^{-1} mg $protein^{-1}$)	44.6^{d}	53.6^{c}	71.3^{a}	62.1^{b}	44.4^{d}	48.1^{cd}	70.5^{a}	36.1^{e}

Table 2.07. Pectin methylesterase activity for TZ-27 and TZ-37. Values represent the average activity for 6 reps. Values in the same row with the same letter represent no significant difference at $p < 0.05$.

Trait	Source of Variation	Degrees of Freedom	Type III SS	Mean Square	F Statistic	p Value
Cooking Time	Genotype	1	13794	13794	1325.6	<0.0001
	Location	8	9660.4	1207.6	116.0	<0.0001
	Genotype*Location	8	5435.6	679.5	65.3	<0.0001
100 Dry Seed Weight	Genotype	1	1357.9	1357.9	166.0	<0.0001
	Location	8	1913.0	239.1	29.2	<0.0001
	Genotype*Location	8	95.5	11.9	1.5	0.2
100 Soaked Seed Weight	Genotype	1	6972.7	6972.7	226.2	<0.0001
	Location	8	7220.1	902.5	29.3	<0.0001
	Genotype*Location	8	322.7	40.3	1.3	0.3
Hydration Capacity	Genotype	1	163.6	163.6	6.9	0.02
	Year	8	1256.5	157.1	6.7	0.0004
	Genotype*Location	8	258.7	32.3	1.4	0.3

Table 2.08. Analysis of variance for cooking time, dry and soaked seed weight, and hydration capacity collected from TZ-27 and TZ-37 in 9 locations. The ANOVA was carried out using SAS 9.4 statistical software package.

	2012		2013		2016		2016		2017		2017	
	Entrican		Entrican		Arusha		Morogoro		Arusha		Morogoro	
Nutrient	TZ-27	TZ-37	TZ-27	TZ-37	TZ-27	TZ-37	TZ-27	TZ-37	TZ-27	TZ-37	TZ-27	TZ-37
Protein (%)	21.4^{def}	28.1^{a}	20.0^{f}	27.7^{ab}	20.8^{ef}	22.6^{def}	22.5^{def}	24.0^{cde}	22.1^{def}	25.8^{abc}	20.8^{f}	24.6^{bcd}
Calcium (mg/g)	1.7^{bc}	0.9^{de}	1.9^{b}	0.9^{de}	2.5^{a}	1.1^{de}	1.3^{cd}	0.8^{e}	1.8^{bc}	1.0^{de}	2.5^{a}	1.3^{cd}
Magnesium (mg/g)	1.9^{bcde}	1.8^{cdef}	2.1^{b}	2.0^{bcd}	1.8^{cdef}	1.6^{ef}	1.9^{bcde}	1.7^{def}	1.7^{def}	1.5^{f}	2.6^{a}	2.0^{bc}
Sulfur (mg/g)	1.7^{cd}	2.0^{b}	2.0^{b}	2.4^{a}	1.6^{cd}	1.6^{cd}	1.4^{d}	1.7^{cd}	1.6^{cd}	1.7^{cd}	1.5^{cd}	1.8^{bc}
Potassium (mg/g)	9.4^{bc}	10.7^{abc}	10.6^{abc}	13.4^{a}	8.0^{cde}	9.0^{bcd}	5.5^{e}	9.7^{bc}	9.0^{bcd}	11.2^{ab}	6.4^{de}	9.4^{bc}
Boron (ppm)	7.0^{ab}	7.5^{a}	6.5^{abc}	7.0^{ab}	5.5^{abcd}	4.5^{abcd}	3.5^{cd}	5.0^{abcd}	5.0^{abcd}	4.0^{bcd}	3.0^{d}	5.5^{abcd}
Iron (ppm)	64.0^{abcd}	68.5^{ab}	66.0^{abcd}	65.5^{abcd}	74.5^{a}	58.5^{bcd}	52.0^{d}	52.0^{d}	67.0^{abc}	53.5^{d}	72.5^{ab}	63.5^{abcd}
Manganese (ppm)	23.0^{c}	17.5^{def}	22.0^{c}	15.5^{fg}	19.5^{cde}	13.5^{g}	27.5^{b}	16.0^{efg}	18.0^{def}	12.5^{g}	32.0^{a}	20.5^{cd}
Zinc (ppm)	32.5^{ab}	33.0^{ab}	34.0^{ab}	36.0^{a}	35.0^{ab}	30.5^{ab}	29.0^{b}	29.0^{b}	29.5^{b}	29.0^{b}	35.0^{ab}	32.5^{ab}
Copper (ppm)	8.5^{ab}	8.5^{ab}	9.5^{a}	9.0^{ab}	7.5^{ab}	7.0^{ab}	6.5^{ab}	7.5^{ab}	6.5^{ab}	5.0^{b}	5.0^{b}	9.0^{ab}
Aluminum (ppm)	ND	ND	ND	ND	3.0^{a}	9.5^{a}	9.0^{a}	7.0^{a}	15.5^{a}	3.5^{a}	5.5^{a}	15.5^{a}
Phytate (mg/g)	12.4^{b}	13.5^{a}	12.6^{b}	13.8^{a}	ND	ND	ND	ND	ND	ND	ND	ND

Table 2.09. Nutrient content of cooked TZ-27 and TZ-37 seed. Values represent 2 replicates. Any values with the same letter within a row are not statistically significant at $p < 0.05$, and values with ND indicate no data was available for that data point.

APPENDIX B

CHAPTER 2 SUPPLEMENTAL FIGURE AND TABLES

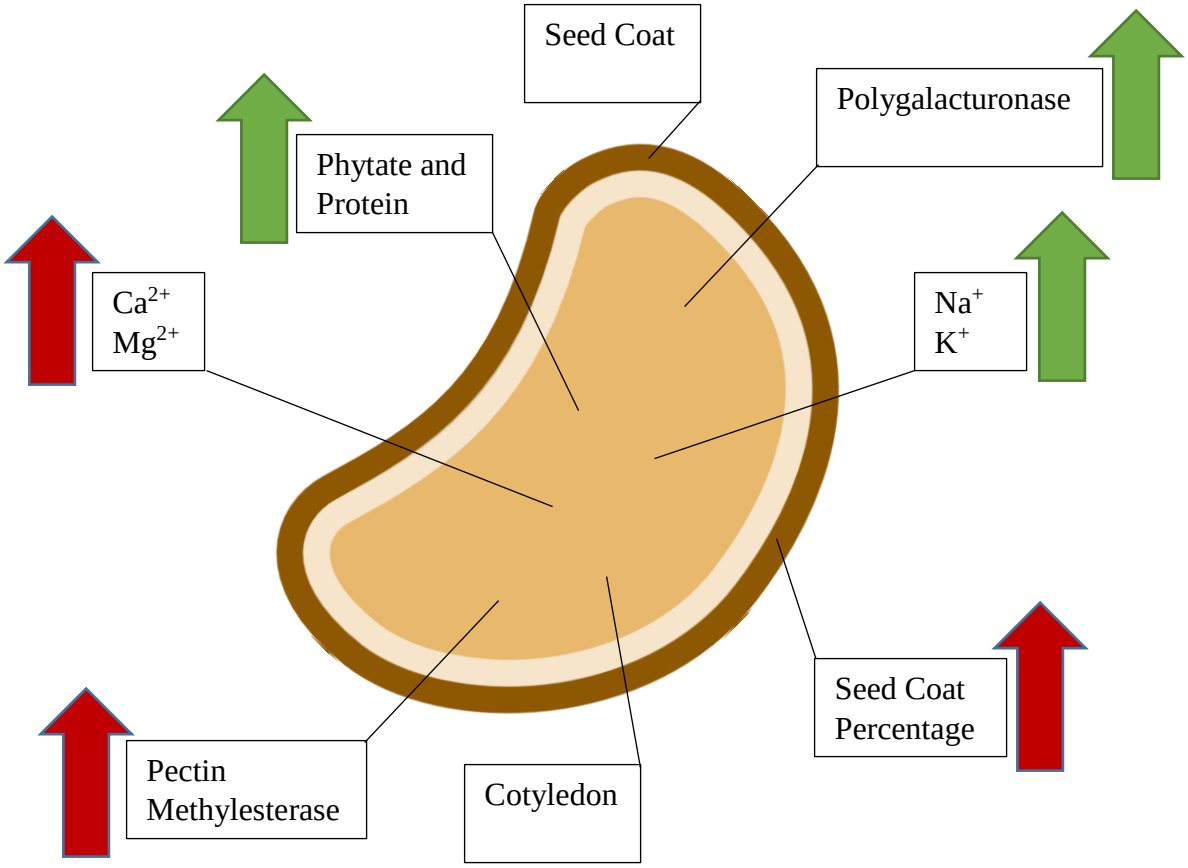

Figure S2.01. Summary of traits that effect cooking time in TZ-27 and TZ-37 when soaked prior to cooking in distilled water. Red arrows indicate factors that lead to longer cooking times, while green arrows show factors that result in shorter cooking times.

Country	Location	Cooking Time (min)			
		Drought		No Drought	
		TZ-27	TZ-37	TZ-27	TZ-37
U.S.A.	**Isabela**	60.0^{b}	34.2^{d}	60.8^{b}	30.2^{de}
U.S.A.	**Pullman**	44.6^{c}	25.3^{ef}	43.2^{c}	23.9^{f}

Table S2.01. Cooking time following a 12 h soak in distilled water for TZ-27 and TZ-37 under drought and no drought conditions. Drought stress was induced by not irrigating, whereas the no drought treatment was irrigated throughout the growing season. Each value is an average of two replicates, and columns or rows with the same letter indicate no significant difference at $p < 0.05$.

	100 Seed Weight	100 Soaked Seed Weight	Hydration Capacity	80% Cooking Time
100 Dry Seed Weight				
100 Soaked Seed Weight	1.0***			
Hydration Capacity	-0.2	-0.1		
80% Cooking Time	-0.4*	-0.4**	-0.4*	

Table S2.02. Pearson correlations of traits measured for TZ-27 and TZ-37 after soaking and cooking in distilled water. Correlations significant at * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.0001$. No asterisk represented no statistical significance.

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CHAPTER 3

QTL ANALYSIS OF AGRONOMIC CHARACTERISTICS AND THEIR RELATIONSHIP WITH DRY BEAN COOKING TIME IN AN ANDEAN RECOMBINANT INBRED LINE POPULATION

ABSTRACT

Cooking time is an important consumer trait in dry bean. A recombinant inbred line population was developed to better understand the inheritance of cooking time in dry bean as there is a lack of research in this area. Days to flower, days to maturity, and seed yield were also examined in the TZ-27 x TZ-37 RIL (TT) population that was grown in Arusha and Morogoro in Tanzania in 2016 and 2017 to determine if cooking time was related to these traits. Morogoro typically has high temperatures around 30 °C, while highs in Arusha are around 25 °C during the growing season. Morogoro also had greater amounts of clay in the soil, but less of every other soil component measured except Mn compared to Arusha. Across growing seasons and environments, days to flower ranged from 28 to 40 days, and days to 50% maturity ranged from 61 to 90 days. Seed yield was also variable within the population with a range of 58.5 to 3374.7 kg ha^{-1}. Yield inversely correlated with cooking time in all but one tested environment. All tested traits varied significantly by genotype ($p < 0.0001$) and location ($p < 0.0001$). Days to flower, for example, was delayed by 3 and 4 days in 2016 and 2017 respectively, compared to Morogoro. The TT population was used to generate a genetic map of 1137.1 cM with one SNP marker every 0.5 cM on average. Multiple QTL were found for each trait and co-localization among some QTL was observed. DF2.1 (days to flower) co-localized with SY2.6 (seed yield). These QTL explained 8.8 and 15.3% of variation for their respective trait, and these traits were inversely correlated with cooking time such that later flowering and

higher yielding lines cooked faster. Also, DM8.2 (days to maturity) and SY8.2 co-localized explaining 9.9 and 14.1% of trait variation respectively. DM11.1 and CT11.1 (cooking time) co-localized. These traits were inversely correlated in both years in Morogoro such that fast cooking lines matured later.

INTRODUCTION

In developing countries, especially in East Africa, legumes are a dietary staple (Bouchenak and Lamri-Senhadjii, 2013; Taiwo et al., 1998, Uebersax, 2006). Cooking time in dry bean is an important consumer trait, and it is often cited as the main factor second only to cost in how often beans are consumed (Chege et al., 2016; Leterme and Muuoz, 2002, Winham, 2017). There is a physical cost to cooking as well in developing countries. Women and children are mainly responsible for cooking and gathering firewood, which is the main fuel source (Hoffman et al., 2015; Munalula and Meincken, 2009; Adkins et al., 2012, Uebersax, 2006). Firewood satisfies 90% of Tanzania's energy requirements followed by 8% from petroleum products, and 2% from electricity (Sosovele, 2010). This demand for firewood is reflected in the high per capita use of 1.8 cubic meters of firewood in Tanzania alone (Drigo, 2006). This necessity for fuelwood has contributed to a deforestation rate as high as 500,000 ha annually in Tanzania (Abdallah and Monela, 2007).

Gathering of firewood can affect education as well. A case study in two Tanzanian villages provided evidence that time spent gathering firewood subtracts from learning time (Levison et al., 2017). This can be caused when the school asks student to sacrifice class time to gather firewood, or when the student cannot attend school because they need to gather firewood for the family (Levison et al., 2017). The task of gathering firewood was also disproportionally assigned to younger girls compared to boys or older girls (Levison et al., 2017). When

gathering firewood, women and children travel up 12.9 km requiring 6 hours per week to collect firewood (Adkins et al., 2012). Combustion of firewood indoors releases many pollutants such as CO, CO_2, and particulates that can cause direct health issues such as inflamed airways, or indirectly harm the person by exacerbating a pre-existing illness such as tuberculosis (Edwards et al., 2004, Wang and Daun, 2005, Saksena et al., 1992, Smith et al., 2000). These smoke related issues lead to 1.5 million deaths per year (World Health Organization, 2006).

The main areas of bean production in Tanzania are in the north (around Arusha), the Great Lakes Region, and the Southern Highlands (Fig. 3.08) because these areas have reliable rainfall and moderate temperatures (Binagwa et al., 2016, Ronner and Giller, 2013). Ranked 7th in the world for bean production, 13% of the amount produced is exported, and of that amount most bean export comes from the northern region both because of the climatic conditions for bean production and because of proximity to an international airport at Kilimanjaro (Ronner and Giller, 2013). Export of beans from Tanzania is to nearby countries such as Malawi, Kenya, and Uganda, but also includes export outside of Africa (Mugisha, 2016). Arusha was chosen as one location to grow the TZ-27 by TZ-37 (TT) recombinant inbred line (RIL) population because it is in a major bean growing region, has moderate climate and reliable rainfall to promote bean production, and it is the site of an internationally renowned research station: Selian Agriculture Research Institute (SARI). Morogoro, in contrast, has low fertility soil, a warmer climate (average 28.5 °C during the growing season), and less reliable rainfall. Morogoro does have an excellent agriculture university (Sokoine University of Agriculture) with research plots available for testing. Morogoro was specifically chosen for these reasons as

it represented a poor production area for beans where traits including cooking time and yield could be assessed under suboptimal conditions.

The TT RIL population was explored for days to flower, days to maturity, and yield with the goal of learning more about the population and identifying one or more mechanisms present in the population that effects cooking time as well as attempt to find traits predictive for cooking time. Days to flower and days to maturity have been indirectly linked to cooking time through protein content. It has been proposed in the literature that beans that require a longer time to flower have additional time in a vegetative state where N fixation can occur, and a longer time to maturity meant the plant had additional time to mobilize N to the seed (Heilig et al., 2017). Higher amounts of protein have been shown to be linked with faster cooking times (Wiesinger et al., 2016, Shimelis and Rakshit, 2005, and Afzal et al., 2003). Therefore, it was of interest to see if this trend held within a RIL population segregating for cooking time and seed protein concentration. Yield was measured because it is an important trait to consider when studying any trait as a fast cooking variety with poor yield would likely not succeed in the marketplace.

MATERIALS AND METHODS

Plant Material

TZ-27 (Incomparable) and TZ-37 (W6 16488) were used as parents of the TT RIL population. Both parental lines were collected in Tanzania, have a type I growth habit, and brown kidney shaped beans. The population was developed in the USDA-ARS greenhouse in East Lansing, MI from 2014-2016. Six generations of single seed descent followed by seed increase were used to develop the population. The TT RIL population and parental lines were grown in 2016 and 2017 in an RCBD with two replications in Arusha and Morogoro in Tanzania. Seed was

planted in 5 m long rows (1 seed every 10 cm) with 50 cm of space between adjacent rows. In 2016, seed was planted in Arusha on Apr 14 and in Morogoro on Apr 11. Harvesting occurred on July 26 in Arusha and July 1 in Morogoro. In 2017, planting at both locations occurred on Apr 5 and harvesting in Arusha occurred on July 19, and in Morogoro, harvesting was performed on July 7. All planting, harvesting, and threshing was done by hand. Within six months of harvest, cooking time was determined from seed stored in ambient conditions. Weather data for both locations were obtained from Sokoine University of Agriculture. Soil data was obtained by sampling 3 random sites at each field location and combining the samples. All soil analyses were performed at Sokoine University of Agriculture.

Seed Yield

Yield was calculated by using the equation below. The resulting unit was kg ha^{-1}.

$$\left(\frac{\left(\frac{\text{Weight of seed (kg)}}{\text{Area of plot (m}^2\text{)}}\right)}{10{,}000\ \text{m}^2}\right) \mathbf{X} \left(\frac{100 - \text{Moisture content of seed}}{100 - 14\%\ \text{Moisture}}\right)$$

Seed weight was obtained on harvested seed and converted into kg. Area of plot was calculated as the area of a row which was 0.5 m wide and 5 m long. In 2016, when 5 m of seed was not available, the length was adjusted as needed. For example, if only 25 seed were available to plant, area of plot was 0.5 m times 2.5 m.

Moisture Content of Seed

Moisture was measured using a GAC 2100 moisture meter (DICKEY-john, Auburn, IL) unless the amount of seed was insufficient for the machine to measure, in which case, a John Deere

Moisture Chek Plus machine (SW08120) (Deere and Company, Moline, IL) was used to measure moisture.

Days to Flower and Maturity

Days to flower was recorded as the number of days from planting when 50% of the plants within a plot had at least one open flower. Days to physiological maturity was recorded when 50% of the plants in a plot began to have yellow pods. In 2016, it was not possible to obtain maturity data for lines in Arusha. In 2017, time to maturity was recorded when a plot had at least 85% of the plants containing yellow pods because physiological maturity time could not be obtained in Arusha.

Cooking Time

Harvested seed was equilibrated before cooking by storing in a controlled atmosphere storage cabinet. A sample of 25 dry seeds were soaked in distilled water for 12 h and cooked in distilled water at 100 ºC with a Mattson bean cooker (also known as a pin drop cooker) (Customized Machining 246 and Hydraulics Co., 247 Winnipeg, Canada). The elapsed cooking time was recorded as the time required for 80% of the pins to pierce the seeds. This technique has been utilized by others to test cooking times (Paredes-López et al., 1988; Alfredo, 1993; Wang and Daun, 2005).

DNA Extraction and GBS

DNA was extracted from young trifoliate leaf tissue from a single plant for each of the 161 RIL lines and the two parental lines (TZ-27 and TZ-37). The Macherey-Nagel NucleoSpin Plant II kit was used to obtain DNA from leaf tissue. Preparation of DNA for genotyping by

sequencing was adapted from the method of Schröder et al. (2016). Barcoded adapters and primers were produced by IDTDNA (Coralville, IA). Specifically, the enzymes Mse I and Taq α1 were used in a double-digest of DNA. Mse I and Taq α1 are methylation insensitive allowing for a more uniform distribution of fragments across euchromatic as well as heterochromatic regions (Schröder et al., 2016). In addition, use of a double digest decreases the chance of generating fragments ligated to only common adapters and barcoded adapters (Schröder et al., 2016). Annealed samples were then amplified pooled (each uniquely barcoded) and amplified via PCR. Validation was performed on a 2% agarose gel with a 100 bp ladder. The final DNA product was analyzed by Michigan State University genomics core facility (East Lansing, MI) using the 200 bp single-end reads on two lanes of an Illumina Hi-Seq 2500.

SNP Calling and Map Construction

FastQC files were cleaned using Cutadapt. Bowtie2 was used for alignment to the reference genome Pvulgaris_442_v2.0 from Phytozome (version 11). NGSEP was used to filter the vcf file data. NGSEP was also used for SNP calling by removing any markers with more than 50% missing data and using a minimum allele frequency (MAF) of 0.05. In addition, markers that were not polymorphic in the parents or that showed segregation distortion (those markers that were greater than 3.125% heterozygous) were removed. Remaining markers were analyzed using order, compare, and ripple commands in MapDisto (version 1.7.7) to generate a genetic map after applying the Kosambi function (Lorieux, 2012).

QTL Analysis

Phenotypic and genotypic data were analyzed to discover QTL using MapDisto (version 1.7.7) (Lorieux, 2012), and the resulting file was analyzed using QTL Cartographer (version 2.5) (Wang, Basten, and Zeng, 2005). Composite Interval mapping with 1000 permutations, forward and backward regression, and a walking speed of 1.0 cM was used to determine threshold at $p < 0.05$. That threshold was used to determine significance of QTL for each trait. Lines that produced no reads, few reads, or no high-quality reads were not analyzed further (lines 60, 127, 141, and lines 145 through 156). Mapchart (version 2.3) was used to display the final map with QTL data included (Voorrips, 2001).

Statistical Analyses

SAS 9.4 (SAS Institute Inc., Cary, NC) was used to perform a proc mixed analysis to generate an ANOVA. The model used for the ANOVA was (Trait) = Line | Location | Year. Significant differences were observed for each year and location, so each location and year was analyzed separately. Proc corr was used to generate Pearson correlations and proc means was used to generate means and ranges.

RESULTS

Weather and Soil Data

Maximum and minimum temperatures were recorded during both bean growing seasons in 2016 and 2017. Except for 4 days in 2017, the maximum temperature in Morogoro was, on average, 5.1 °C and 4.3 °C higher than Arusha in 2016 and 2017 respectively (Fig. 3.01). The

minimum temperature in Arusha was, on average, 2.4 °C and 3.6 °C lower than Morogoro in 2016 and 2017 respectively except for 11 days across the two years of the study (Fig. 3.01).

Morogoro received more rain (712.7 mm) than Arusha in 2016 (124.4 mm) to the point where the field was flooded shortly after planting. Additionally, rainfall was more consistent across the growing season in Morogoro compared to Arusha in 2016 (Fig. 3.02). In 2017, both locations received a similar amount of rain (453.4 mm in Arusha and 439.2 mm in Morogoro).

Soil quality in Morogoro was lower in Arusha for nearly every soil component tested (Table 3.06). For example, Na (a monovalent cation) was 2.5 to 3.7 times greater in Arusha than Morogoro in 2016 and 2017 respectively (Table 3.06). Nitrogen in the soil was also higher in Arusha (1.5 times greater on average) than Morogoro. In addition, other measures of soil fertility including electrical conductivity and cation exchange capacity were 2.0 and 2.1 times greater, on average, in Arusha than in Morogoro (Table 3.06). Morogoro only had two soil components that were higher than Arusha which was clay percentage (4% greater in Morogoro), and Mn (3.1 to 4.5 times greater in Morogoro) (Table 3.06).

Days to Flower

TZ-27 and TZ-37 were not significantly different for days to 50% flower. Both lines required 30 to 36 days to flower. Flowering time was variable within the TT RIL population with some lines flowering 7 to 10 days sooner than later flowering lines (Fig. 3.04, Table 3.02). Flowering time was consistent across years such that both locations contained lines that flowered one day sooner in 2017 than 2016 (Table 3.02). Additionally, flowering time began 4 days later in Arusha than Morogoro in both years (Fig. 3.04, Table 3.02). Days to flower were

inversely correlated with cooking time in Morogoro in 2016 and 2017 (r = -0.2 for both years) (Table 3.05).

Days to Maturity

Days to 50% maturity could only be assessed for the Morogoro location in both years, while in Arusha, it could not be measured in 2016 and 85% maturity was recorded in 2017. TZ-37 matured in 71 days in both years, while TZ-27 took 65.5 days in 2016 and 63.5 days to reach maturity in 2017 (Table 2). Parental lines were significantly different for maturity in Morogoro in 2017. Maturation time was significant within the population, but days to maturity was not significant across years possibly because 50% maturity could not be measured in Arusha in either year. (Fig. 3.05, Table 3.01, Table 3.02). The average for maturity times were almost identical in the population in Morogoro (69.1 days in 2016 and 68.8 days in 2017) (Table 3.02). Days to 50% maturity inversely correlated with cooking time with r = -0.3 in 2016 and r = -0.2 in 2017.

Seed Yield

Seed yield was significantly different between the parents with TZ-37 having a higher yield than TZ-27 in each environment tested (Table 3.02). The largest difference in yield was in Morogoro in 2017 where TZ-27 yielded 72.1 kg ha^{-1} and TZ-37 had a yield of 469.1 kg ha^{-1} (Table 3.02). The RIL population was also significantly different in each location and year (Fig. 3.06, Table 3.02). Specifically, average seed yield was 390.3 kg ha^{-1} and 768.7 kg ha^{-1} in Arusha and Morogoro respectively in 2016 (Table 3.02). That result could be because Morogoro received rain later in the growing season in 2016 that did not occur in 2017 (Fig. 3.02). In 2017, the average yield was 2394.3 kg ha^{-1} and 398.2 kg ha^{-1} in Arusha and

Morogoro, respectively. Yield inversely correlated with cooking time in every environment except Arusha 2016 (r = -0.1 to -0.3) (Table 3.05).

Cooking Time

Regardless of location or year, TZ-27 always required more time to cook than TZ-37 (Table 3.02). Except for Arusha in 2017 (41.0 min), TZ-27 required at least an hour to cook, while TZ-37 reached an 80% cooking time in 27.7 to 35.6 min (Table 2). A range of cooking times were observed in the TT population with some lines cooking faster than TZ-37, while others required more time than TZ-27 (Fig. 3.03, Table 3.02). The population showed variation in cooking time for each year and location studied where lines grown in Morogoro in both years resulted in a 15 to 18 minute increase in mean cooking time compared to lines grown in Arusha (Fig. 3.03, Table 3.02).

QTL Analysis

Linkage Map

A total of 48,244 SNPs were discovered over the entire genome. SNPs within repetitive regions were removed as those regions mapped to more than one area of the genome, and after excluding those SNPs, 12,220 unique SNPs remained. Additional filtering removed monomorphic SNPs as well as ones that were not informative. A total of 2,427 SNP markers remained still allowing for dense coverage of the genome (Table 3). The average density was 1 marker every 0.5 cM (Table 3.03), and marker density ranged from 1 marker every 0.2 cM on chromosome Pv02 to 1 marker every 1.2 cM on chromosome Pv01 (Table 3.03). The genetic map developed was 1137.14 cM in size (Table 3.03).

Days to Flower

Six flowering time QTL were found on chromosome Pv02, Pv03, Pv05, and Pv08 (Table 3.04). Each QTL was discovered in environment. DF2.1 was identified in Arusha in 2016 and spanned a physical region of 27,079,491 to 31,152,329. The peak LOD score was 8.8, the additive effect was 0.4 days, and DF2.1 explained 8.8% of variation for flowering time (Table 3.04). DF2.2 explained 18.2% of variation for cooking time in Morogoro in 2016. The peak LOD score was 6.8 with an additive effect of 0.7 days, and a physical size of 33,517,125 to 34,443,830 bases. DF3.3 was identified in Morogoro in 2017, explained 5.9% of variation for flowering time, and had a peak LOD score of 2.3 (Table 3.04). DF3.3 had an additive effect of 2.1 days and the QTL spanned the region from 2,053,860 to 4,652,206 bases. DF5.5 was identified in Morogoro in 2017 and explained 8.0% of flowering time variation. The LOD score was 3.3 and the additive effect was 2.5 days (Table 3.04). The QTL spanned a region from 19,480,437 to 37,281,421 bases. DF8.1 explained 9.0% of phenotypic variation in Arusha in 2016, and the DF8.1 spanned a region from 11,800,414 to 14,014,183 bases. The peak LOD score was 9.0 and the additive effect was 1.5 days (Table 3.04). DF8.2 explained 8.1% of flowering time variation in Arusha in 2016, and the QTL spanned a distance of 16,158,103 to 17,014,191 bases. The peak LOD score was 3.9 and the additive effect was 1.1 days (Table 3.04). QTL for flowering time explained between 5.9 and 18.2% of the variation observed for this trait (Table 3.04). Co-localization was discovered for DF2.1 and SY2.6 as well as DF8.1 and SY8.1, which could explain the inverse correlation in Morogoro in 2016 such that lines that flowered sooner had higher yields (Fig. 3.07, Table 3.05).

Days to Maturity

Three QTL for days to maturity (DM8.2, DM9.1, and DM11.1) were discovered on chromosomes Pv08, Pv09, and Pv11 and all were found in Morogoro in 2016 (Table 3.04). DM8.2 explained 9.9% of variation for maturation time, had a peak LOD score of 3.9, and the additive effect was 1.1 days. The physical distance of this QTL was from 16,870,937 to 17,014,191 bases (Table 3.04). DM9.1 had a peak LOD score of 3.6, an additive effect of 1.0 days, and explained 8.5% of phenotypic variation. The physical distance of DM9.1 was from 16,197,475 to 16,641,845 bases (Table 3.04). DM11.1 spanned a region from 3,153,228 to 3,635,923 bases. The QTL had an LOD score of 4.6, explained 10.9% of variation, and had an additive effect of 1.2 days (Table 3.04). In contrast to flowering time QTL, all days to maturity QTL covered smaller physical distances on the *P. vulgaris* genome (less than 0.5 Mb in size) (Table 3.04). DM8.2 and SY8.2 were overlapping QTL that were both discovered in Morogoro in 2016 (Fig. 3.07, Table 3.04). A direct correlation was discovered between yield and days to maturity in Morogoro in 2017 such that later maturing lines had greater yield (Table 3.05).

Seed Yield

Chromosomes Pv02, Pv04, Pv08, and Pv11 contained a total of 9 QTL for seed yield of which two-thirds came from the fast cooking parent (TZ-37) (Table 3.04). SY2.4 and SY2.5 were found in Arusha in 2017. SY2.4 explained 20.7% of variation for seed yield, had an LOD score of 9.7, and an additive effect of 262.2 kg ha^{-1}. The physical region of the QTL was from 12,896,409 to 13,046,995 bases. SY2.5 spanned a physical distance of 14,919,797 to 23,553,052 and had a peak LOD score of 8.9 (Table 3.04). SY2.5 explained 19.0% of seed yield variation and an additive effect of 249.3 kg ha^{-1}. SY2.6 was identified in Morogoro in

2017 and explained 15.3% of phenotypic variation. The QTL had an LOD score of 6.0, an additive effect of 145.1 kg ha^{-1}, and a physical distance of 26,833,150 to 29,343,972 bases (Table 3.04). SY2.4 through SY2.6 could be part of one large QTL as SY2.4 and SY2.5 were separated by 187.3 kb and SY2.5 and SY2.6 were separated by 328.0 kb (Table 3.04). SY4.2 was found in Arusha in 2017, had a peak LOD score of 5.1, and explained 9.7% of variation for the trait. SY4.2 had an additive effect of 289.4 kg ha^{-1} and spanned a region from 13,160,833 to 16,849,199 bases (Table 3.04). SY8.1 was identified in Arusha in 2016 and explained 6.8% of variation of seed yield. The peak LOD score was 2.9, the additive effect was 152.7 kg ha^{-1}, and the QTL spanned a distance of 11,218,958 to 12,970,064 bases (Table 3.04). SY8.2 explained 14.1% of variation for seed yield in Morogoro in 2016. The QTL explained 14.1% of phenotypic variation, had a peak LOD score of 3.3, and an additive effect of 204.9 kg ha^{-1}. SY8.2 spanned a distance of 61,705,467 to 62,203,862 bases (Table 3.04). SY11.1 was identified in Arusha in 2017 and had a physical location from 797,504 to 1,323,151 bases. The QTL explained 7.8% of variation for seed yield, had an additive effect of 159.1 kg ha^{-1}, and a peak LOD score of 3.6 (Table 3.04). SY11.3 and SY11.4 were identified in Morogoro in 2016. SY11.3 spanned a physical distance of 8,689,907 to 9,060,175 bases, explained 14.1% of seed yield variation, had an LOD score of 2.5, and an additive effect of 333.8 kg ha^{-1} (Table 3.04). SY11.4 explained 13.2% of seed yield variation, had an additive effect of 323.7 kg ha^{-1}, an LOD score of 2.7, and a physical location from 39,691,193 to 40,394,549 bases (Table 3.04).

Cooking Time

DF3.3 was less than 1 Mb from CT3.1 (Table 3.04), and DF5.5 was separated from CT5.1 by 6.1 cM. These close relationships could explain the inverse correlation seen in Morogoro between cooking time and flowering time (Table 3.05) even though the QTL for flowering and

cooking time did not overlap. DM11.1 and CT11.1 co-localized and explained 10.9 and 15.0% of variation for the respective traits (Table 3.04). No co-localization was observed for seed yield and cooking time though SY11.2 and SY11.3 flanked CT11.1 (Fig. 3.07). Seed yield correlated with cooking time in 3 of 4 environments suggesting that though overlap was not observed, linkage between the two traits could still exist.

DISCUSSION

Environmental Differences

The maximum temperature was almost always higher in Morogoro than Arusha and the same trend was noted for minimum temperatures at both locations. Specifically, the maximum temperature in Morogoro climbed to 32 °C at times during the growing seasons of 2016 and 2017. Multiple lines of independent research point to seed being stored at higher temperatures resulting in longer cooking times, so it is possible that this phenomenon would apply to seed that is developing at higher temperatures (Hentges et al., 1990, Maurer et al., 2004, Coelho et al., 2007). The higher temperature in Morogoro could explain why lines grown in Morogoro required 1.4 to 1.5 times as long to cook compared to lines grown in Arusha on average.

The Morogoro study site also serves as a location to assess how this population can perform in harsher growing conditions such as high temperature and inconsistent rainfall. Current models of climate change predict a global temperature increase of 1.5 to 2.0 °C by 2040 to 2050 depending on the model (Karmalkar and Bradley, 2017). In Tanzania, that change in temperature can result in a greater than 20% loss of agricultural productivity by 2050 in many areas of the country where beans are primarily produced (Thornton et al., 2009). The discovery of lines that can yield over 1000 kg ha^{-1} and can also cook quickly could result in outcomes

such as less wood burned and decreased deforestation (contributors to global warming), increased bean consumption and decreased inhalation of combustion products (health benefits), and increased selling and export of seed (financial benefits). Increasing yield in Tanzania is important as demand is predicted to outpace supply by 100,000 metric tonnes by 2020 (Binagwa et al., 2016).

Growth in Arusha showed how well this population could perform in a bean growing region of the country. With yields as high as nearly 3400 kg ha^{-1} and cooking times less than half an hour, the population showed that a highly desired consumer trait and an important agronomic trait can be expressed together, which can lead to the production of varieties preferred by farmers and desired by consumers. All dry bean production in Tanzania averaged to 1035 kg ha^{-1} (FAOSTAT, 2018) so lines yielding greater amounts than that would likely be preferred by growers. As a comparison, the world dry bean production average is 913 kg ha^{-1} and the United States averaged 2013 kg ha^{-1} in 2016 (FAOSTAT, 2018). The highest yielding lines in this study exceeded average production in the US, showing an improvement of the average yield in Tanzania. It should be noted that the average seed yield in Morogoro outperformed seed yield in Arusha in 2016. This was an unexpected result that was likely caused by an unusual amount of precipitation in Morogoro in 2016. An average of 34 years of data leading up to the late 1990's revealed that typical rainfall is 17 mm for the entire month of June (Mahoo et al., 1999). In June of 2016, 485.9 mm of rain was recorded in Morogoro (28.6 times greater than the average). The additional precipitation during the growing season likely caused the increased yield in Morogoro compared to Arusha, which received 11.2 mm of rain that same June.

Multiple differences in soil components were also observed (Table 3.06). Electrical conductivity, pH, % silt, % sand, Zn, Mn, Cu, N, cation exchange capacity, Mg, K, and Na were all higher in Arusha than Morogoro for both years studied, while Morogoro only had a greater percentage of clay than Arusha in both years. These findings point to Arusha being a more fertile and nutrient rich environment, while Morogoro contains less nutrients, which agrees with work by Amijee et al. (1998), and the increased clay content increases the chance of field waterlogging and decreases porosity of the soil. In winter wheat, waterlogging of a clay soil led to oxygen deprivation that was more severe than in sandy soil, and components of yield were more severely reduced in waterlogged clay soil compared to sandy soil (Cannell et al., 1984). Seed yield was also reduced in mung bean when waterlogged. In susceptible genotypes, 3 days of waterlogging resulted in a 20 to 40% loss of yield, whereas 9 days of waterlogging led to yield losses from 60 to 80% (Kumar, 2012). Waterlogging issues could be more prevalent in Morogoro as the clay content is greater there than in Arusha. Research into fertilizers and cooking time in chickpea showed that one of the fastest cooking times obtained in the study was in plots that were provided with the most nutrient dense fertilizer included in the study (Mohammadi et al., 2011). Though a plateau effect would likely be observed as the concentration of nutrients increased, the differences in nutrients available in the two locations in the current study could explain why lines grown in Morogoro took 16 – 19 min longer to cook, on average, than lines grown in Arusha.

Days to Flower

A weak inverse correlation of -0.2 was founds in Morogoro for both growing seasons between days to flower and cooking time, which could be explained by the proximity of CT3.1 and DF3.3 (693.0 kb between the two QTL). This correlation agrees with Khattak et al. (2006) who

found an inverse correlation between cooking time and flowering time in chickpea, but contrasts with work by Hamdi et al. (1987) who did not find a significant relationship between flowering time and cooking time in lentil. Heilig (2015) noted that earlier flowering time for beans grown in Puerto Rico compared to East Lansing Michigan also had lower N yield. The explanation given for this result was that a shorter vegetative phase meant the plant did not have as much time to fix N before flower and seed development began acting as a N sink (Heilig et al., 2017). This relationship between flowering time and N is important because current work also showed a relationship between cooking time and protein concentration such that as cooking time decreased, amount of protein increased (Chapter 4).

QTL for flowering time were contributed by TZ-27 except for DF3.3. Candidate genes within the flowering time QTL included cytochrome genes and MADS box proteins, both of which have been related to flowering time in Arabidopsis (Salomé et al., 2011). Though being able to reduce a population based on flowering time as a proxy for cooking time could decrease the number that need to be phenotyped through cooking, current research and contrasting results in the literature suggest this trait is not a reliable predictor for cooking time. At best, the relationship between cooking time and flowering time appears to be environment specific.

Days to Maturity

In every environment where days to 50% maturity was measured, cooking time had a significant inverse correlation (-0.2 [$p < 0.0001$] to -0.3 [$p < 0.01$]) with days to maturity such that longer maturing lines tended to cook faster than lines that matured quickly. This could be seen as an undesirable outcome as lines that require additional time to mature require more time in the field. Sudden weather changes, pests, and pathogens have more time to reduce

yield through temperature swings, drought, and flooding, or consumption or destruction of plant tissue. However, longer maturing lines were found to have increased amounts of N (Heilig et al., 2017). Current work was able to show that increasing amount of protein meant faster cooking times (chapter 4).

QTL for this trait were all found in Morogoro as it was not possible in either year to measure 50% maturity in Arusha. Possible genes that could control maturation time include a cold regulated protein within DM11.1 and a cytochrome gene on DM8.2. Days to maturity had also been found on Pv08, Pv09, and Pv11 by other researchers (Mukeshimana et al., 2014, Papa, 2007, Kelly et al., 2003). Additional research in this area is needed as 50% maturation time could not be recorded in either year in Arusha. If it had been, additional QTL might have been discovered that were in close proximity or overlapped with cooking time QTL. This trait also warrants additional exploration as it was significantly inversely correlated with cooking time in Morogoro for both years.

Seed Yield

Seed yield had weak to moderate inverse correlations (-0.1 to -0.3) with cooking time in every environment except Arusha in 2016. This agrees with other work on this population that showed an inverse correlation (-0.3 to -0.6) between cooking time and seed weight, which is a component used to calculate seed yield.

The greatest number of QTL were found for seed yield and most were contributed by TZ-37. A gene for cellulose synthesis was found on two QTL for seed yield and 4 different QTL contained genes for aquaporins, both of which could increase the weight and effect seed yield (data not shown). This trait did not co-localize with cooking time, but yield QTL were in

proximity with cooking time QTL, which could explain how seed yield and cooking time appear to be inherited together.

Seed yield is an important trait to consider in breeding programs (Beaver and Osorno, 2009). TZ-37 was one of the parental lines used to generate the TT population because of its fast cooking time. It also had one of the highest 100 seed weight in the population. Previous research has suggested that the availability of germplasm that cooks quickly and has high yield can limit the progress of a breeding program (Kelly et al., 1998), but current work identified a germplasm resource that can be used to integrate these traits into a breeding program.

CONCLUSION

The relationship between field traits and cooking time was explored to further understand the TT population and possibly identify one or more traits that could be a predictor of cooking time. Possibly due to the higher growing season temperatures in Morogoro (nearly 29 °C and 5 °C warmer than Arusha on average) cooking time was 15 to 18 minutes longer in Morogoro than in Arusha. With global temperatures possibly increasing by 2 °C in the next 20 years, that could lead to a cycle of increased cooking times resulting in greater firewood consumption for cooking which would then exacerbate global warming further. Increased temperatures can also reduce yield in beans so finding lines in the population that yield high and cook fast in Morogoro could help mitigate issues of yield loss and longer cooking times in areas currently more amenable to bean production.

Days to maturity should be explored further as it was not possible to obtain measurements for every environment. During both years in Morogoro a relationship between cooking time and 50% maturity were found phenotypically with inverse correlations. Genotypically, DM8.2

contained a gene for Pectinesterase, which has been shown to effect cooking time (Garcia et al., 1998), and DM11.1 and CT11.1 co-localized. Seed yield is one of the most important traits a breeder considers when running a breeding program, and the observation that lines with higher yield tend to cook faster should be followed with additional experiments in other environments to better understand the link between these two traits.

APPENDIX

APPENDIX

Chapter 3 Figures and Tables

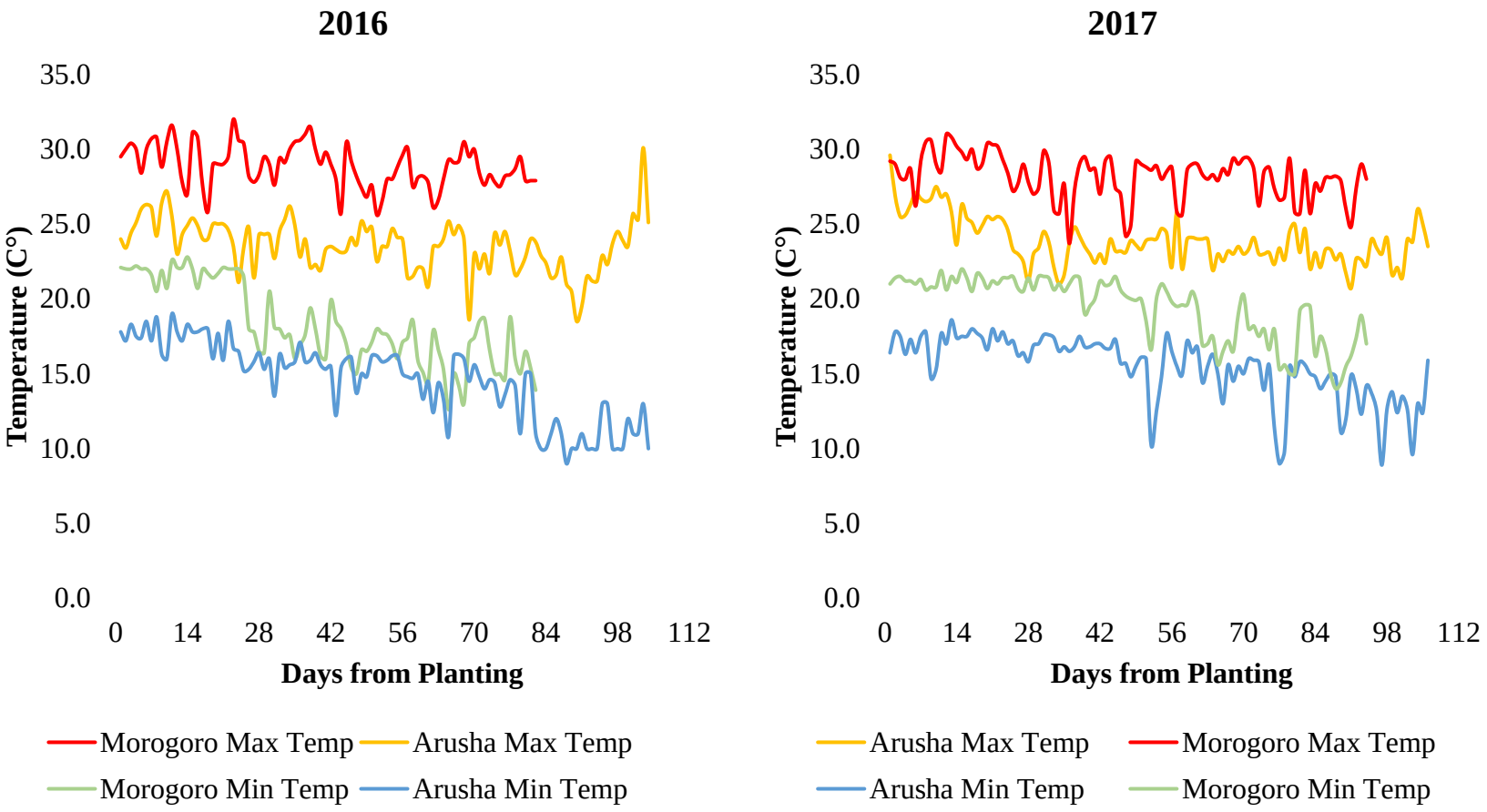


Figure 3.01. **Maximum and minimum temperatures in Arusha and Morogoro in 2016 and 2017.** Weather data was obtained from Sokoine University of Agriculture. Day 0 is the day seed was planted, and measurements were recorded until the day the last lines were harvested at each location.

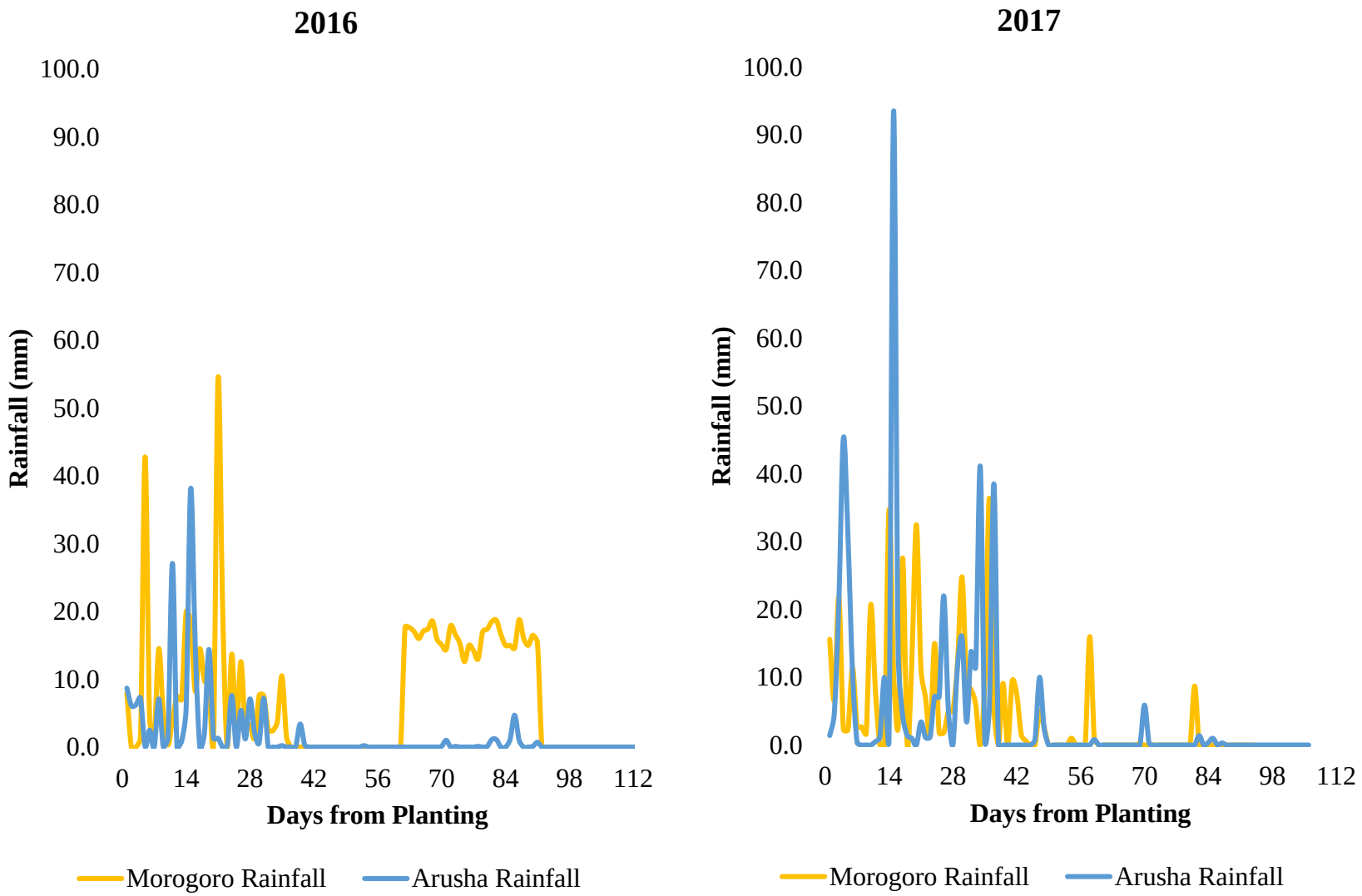


Figure 3.02. **Rainfall in Arusha and Morogoro in 2016 and 2017.** Weather data was obtained from Sokoine University of Agriculture. Day 0 is the day seed was planted, and measurements were recorded until the day the last lines were harvested at each location.

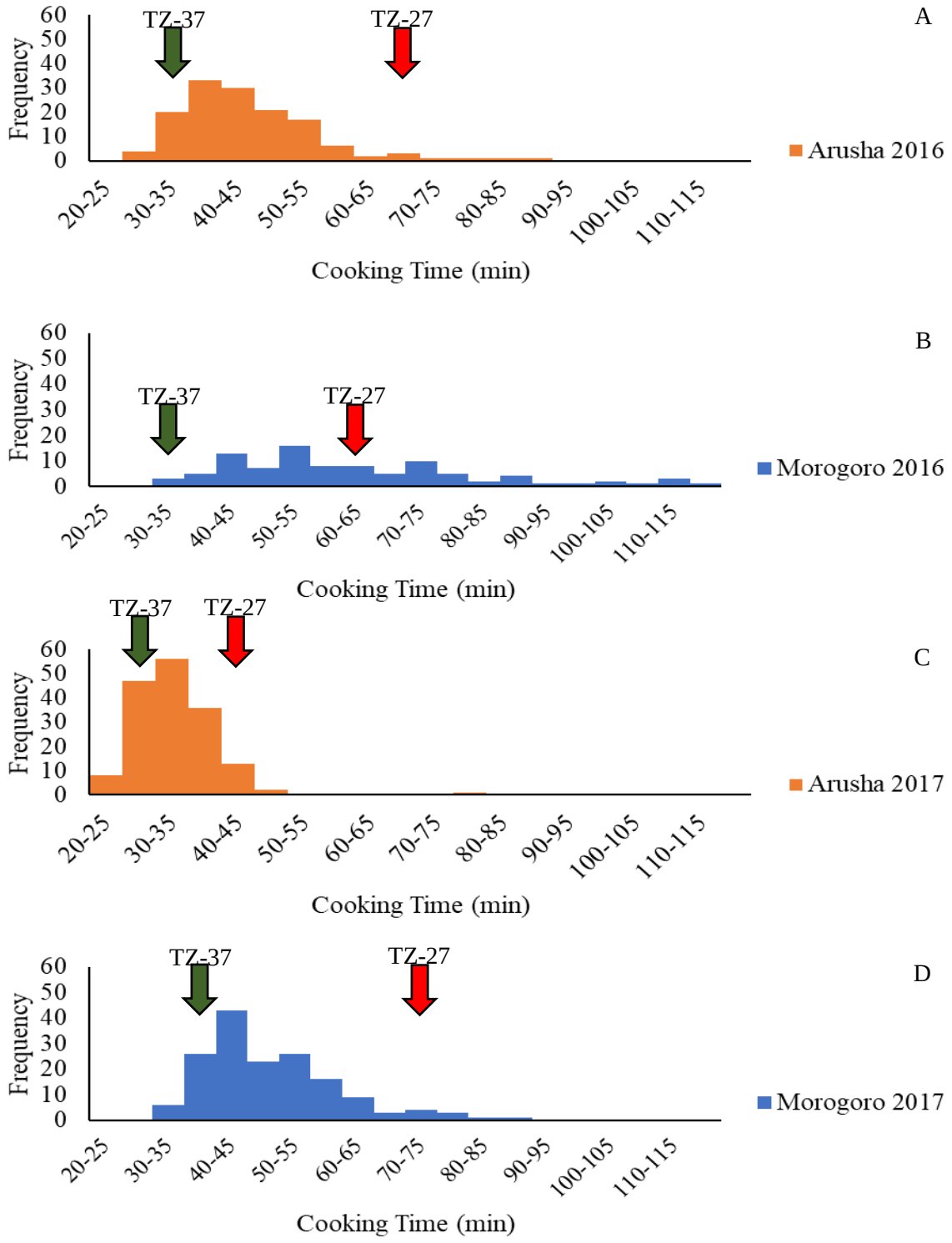


Figure 3.03. Cooking time histograms for beans grown in Arusha and Morogoro in 2016 and 2017. Distribution of cooking times for beans grown in Arusha (A and C) and Morogoro (B and D) in Tanzania in 2016 (A and B) and 2017 (C and D). Frequencies were determined by using the average of 2 replications for each line. Arrows indicate parental values for this trait.

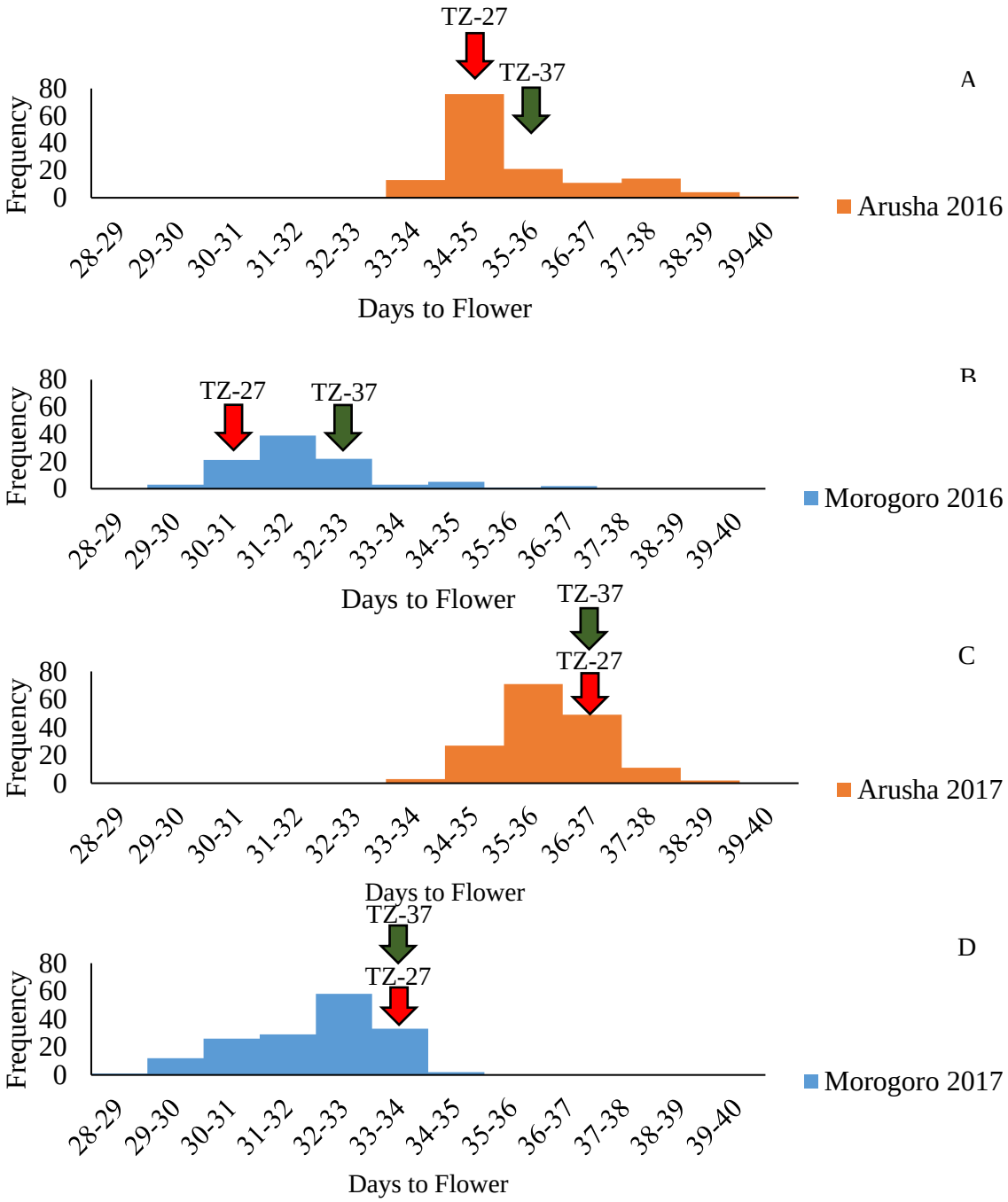


Figure 3.04. Days to flower histograms for beans grown in Arusha and Morogoro in 2016 and 2017. Distribution of flowering times for beans grown in Arusha (A and C) and Morogoro (B and D) in Tanzania in 2016 (A and B) and 2017 (C and D). Frequencies were determined by using the average of 2 replications for each line. Arrows indicate parental values for this trait.

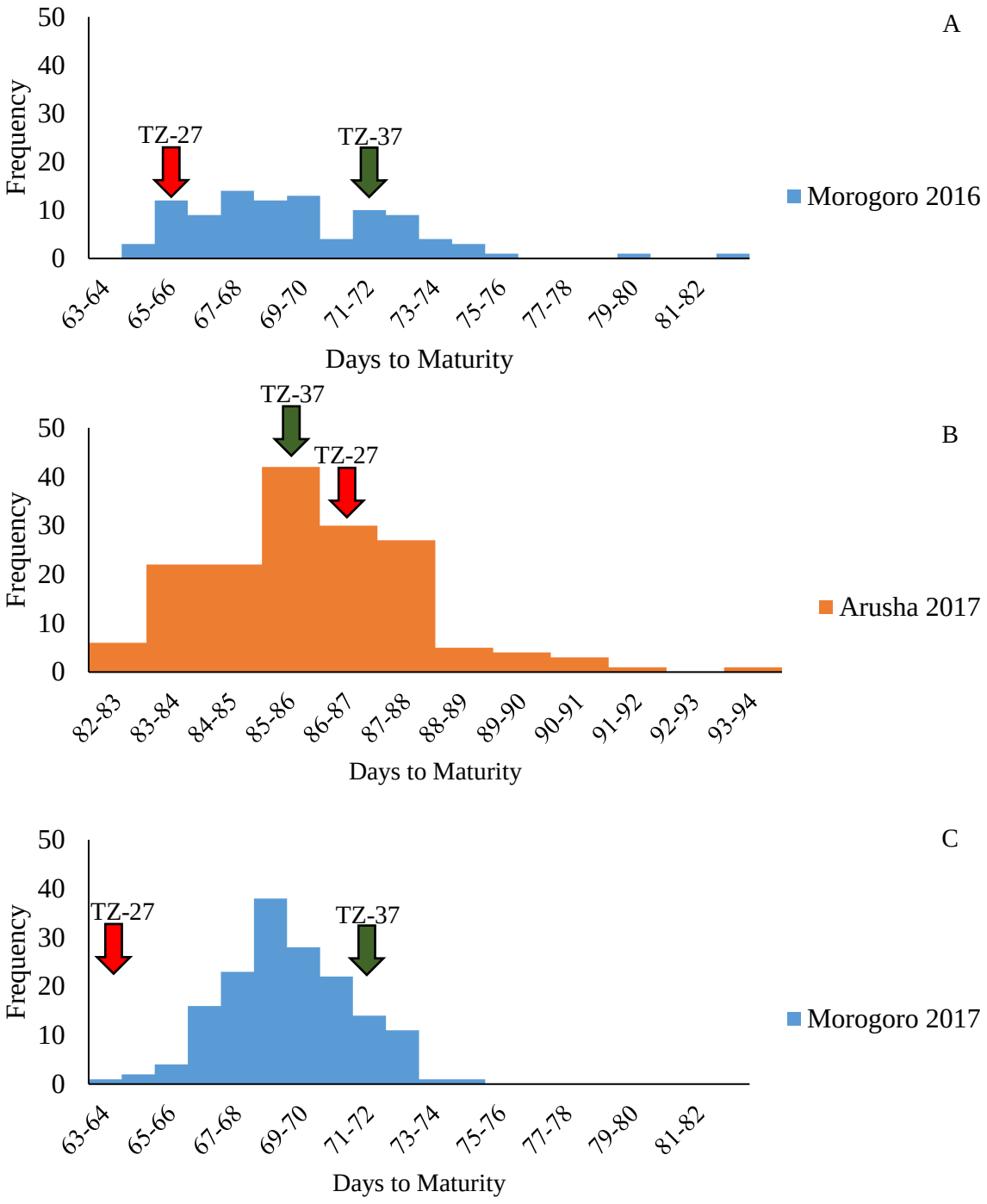


Figure 3.05. Days to maturity histograms for beans grown in Arusha and Morogoro in 2016 and 2017. Distribution of maturity times for beans grown in Arusha (B) and Morogoro (A and C) in Tanzania in 2016 (A) and 2017 (B and C). In Arusha, days to maturity was measured when 85% of the plants reached maturity, while in Morogoro, maturity was recorded when 50% of plants reached maturity. Frequencies were determined by using the average of 2 replications for each line. Arrows indicate parental values for this trait.

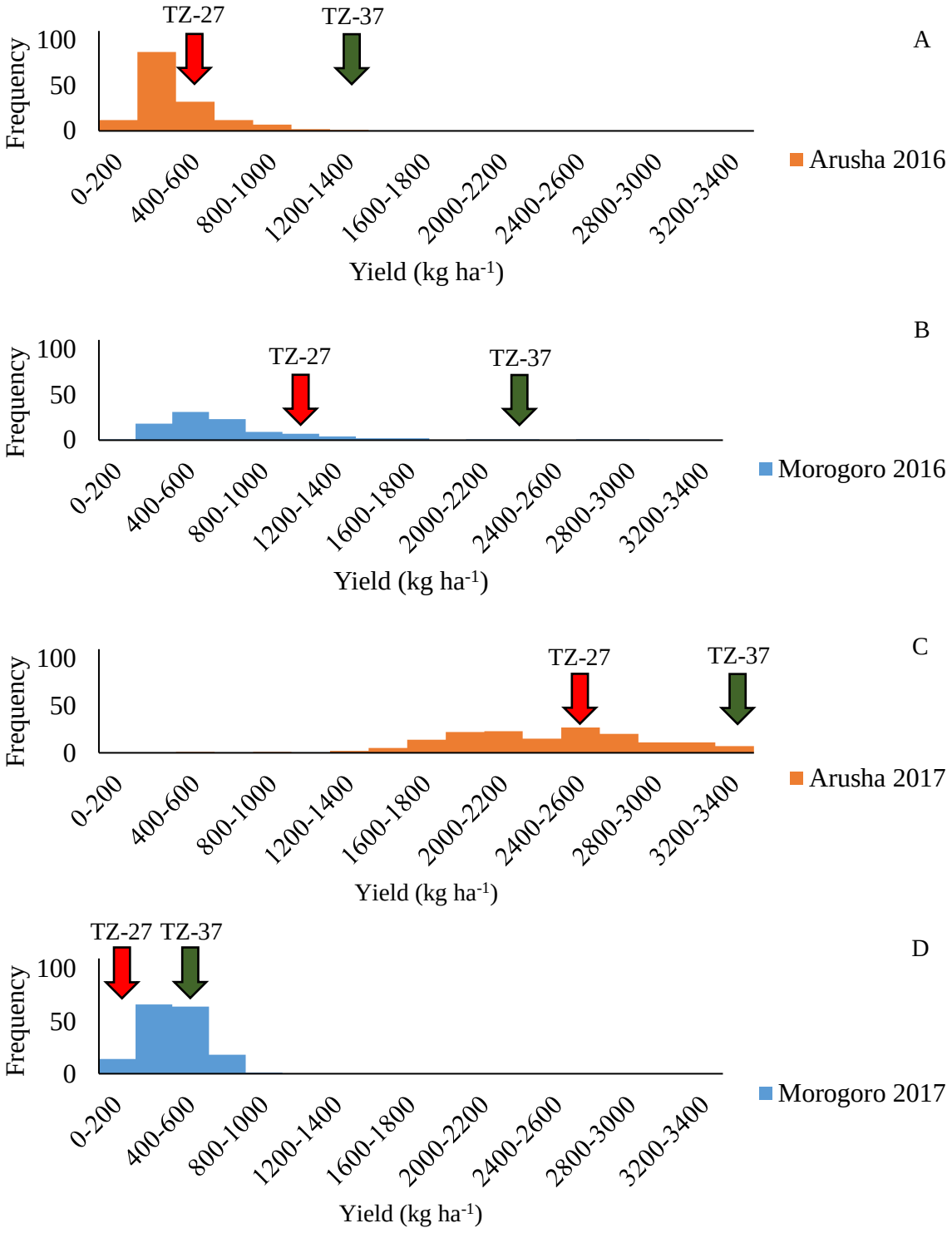


Figure 3.06. Seed yield histograms for beans grown in Arusha and Morogoro in 2016 and 2017. Distribution of yield for beans grown in Arusha (A and C) and Morogoro (B and D) in Tanzania in 2016 (A and B) and 2017 (C and D). Frequencies were determined by using the average of 2 replications for each line. Arrows indicate parental values for this trait.

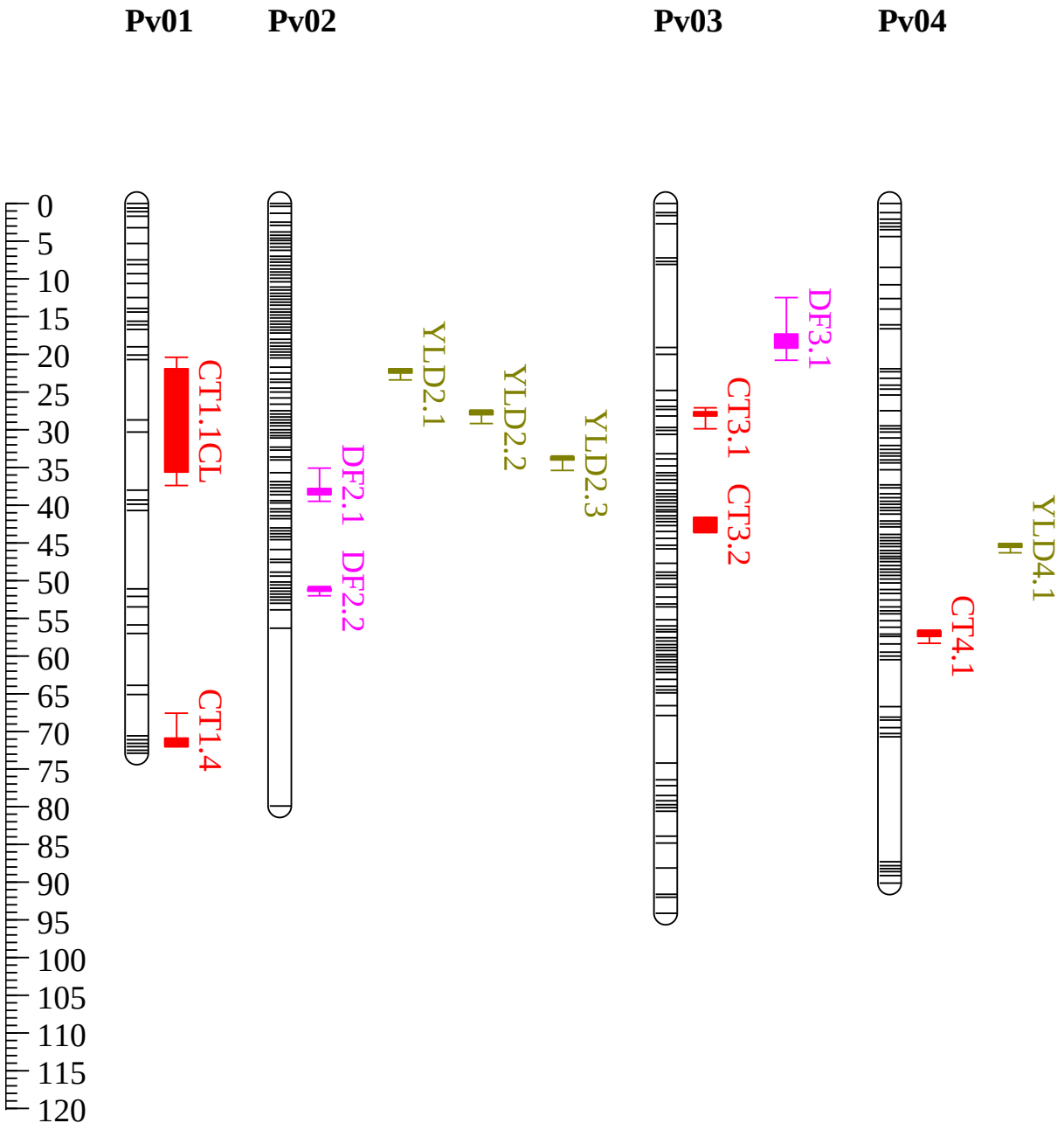


Figure 3.07. QTL map for cooking time (CT), days to flower (DF), days to maturity (DM), and seed yield (SY) in the TZ-27/TZ-37 (TT) RIL population. The 0 – 120 scale is in cM. Linkage group 11 begins at 0 cM at the top of Pv11a and proceeds through to the bottom of Pv11b, which ends at 240 cM. The ruler on the left indicates size in cM.

Figure 3.07 (cont'd)

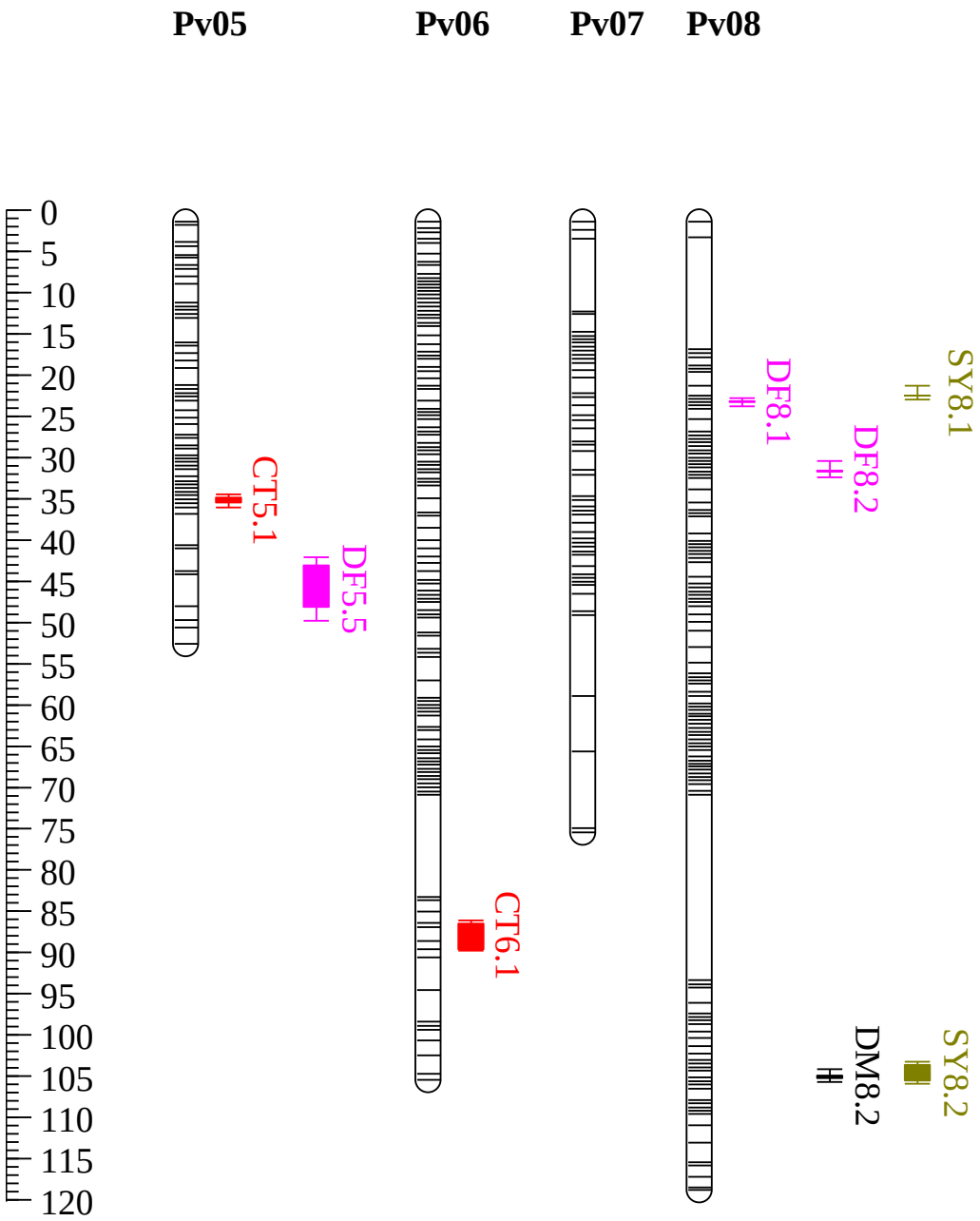
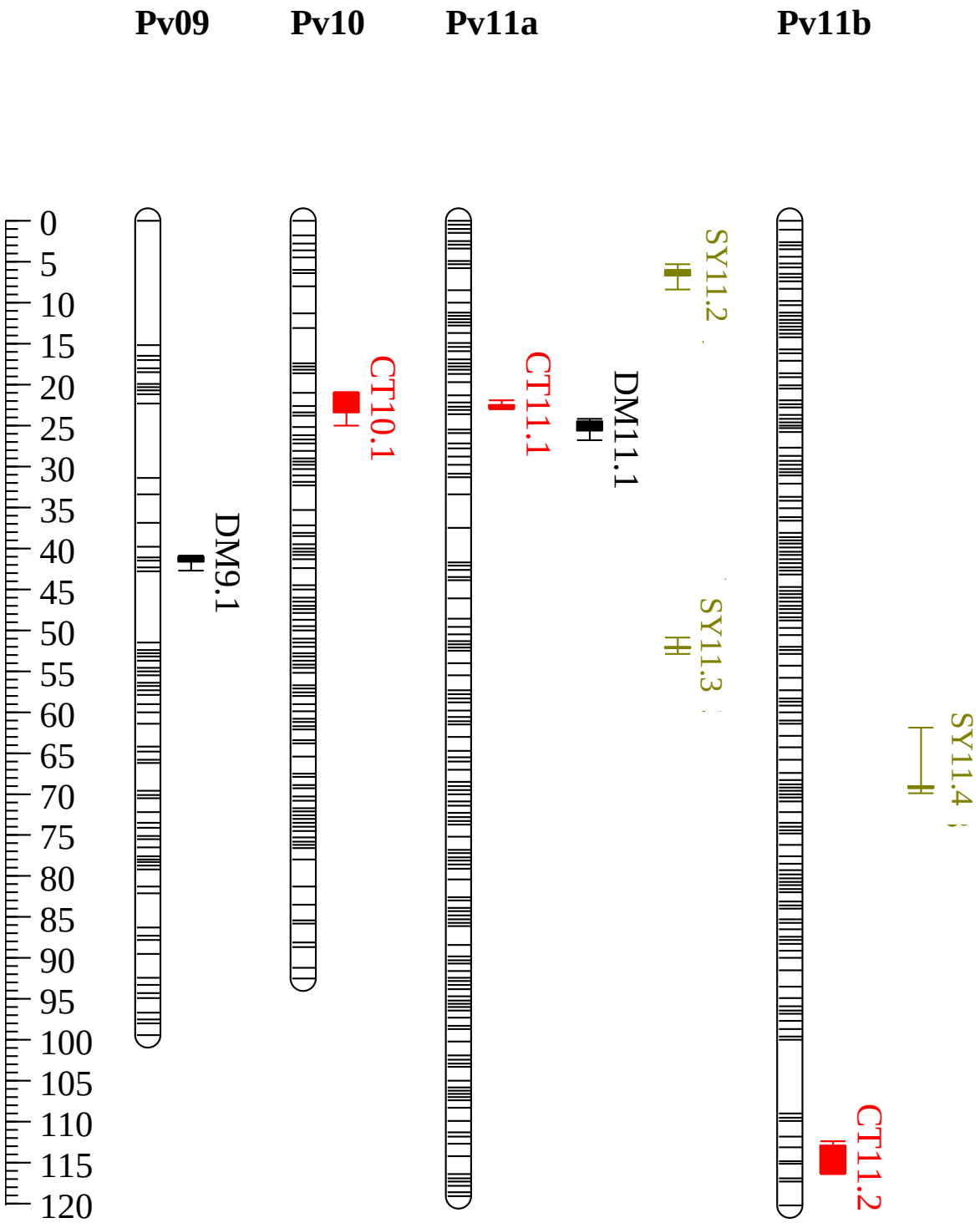


Figure 3.07 (cont'd)



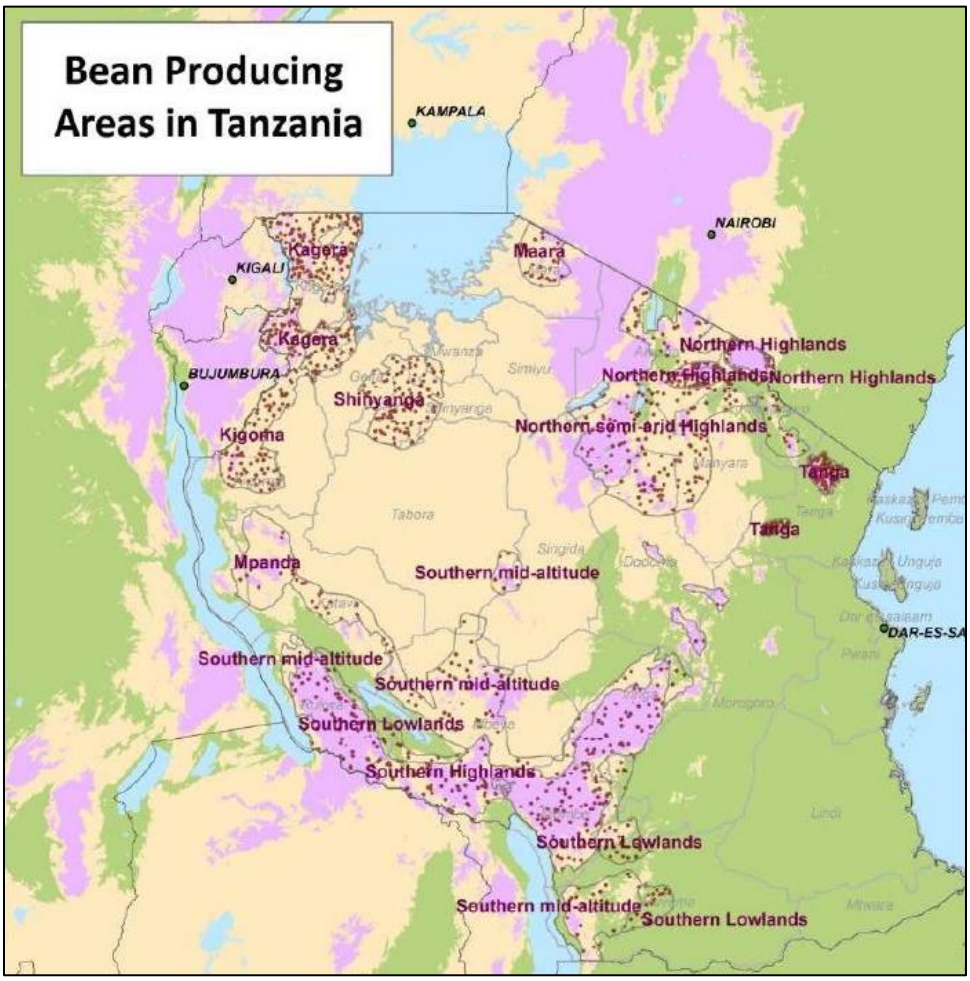


Figure 3.08. Bean producing areas in Tanzania. Pink areas are highlands, green areas are lowlands, and yellow regions are at a mid-altitude. Each dot represents 500 ha of bean production (Binagwa et al., 2016).

Trait	Source of Variation	Degrees of Freedom	Type III SS	Mean Square	F Statistic	p Value
Cooking Time	Genotype	162	86388	533.3	12.6	<0.0001
	Year	1	31943	31943	756.9	<0.0001
	Location	1	60965	60965	1444.6	<0.0001
	Genotype*Year	150	24280	161.9	3.8	<0.0001
	Genotype*Location	162	30855	190.5	4.5	<0.0001
	Genotype*Location*Year	82	15469	188.6	4.5	<0.0001
Days to Flower	Genotype	162	639.1	3.9	2.4	<0.0001
	Year	1	40.4	40.4	24.1	<0.0001
	Location	1	2531.1	2531.1	1507.9	<0.0001
	Genotype*Year	150	403.9	2.7	1.6	<0.0001
	Genotype*Location	162	372.5	2.3	1.4	0.0050
	Genotype*Location*Year	83	150.4	1.8	1.1	0.3
Days to Maturity	Genotype	162	2277.5	14.1	2.2	<0.0001
	Year	1	12.2	12.2	1.9	0.2
	Location	0	0	0	ND	ND
	Genotype*Year	93	762.0	8.2	1.3	0.1
	Genotype*Location	0	0	0	ND	ND
	Genotype*Location*Year	0	0	0	ND	ND
Seed Yield	Genotype	162	10241071	63216	3.6	<0.0001
	Year	1	20652611	20652611	1189.8	<0.0001
	Location	1	19479598	19479598	1122.2	<0.0001
	Genotype*Year	150	5996038	39974	2.3	<0.0001
	Genotype*Location	162	33407182	33407182	1.8	<0.0001
	Genotype*Location*Year	81	2465921	30443	1.8	0.0002

Table 3.01. Analysis of variance for cooking time and agronomic traits for 163 genotypes grown in Arusha and Morogoro in Tanzania during 2016 and 2017. The ANOVA analysis was carried out using SAS 9.4 statistical software package.

Traits	Parents	Arusha			Morogoro			RILs	Arusha		Morogoro		
	Years	TZ-27	TZ-37	p value	TZ-27	TZ-37	p value	Mean	Range	p value	Mean	Range	p value
Cooking Time	2016	66.9	35.6	***	62.9	33.3	**	44.0	23.4 – 94.5	***	62.8	28.4 – 134.6	***
	2017	41.0	27.7	***	70.4	35.3	***	33.1	21.4 – 79.1	***	48.7	30.0 – 100.7	***
Days to Flower	2016	34	35.5	NS	30.5	32	NS	34.8	33.0 – 40.0	**	31.6	29.0 – 39.0	*
	2017	36	36	NS	33.5	33	NS	35.6	32.0 – 40.0	NS	31.7	28.0 – 39.0	***
Days to Maturity	2016	NA	NA	NA	65.5	71	NS	NA	NA	NA	69.1	62.0 – 90.0	**
	2017	86	85.5	NS	63.5	71	**	85.6†	81.0 – 95.0†	**†	68.8	61.0 – 77.0	**
Yield	2016	486.6	1278.9	***	1011.1	2202.0	*	390.3	58.5 – 1278.9	***	768.7	116.6 – 2820.5	**
	2017	2406.1	3374.7	*	72.1	469.1	*	2394.3	595.2 – 3374.7	***	398.2	72.1 – 804.5	***

Table 3.02. Means and ranges of 2 replications of four traits measured in the TT RIL population and the parental lines, TZ-27 and TZ-37.

Chromosome #	# of Markers	Chromosome Size (cM)	Marker Density (Average Distance between Markers [in cM])
1	59	72.9	1.2
2	364	79.9	0.2
3	170	94.1	0.6
4	233	90.1	0.4
5	201	69.5	0.4
6	263	105.1	0.4
7	81	74.5	0.9
8	302	118.6	0.4
9	129	99.5	0.8
10	149	92.5	0.6
11	476	240.4	0.5
Total	2427	1137.1	0.5

Table 3.03. Linkage map information. Over 2,427 markers were discovered resulting in an overall genome size of 1137.1 cM. Marker density varied by linkage group with the average coverage across the entire genome being 1 marker every 0.5 cM.

QTL Name	LG	Trait	Year	Location	LOD Threshold	LOD Max	R^2 (%)	Additive	Physical Location	Flanking Markers	QTL range (cM)	Number of SNPs within QTL
CT1.1[CL, TT]	Pv01	Cooking Time	2016	Arusha	2.76	2.8	5.4	-2.8	4687531 - 4776925	Chr01_4687531, Chr01_4776925	26.3 - 34.6	4
CT1.1[CL, TT]	Pv01	Cooking Time	2017	Arusha	2.55	2.7	4.1	-1.5	4687531 - 4776925	Chr01_4687531, Chr01_4776925	30.2 - 37.4	4
CT1.4[TT]	Pv01	Cooking Time	2017	Morogoro	2.69	3.1	4.1	-2.25	50446775 - 51415447	Chr01_50446775, Chr01_51415447	67.6 – 72.0	10
CT3.1[TT]	Pv03	Cooking Time	2017	Morogoro	2.69	6.3	10.1	-3.5	5345202 - 5684988	Chr03_5345202, Chr03_5684988	27.1 – 29.9	5
CT3.2[TT]	Pv03	Cooking Time	2017	Morogoro	2.69	10.2	15.3	-4.2	14820087 - 16876828	Chr03_14820087, Chr03_16876828	41.6 – 43.6	10
CT3.2[TT]	Pv03	Cooking Time	2017	Arusha	2.55	8.0	20.5	-14.3	14931976 - 16827835	Chr03_14931976, Chr03_16827835	42.0 – 43.2	7
CT4.1[TT]	Pv04	Cooking Time	2017	Arusha	2.55	2.9	3.5	-1.4	40823247 - 41649034	Chr04_40823247, Chr04_41649034	56.6 – 58.3	6
CT5.1[TT]	Pv05	Cooking Time	2017	Arusha	2.55	3.3	3.9	-1.3	6659800 - 6983523	Chr05_6659800, Chr05_6983523	33.4 – 35.0	14
CT6.1[TT]	Pv06	Cooking Time	2017	Arusha	2.55	9.7	14.5	-2.6	24610619 - 24964521	Chr06_24610619, Chr06_24964521	85.6 – 87.5	7
CT6.1[TT]	Pv06	Cooking Time	2017	Morogoro	2.69	6.9	9.7	-3.4	24945213 - 25311110	Chr06_24945213, Chr06_25311110	87.0 – 89.3	4
CT10.1[TT]	Pv10	Cooking Time	2017	Morogoro	2.69	4.2	6.3	-2.8	5660338 - 6984937	Chr10_5660338, Chr10_6984937	20.9 – 25.0	7
CT10.1[TT]	Pv10	Cooking Time	2016	Morogoro	2.91	3.5	7.8	-5.9	5660338 - 6984937	Chr10_5660338, Chr10_6984937	20.9 – 25.0	7
CT11.1[TT]	Pv11	Cooking Time	2016	Arusha	2.76	6.3	15.0	-6.2	3077505 - 3153110	Chr11_3077505, Chr11_3153110	21.9 – 23.0	9

Table 3.04. QTL information for cooking time (CT), days to flower (DF), days to maturity (DM), and seed yield (SY). The largest LOD, r^2, and additive score within the QTL were reported. Positive additive values indicated the allele was contributed by TZ-27, and negative values indicated TZ-37 contributed the allele. The threshold LOD was generated by running 1000 permutations and represents the minimum LOD score significant at $p < 0.05$. Flanking markers are named for the physical placement on the named linkage group. TT represents the TZ-27 x TZ-37 RIL population.

Table 3.04 (cont'd)

CT11.2[TT]	Pv11	Cooking Time	2017	Arusha	2.55	3.5	5.1	-1.6	50757624 - 51161990	Chr11_50757624, Chr11_51161990	232.5 – 236.5	7
DF2.1[TT]	Pv02	Days to Flower	2016	Arusha	2.90	3.5	8.8	-0.4	27079491 – 31152329	Chr02_27079491, Chr02_31152329	35.1 – 39.5	43
DF2.2 [TT]	Pv02	Days to Flower	2016	Morogoro	2.76	6.8	18.2	-0.7	33517125 – 34443830	Chr02_33517125, Chr02_34443830	50.8 – 52.0	9
DF3.3 [TT]	Pv03	Days to Flower	2017	Morogoro	2.21	2.3	5.9	2.1	2053860 - 4652206	Chr03_2053860, Chr03_4652206	12.5 – 20.8	5
DF5.5 [TT]	Pv04	Days to Flower	2017	Morogoro	2.21	3.3	8.0	-2.5	19480437 - 37281421	Chr05_19480437, Chr05_37281421	41.1 – 48.9	9
DF8.1 [TT]	Pv08	Days to Flower	2016	Arusha	2.90	3.4	9.0	-1.5	11800414 - 14014183	Chr08_11800414, Chr08_14014183	21.6 – 22.6	21
DF8.2 [TT]	Pv08	Days to Flower	2016	Arusha	2.90	3.0	8.1	-1.0	16158103 - 17014191	Chr08_16158103, Chr08_17014191	29.3 – 31.3	8
DM8.2 [TT]	Pv08	Days to Maturity	2016	Morogoro	2.70	3.9	9.9	-1.1	61870937 - 62203862	Chr08_61870937, Chr08_62203862	103.8 – 105.4	6
DM9.1 [TT]	Pv09	Days to Maturity	2016	Morogoro	2.70	3.6	8.5	-1.0	16197475 - 16641845	Chr09_16197475, Chr09_16641845	40.9 – 42.7	12
DM11.1 [TT]	Pv11	Days to Maturity	2016	Morogoro	2.70	4.6	10.9	1.2	3153228 - 3635923	Chr11_3153228, Chr11_3635923	24.2 – 26.8	8
SY2.4 [TT]	Pv02	Seed Yield	2017	Arusha	2.92	9.7	20.7	-262.2	12896409 - 13046995	Chr02_12896409, Chr02_13046995	21.9 – 23.4	5
SY2.5 [TT]	Pv02	Seed Yield	2017	Arusha	2.92	8.9	19.0	-249.3	14919797 - 23553052	Chr02_14919797, Chr02_23553052	27.4 – 29.2	14
SY2.6 [TT]	Pv02	Seed Yield	2017	Morogoro	2.94	6.0	15.3	-145.1	26833150 - 29343972	Chr02_26833150, Chr02_29343972	33.5 – 35.4	4
SY4.2 [TT]	Pv04	Seed Yield	2017	Arusha	2.92	5.1	9.7	289.4	13160833 - 16849199	Chr04_13160833, Chr04_16849199	45.1 – 46.3	11
SY8.1 [TT]	Pv08	Seed Yield	2016	Arusha	2.82	2.9	6.8	152.7	11218958 - 12970064	Chr08_11218958, Chr08_12970064	20.1 – 21.8	12
SY8.2 [TT]	Pv08	Seed Yield	2016	Morogoro	2.44	3.3	14.1	-204.9	61705467 - 62203862	Chr08_61705467, Chr08_62203862	102.9 – 105.6	8
SY11.2 [TT]	Pv11	Seed Yield	2017	Arusha	2.92	3.6	7.8	-159.1	797504 - 1323151	Chr11_797504, Chr11_1323151	5.3 – 8.4	5
SY11.3 [TT]	Pv11	Seed Yield	2016	Morogoro	2.44	2.5	14.1	-333.8	8689907 - 9060175	Chr11_8689907, Chr11_9060175	50.9 – 52.9	10
SY11.4 [TT]	Pv11	Seed Yield	2016	Morogoro	2.44	2.7	13.2	323.7	39691193 - 40394549	Chr11_39691193, Chr11_40394549	182.0 – 190.0	12

A\B	80% Cooking Time	Days to Flower	Days to Maturity	Yield	C\D	80% Cooking Time	Days to Flower	Days to Maturity	Yield
80% Cooking Time		-0.2*	-0.3**	-0.2**	80% Cooking Time		-0.2**	-0.2***	-0.3***
Days to Flower	-0.1		0.6***	-0.2**	Days to Flower	0.1		0.03	-0.1
Days to Maturity	NA	NA		0.03	Days to Maturity	-0.03†	0.2**†		0.2***
Yield	-0.01	0.0	NA		Yield	-0.1*	-0.01	0.04†	

Table 3.05. Pearson correlations of traits measured for the TT RIL population at Arusha (A and C) **and Morogoro** (B and D) **in Tanzania during the 2016** (A and B) **and 2017** (C and D) **growing seasons†.**
Correlations significant at * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.0001$. No asterisk represented no statistical significance.
†Days to Maturity was recorded at 85% maturity rather than 50% maturity.

	2016		2017	
Soil Component	Arusha	Morogoro	Arusha	Morogoro
pH	6.4	6.1	7.0	5.6
Electrical Conductivity (mS/cm)	0.4	0.1	0.1	0.1
Particle Size Distribution (% Clay)	31.6	49.6	26.8	43.8
Particle Size Distribution (% Silt)	21.3	7.3	19.9	6.9
Particle Size Distribution (% Sand)	47.1	43.1	53.2	49.2
Zinc (ppm)	2.4	1.3	3.5	1.3
Manganese (ppm)	21.1	64.5	21.9	104.5
Copper (ppm)	1.5	1.0	2.3	1.6
Iron (ppm)	26.9	26.4	38.2	38.2
Total Kjeldahl Nitrogen (%)	0.2	0.1	0.2	0.1
Cation Exchange Capacity	15.8	6.2	24.8	16.8
Calcium (cmol kg^{-1})	15.1	3.9	6.5	18.7
Magnesium (cmol kg^{-1})	3.8	2.6	4.3	2.8
Potassium (cmol kg^{-1})	4.8	0.8	5.5	0.6
Sodium (cmol kg^{-1})	1.4	0.6	0.4	0.1
Aluminum (ppm)	0.0	0.0	0.0	0.0

Table 3.06. Soil Component Analysis for Arusha and Morogoro in 2016 and 2017. Soil components were measured for both years at Sokoine University of Agriculture in Morogoro, Tanzania.

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LITERATURE CITED

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CHAPTER 4

QTL ANALYSIS OF COOKING TIME, HYDRATION CAPACITY, SEED COAT PERCENTAGE, AND AGRONOMIC TRAITS IN AN ANDEAN RECOMBINANT INBRED LINE POPULATION

ABSTRACT

Cooking time is an important consumer trait in dry bean (*Phaseolus vulgaris*). Long cooking times deter greater utilization of beans. While genetic variability for cooking time is present within the species, understanding the genetic control and breeding faster cooking beans has not been a priority of many bean breeding programs. In order to understand the genetic control of cooking time, a RIL population was developed between TZ-27, a genotype that takes about 70 minutes to cook, and TZ-37, a genotype that requires around 30 minutes to cook. The TZ-27 x TZ-37 (TT) RIL population of 161 lines was grown in Arusha and Morogoro in Tanzania in 2016 and 2017. Morogoro typically has higher temperatures, lower altitude, and lower soil fertility soil than Arusha, which could explain why lines grown in Morogoro required 15 minutes longer to cook, on average. The range of cooking times for the TT population was from 21.4 min in Arusha in 2017 to 134.6 min in Morogoro in 2016. Seed characteristics including hydration capacity, seed coat percentage, protein concentration, and seed weight were also measured in the population in each location and year. All traits examined, except hydration capacity, correlated with cooking time. Larger seed weight was correlated with faster cooking times in all 4 environments ($r = -0.3$ [$p < 0.0001$] to -0.6 [$p < 0.0001$]), and higher amounts of protein were correlated with faster cooking times in 3 of the 4 environments ($r = -0.2$ [$p < 0.05$] to -0.4 [$p < 0.0001$]). Ten QTL were identified for cooking time on chromosomes Pv01, Pv03, Pv04, Pv05, Pv06, Pv10, and Pv11. CT11.1 (cooking time) was identified in Arusha in 2016, while CT4.1, CT5.1, and CT11.2 were identified in Arusha in

2017. CT1.4 and CT3.1 were identified in Morogoro in 2017, and the remaining four QTL were found across both locations or in both years. The four QTL found in more than one environment explained 41.8% of variation for the trait collectively, and genotypes with those four QTL cooked 16 min faster than genotypes with none of those QTL. One QTL for cooking time, CT6.1, co-localized with QTL for hydration capacity, seed coat percentage, and 100 seed weight, which could allow one to manipulate multiple beneficial traits at once by using markers within this QTL. In addition, candidate genes including pectate lyase (CT1.4 and CT6.1), polygalacturonase (CT1.4 and CT10.1), and an inhibitor of pectin methylesterase (PMEi) (CT10.1) suggest an enzymatic role in cooking time in dry bean, which has been observed in other work. These QTL could be used in marker assisted selection to develop faster cooking bean varieties.

INTRODUCTION

Dry bean is a dietary staple in many parts of the world including Africa and Latin America (Bouchenak and Lamri-Senhadjii, 2013; Taiwo et al., 1998, Uebersax, 2006). In Tanzania specifically, beans are estimated to be consumed at a rate of 15.3 kg per capita and pulses, as a group, contribute 14% of per capita protein intake. Beans contain high amounts of protein leading it to be called “the poor man’s meat” in some parts of the world (Uebersax, 2006; Chianu et al., 2010). Despite its nutritive value, the principle drawback in dry bean consumption is the length of time required for cooking (Chege et al., 2016; Leterme and Muuoz, 2002, Winham, 2017). Cooking time in dry bean has been studied for decades, however, research in this area has focused on storage conditions, field conditions during the growing season, or seed treatment immediately prior to or during cooking (Jackson and Varriano-Marston, 1981; Alfredo, 1993; and Coelho et al., 2007).

A few studies have attempted to elucidate a genetic cause behind cooking time differences in dry bean. Early work on the genetics of cooking time indicated that the cooking time trait has a high heritability of 0.9 (Elia et al., 1997). In addition to heritability estimates, some research has been conducted on marker analyses related to cooking time. Jacinto-Hernandez et al. (2003) used random amplified polymorphic DNA (RAPD) markers in their research and found a single RAPD associated with shorter cook time, which was named UNAM 16. Parental lines with contrasting cooking times (Bay Mecentral and Bayo Victoria) were used to develop 104 RILs. The RILs were tested in the F_6 through F_8 generation (Jacinto-Hernandez et al., 2003). A more recent study evaluated cooking time on an $F_{2:5}$ population of 140 families derived from a cross between CNFM 7875 and Laranja and mapped with SSR markers (Garcia et al., 2012). Few loci were found in this study similar to the study of Jacinto-Hernandez et al. (2003). QTL were discovered on chromosomes Pv01 and Pv09 (Garcia et al., 2012). The most promising locus found was CT1.1 as it was found in two successive generations and explained up to 21% of the variation in cook time (Garcia et al., 2012). Another study with an Andean diversity panel of 206 lines, found SNPs associated with cook time on Pv02, Pv03 and Pv06 that explained phenotypic variation between 4% and 8.7% (Cichy et al., 2015). These three studies combined, reveal QTL on chromosomes 1, 2, 3, 6, and 9 to need be explored further for their effects on cooking time.

The goal of the current research was to elucidate genetic control of cooking time through QTL analysis in a biparental recombinant inbred line (RIL) population developed from two closely related Andean bean genotypes from Tanzania with similar seed type and color but contrasting cooking times. One genotype (TZ-37) cooks approximately twice as fast as the other genotype (TZ-27). This TZ-27 x TZ-37 (TT) RIL population was developed for 6 generations via single

seed descent. The TT RIL population was used to discover QTL for cooking time and other traits that have been linked to cooking time in the literature.

MATERIALS AND METHODS

Plant Material

Parental lines Incomparable (TZ-27) and W6 16488 (TZ-37) were used as parents of the TT population. Both parental lines were collected in Tanzania (TZ-27 was collected in 1943 and TZ-37 was collected by Gaylord Mink), had a type I growth habit, and produced brown kidney shaped beans. The population was developed in the USDA-ARS greenhouse in East Lansing, MI from 2014-2016. Five generations of single seed descent followed by seed increase in the F_6 generation were used to develop the population. The TT RIL population and parental lines were grown in 2016 and 2017 in an RCBD with two replications at the Selian Agriculture Institute in Arusha, Tanzania and at Sokoine University of Agriculture in Morogoro, Tanzania. Both field sites were in Tanzania. Seed was planted in 5 m long rows (1 seed every 10 cm) with 50 cm of space between adjacent rows. In 2016, seed was planted in Arusha on Apr 14 and in Morogoro on Apr 11. Harvesting occurred on July 26 in Arusha and July 1 in Morogoro. In 2017, planting at both locations occurred on Apr 5 and harvesting in Morogoro occurred on July 7. In Arusha, harvesting was performed on July 19. All planting, harvesting, and threshing was done by hand both years. No fertilizer was used at either location. Morogoro was irrigated as needed, but Arusha was not irrigated. Seed was treated with Actellic Gold Dust (Syngenta) to reduce bean weevil damage to a minimum by affecting the nervous system of pests through the actions of Pirimiphos-methyl and Thiamethoxam. Within six months of harvest, cooking time was determined from seed stored at 4 °C.

Hydration Capacity

Before soaking, dry seed was weighed. The same seed was weighed after 12 hours of soaking. Hydration capacity was calculated by subtracting the weight of soaked seed from the weight of dry seed, which was then divided by the weight of the dry seed and multiplied by 100 to determine the grams of water taken up by 100 g of seed. This method is adapted from AACC method 56-35.01 though seed was soaked for 12 h instead of 16 h (AACC International, 2012).

Cooking Time

Harvested seed was equilibrated to a moisture content of 10-12% before cooking. A sample of 25 dry seeds were soaked in distilled water for 12 h and cooked in distilled water at 100 ºC with a Mattson bean cooker (also known as a pin drop cooker) (Customized Machining 246 and Hydraulics Co., 247 Winnipeg, Canada). The elapsed cooking time was recorded as the time required for 80% of the pins to pierce the seeds. This technique has been utilized by others to test cooking times (Paredes-López et al., 1988; Alfredo, 1993; Wang and Daun, 2005).

Seed Weight

Seed weight of 100 seeds was measured by weighing 100 seed once the seed had a moisture content between 10 and 12%. Raw seed weight was measured prior to soaking seed, and after 12 hours of soaking in distilled water, soaked seed weight was measured.

Seed Coat Percentage

The seed coats of 5 seed were removed and the seed coats and cotyledons were placed in an Isotemp convection oven (Thermo Fisher Scientific, Pittsburgh, PA) at 105 °C for 24 hours. The cotyledons and seed coats were then weighed, and the seed coat percentage was calculated by taking the seed coat weight divided by the total seed weight (seed coats + cotyledons) and multiplying by 100. This method was adapted from Escribano et al. (1997).

Protein Analysis

Cooked samples (25 seed) were freeze dried using a Virtis Genesis Freeze Drier (The Virtis Company, Gardiner, NY) for one week (until constant readings were achieved). The freeze-dried samples were then weighed followed by milling in a Polymix PX-MFC 90D (Kinematica AG, Luzern, Switzerland). Total N concentration was determined in 1 g of lyophilized powder from cooked samples by the Dumas combustion method at A & L Great Lakes Laboratories (Fort Wayne, IN, USA). The resulting N content was converted to a protein percentage by multiplying by 6.25.

DNA Extraction and GBS

DNA was extracted from young trifoliate leaf tissue from a single plant for each of the 161 RIL lines and the two parental lines (TZ-27 and TZ-37). The Macherey-Nagel NucleoSpin Plant II kit was used to obtain DNA from leaf tissue. Preparation of DNA for genotyping by sequencing was adapted from the method of (Schröder et al., 2016). Barcoded adapters and primers were produced by IDTDNA (Coralville, IA). Specifically, the enzymes Mse I and Taq α1 were used in a double-digest of DNA. Mse I and Taq α1 are methylation insensitive allowing for a more uniform distribution of fragments across euchromatic as well as

heterochromatic regions (Schröder et al., 2016). In addition, use of a double digest decreases the chance of generating fragments ligated to only common adapters or barcoded adapters (Schröder et al., 2016). Annealed samples were then amplified via PCR and pooled (each uniquely barcoded). Validation was performed on a 2% agarose gel with a 100 bp ladder. The final DNA product was analyzed by Michigan State University genomics core facility (East Lansing, MI) using the 200 bp single-end reads on two lanes of an Illumina Hi-Seq 2500.

SNP Calling and Map Construction

FastQC files were cleaned using Cutadapt. Bowtie2 was used for alignment to the reference genome Pvulgaris_442_v2.0 from Phytozome (version 11). NGSEP was used to filter the vcf file data. NGSEP was also used for SNP calling by removing any markers with more than 50% missing data and using a minimum allele frequency (MAF) of 0.05. In addition, markers that were not polymorphic in the parents or that showed segregation distortion were removed. Remaining markers were analyzed using order, compare, and ripple commands in MapDisto (version 1.7.7) to generate a genetic map after applying the Kosambi function (Lorieux, 2012).

QTL Analysis

Phenotypic and genotypic data were analyzed MapDisto (version 1.7.7) (Lorieux, 2012), which generated a linkage map. The resulting file was analyzed using QTL Cartographer to discover QTL within the *P. vulgaris* genome (version 2.5) (Wang, Basten, and Zeng, 2005). Composite Interval mapping with 1000 permutations, forward and backward regression, and a walking speed of 1.0 cM was used to determine threshold at $p < 0.05$. That threshold was used to determine significance of QTL for each trait. Lines that produced no reads, few reads, or no high-quality reads were not analyzed further (lines 60, 127, 141, and lines 145 through 156).

Mapchart (version 2.3) was used to display the final map with QTL data included (Voorrips, 2001).

Statistical Analyses

SAS 9.4 (SAS Institute Inc., Cary, NC) was used to perform a proc mixed analysis to generate an ANOVA for all studied traits. Significant differences were observed for each year and location, so each location and year was analyzed separately. Proc corr was used to generate Pearson correlations and proc means was used to generate means and ranges.

RESULTS

Cooking Time

TZ-37 required 27.7 to 35.6 minutes to cook across locations and years, while TZ-27 always needed more time to cook with a range of 41.0 to 70.4 minutes in both years and locations (Table 4.02). A range of cooking times (21.4 to 134.6 min) were observed in the TT population with transgressive segregation observed for this trait (Fig. 4.01, Table 4.02). The population showed a normal distribution and significant variation in cooking time for each year and location studied (Table 4.01, Table 4.02). Compared to 2017, the population needed 1.3 times longer to cook 2016, on average (Fig. 4.01, Table 4.02). In addition, lines grown in Morogoro required 1.5 times longer to cook than lines grown in Arusha, on average (Fig. 4.01, Table 4.02). Cooking time was inversely correlated ($r = -0.3$ to -0.6 [$p < 0.0001$ for each correlation]) with seed weight regardless of year or location such that larger seed cooked more quickly (Table 4.04). Cooking time was also significantly correlated directly with seed coat percentage ($r = 0.4$ to 0.5 [$p < 0.0001$ for each correlation]) and inversely with protein

concentration (r = -0.2 [$p < 0.05$] to -0.4 [$p < 0.0001$]) though significance was not observed for Arusha in 2016 for these two traits.

100 Seed Weight

TZ-37 weighed between at least 41.3 g, while TZ-27 weighed at least 27.5 g, which was significant in each year and location studied (Table 4.02). The greatest difference between the two lines was recorded in Morogoro in 2017 with TZ-27 weighing 27.5 g and TZ-37 weighing 41.3 g per 100 seed. Across the years and locations studied, 100 seed weight in the RIL population ranged from 25.4 g in Morogoro in 2016 to 66.4 g in Arusha in 2017 (Fig. 4.03, Table 4.02). 100 seed weight was also significant in both locations and years (Table 4.01, Table 4.02). The mean seed weight from Arusha was greater than seed from Morogoro in 2016 and 2017.

Seed Coat Percentage

The seed coat percentage showed how much of a seed's mass was comprised of the seed coat. TZ-27 had at least a 1.2% greater percentage of the total seed weight come from the seed coat than TZ-37 in every environment (Fig 4.04., Table 4.02). In addition, the parental lines from the Morogoro field had 0.3 to 2.0% higher seed coat percentages than Arusha in both years (Fig. 4, Table 2). Within the population, ranges for seed coat percentage were greater in Morogoro than Arusha in both years with the largest range observed in 2017 (8.1 to 16.6%) (Table 4.02). The seed coat percentage trait was inversely correlated with seed weight indicating that as seed mass increased, the percentage of seed coat to total seed mass decreased (Table 4.04).

Hydration Capacity

The parental lines tended to cluster near the middle of the distribution curve for hydration capacity within the population (Fig. 4.02). Differences in hydration capacity between the parents were not significant with the largest difference between the two parental lines being 7.7 g water 100 g seed^{-1} (Table 4.02). The distribution of hydration capacities showed less variability compared to other traits in this study though transgressive segregation was still observed (Fig. 4.02). On average, seed took up 1.03 to 1.06 times more water in 2016 compared to 2017 in Arusha and Morogoro respectively (Table 4.02). The population showed variation in hydration for year and also genotype, but each location was not statistically different (Table 4.01). This was especially evident in 2017 where the mean for hydration capacity in Arusha and Morogoro was identical at 96.4 g water 100 g seed^{-1} (Table 4.02).

Protein Concentration

Cooked bean protein concentration was higher in TZ-37 than TZ-27 in all environments tested. TZ-37 had 25.8 and 24.6% protein in Arusha and Morogoro, respectively, in 2017, while TZ-27 had only 20.8 and 22.5% protein in those locations that year (Table 4.02). Protein concentration was variable within the TT RIL population. In each environment, the highest protein concentration was at least 1.5 times greater than the lowest protein concentration (Fig. 4.05, Table 4.02). Transgressive segregation for this trait was observed with lines having over 30% cooked protein in Morogoro in 2016 and Arusha in 2017 (Fig. 4.05, Table 4.02).

QTL Analysis

Linkage Map

A total of 48,244 SNPs were discovered over the entire genome. SNPs were characterized based on their location in the genome with 36,024 in repetitive regions and 12,220 were in non-repetitive regions. SNPs within repetitive regions were removed from further analysis because their location could not be discerned as the region could not be mapped to a unique location in the genome. Additional filtering removed monomorphic SNPs as well as ones that were not informative. Remaining SNPs still allowed for dense coverage of the genome with 2,427 SNP markers (Table 4.04). The average density was one marker every 0.5 cM (Table 4.04), and marker density ranged from one marker every 0.2 cM on Pv02 to one marker every 1.2 cM on Pv01. The genetic map developed was 1137.14 cM in size.

Cooking Time

QTL for cooking time were identified on chromosomes Pv01, Pv03, Pv04, Pv05, Pv06, Pv10, and Pv11 (Fig. 4.06). All fast cooking QTL were contributed by TZ-37 (Table 4.03). CT1.1 was identified in Arusha in 2016 and 2017 and spanned a physical region of 4,687,531 to 4,776,9295 bases on chromosome Pv01. The LOD score associated with the QTL was 2.8, additive effect of 2.8 min, and explained 5.4% of variation for cooking time. CT1.4 was found in Morogoro in 2017 and explained 4.1% of phenotypic variation. The QTL peak LOD score was 3.1 and it spanned a region of 50,446,775 to 51,415,447 bases. The additive effect was 2.3 min. CT3.1 had a peak LOD of 6.3 and an additive effect of 3.5 min. The QTL was identified in Morogoro in 2017, explained 10.1% of variation for cooking time, and spanned a region of 5,345,202 to 5,684,988 bases. CT3.2 was identified in Morogoro and Arusha in 2017, had a

peak LOD of 10.2, and explained 17.9% of cooking time variation on average. The average additive effect was 9.3 min, and the QTL spanned a region from 14,820,087 to 16,876,828 bases. CT4.1 was identified in Arusha in 2017 with a peak LOD of 2.9. The additive effect was 1.4 min, and the QTL explained 3.5% of variation for the trait. The QTL spanned a region from 40,823,247 to 41,649,034 bases. CT5.1 explained 3.9% of variation for the trait and was identified in Arusha in 2017. The QTL had a peak LOD of 3.3 and an additive effect of 1.3 min. The physical distance of this QTL was 6,659,800 to 6,983,523 bases. CT6.1 was found in Arusha and Morogoro in 2017 and explained 12.1% of cooking time variation on average. The peak LOD score was 9.7 and the average additive effect was 3.0 min. The physical region of the QTL spanned from 24,610,619 to 25,311,110 bases. CT10.1 was identified in Morogoro in 2016 and 2017 spanning 5,660,338 to 6,984,937 bases. The average additive effect was 4.4 min, the peak LOD score was 4.2, and this QTL explained 7.1% of variation for cooking time. CT11.1 was identified in Arusha in 2016, had a peak LOD score of 6.3, and explained 15% of variation for the trait. The additive effect was 6.2 min and the QTL spanned the region of 3,077,505 to 3,153,110 bases. CT11.2 explained 5.1% of variation for cooking time and was found in Arusha in 2017. The LOD score was 3.5, and the additive effect was 1.6 min. The physical distance of this QTL was from 50,757,624 to 51,161,990 bases. The four most robust QTL were found in more than one environment and included CT1.1 (Arusha in 2016 and 2017), CT0.1 (Morogoro in 2016 and 2017), and CT3.2 and CT6.1 (Arusha and Morogoro in 2017). These four QTL explained 41.8% of variation for the trait collectively, and lines with those four QTL cooked 16 min faster than genotypes with none of those QTL. CT6.1 co-localized with QTL for hydration capacity (HC6.3), seed coat percentage (SCP6.1), 100 seed weight (SW6.1), and protein concentration (PT6.1).

Hydration Capacity

Ten QTL for hydration capacity were identified on Pv01, Pv03, Pv05, and Pv06 (Table 4.05, Fig. 4.06). HC1.1 was identified in Morogoro in 2017 and spanned a region of 1,344,330 to 2,835,419 bases. The QTL had a peak LOD score of 7.3, an additive effect of 1.6 g water 100 g seed^{-1}, explained 7.9% of variation for hydration capacity (Table 4.05). HC3.1 explained 15.3% of phenotypic variation and was identified in Morogoro in 2016. The QTL was located from 7,222,712 to 9,239,838 bases, had an LOD score of 3.9, and an additive effect of 5.4 g water 100 g seed^{-1} (Table 4.05). HC3.2 was found in Morogoro in 2017, had a peak LOD score of 4.1, and explained 9.7% of hydration capacity variation. The physical distance of the QTL spanned 16,827,844 to 17,859,277 bases and had an additive effect of 1.8 g water 100 g seed^{-1} (Table 4.05). HC3.3 was identified in Arusha in 2017 and spanned a region of 46,933,182 to 49,610,607 bases. The QTL had a peak LOD score of 2.9, an additive effect of 3.5 g water 100 g seed^{-1} and explained 7.0% of variation for hydration capacity (Table 4.05). HC5.1 had a peak LOD score of 3.6 and explained 8.1% of hydration capacity variation. HC5.1 was identified in Arusha in 2017, had an additive effect of 3.8 g water 100 g seed^{-1}, and spanned a distance of 1,494,629 to 1,875,228 bases (Table 4.05). HC5.2 explained 8.9% of phenotypic variation and was identified in Arusha in 2016. The QTL was located from 6,831,009 to 13,825,799 bases, had an LOD score of 3.1, and an additive effect of 4.0 g water 100 g seed^{-1} (Table 4.05). HC6.1 and HC6.2 were identified in Arusha in 2017. HC6.1 spanned a physical distance of 4,786,327 to 4,908,107 bases and explained 18.5% of variation for the trait. The peak LOD score for this QTL was 7.7 and an additive effect of 5.5 g water 100 g seed^{-1} (Table 4.05). HC6.2 explained 17.0% of hydration capacity variation, had an additive effect of 5.2 g water 100 g seed^{-1}, and a peak LOD score of 7.0. The QTL spanned a region from 7,046,027 to

7,337,422 (Table 4.05). HC6.3 and HC6.4 were identified in Morogoro in 2017. HC6.3 had a peak LOD score of 5.4 and explained 13.1% of hydration capacity variation. The physical distance of the QTL spanned 24,610,619 to 24,964,521 bases and had an additive effect of 1.9 g water 100 g seed^{-1} (Table 4.05). HC6.4 had an additive effect of 1.7 g water 100 g seed^{-1}, explained 9.8% of phenotypic variation, and a peak LOD score of 3.7. The physical location of this QTL was from 25,311,110 to 27,373,518 bases (Table 4.05). QTL HC6.3 and HC6.4 may be a single QTL as the distance between them is 346.6 kb. HC5.2 co-localized with CT5.1, and HC6.3 overlapped with CT6.1 (Fig. 4.06).

Seed Coat Percentage

Nine QTL were identified for the seed coat percentage trait on chromosomes Pv02, Pv03, Pv06, Pv08, and Pv10 (Fig. 4.06). SCP2.1 and SCP2.2 were identified in Arusha in 2016. SCP2.1 spanned a region of 5,504,885 to 5,776,592 bases. The QTL had a peak LOD score of 4.5, an additive effect of 0.5% and explained 9.2% of variation for seed coat percentage (Table 4.05). (Table 4.05). SCP2.2 had a peak LOD score of 4.1, explained 9.7% of seed coat percentage variation, had a physical distance of 9,136,872 to 9,459,188 bases, and had an additive effect of 0.5% (Table 4.05). SCP2.3 explained 8.0% of variation for the trait and was identified in Arusha in 2017. The QTL had a peak LOD of 6.4 and an additive effect of 0.2%. The physical distance of this QTL was 12,204,074 to 12.776.363 bases. SCP3.1 spanned a physical distance of 17,258,583 to 17,859,277 bases and explained 12.8% of variation for the trait. The peak LOD score for this QTL was 4.5 and an additive effect of 0.6% (Table 4.05). SCP6.1 and SCP6.2 were identified in Arusha in 2016. SCP6.1 had a peak LOD of 7.5, the additive effect was 0.4%, and the QTL explained 18.9% of variation for the trait. The QTL spanned a region from 1,469,365 to 2,737,435 bases. SCP6.2 had a peak LOD score of 6.8,

explained 17.4% of seed coat percentage variation, had a physical distance of 3,805,946 to 4,721,385 bases, and had an additive effect of 0.4% (Table 4.05). SCP6.1 and SCP6.2 could also be part of the same QTL as they were separated by 1.1 Mb. SCP6.3 was identified in Arusha in 2017 and explained 9.0% of phenotypic variation. The physical distance of the QTL spanned from 7,847,018 to 7,945,276 bases, the additive effect was 0.2%, and the peak LOD score was 7.2 (Table 4.05). SCP8.1 was identified in Arusha in 2016, had a peak LOD score of 3.1, and explained 6.0% of seed coat percentage variation. The QTL had a physical distance of 46,269,208 to 46,441,851 bases and had an additive effect of 0.2% (Table 4.05). SCP10.1 explained 34.3% of variation for the trait and was identified in Morogoro in 2016. The QTL had a peak LOD of 10.1 and an additive effect of 0.5%. The physical distance of this QTL was 35,246,620 to 35,646,609 bases. Though no QTL for seed coat percentage overlapped with cooking time or hydration capacity, SCP6.2 was 64.9 kb from HC6.1, and SCP3.1 was 430.7 kb from CT3.1, which warrant further investigation.

Protein Concentration

Eight QTL for cooked seed protein on chromosomes Pv03, Pv06, Pv07, and Pv08 were identified (Fig. 4.06, Table 4.05). PT3.1 was identified in Morogoro in 2016 and spanned a physical region of 22,619,427 to 29,362,352 bases. The LOD score associated with the QTL was 8.4, additive effect of 0.9%, and explained 17.6% of variation for protein concentration. PT3.2 was found in Arusha in 2016 and explained 13.4% of phenotypic variation. The QTL peak LOD score was 7.3 and it spanned a region of 29,365,504 to 29,527,739 bases. The additive effect was 0.7%. PT3.1 and PT3.2 were 3.2 kb apart suggesting that they could be part of a larger QTL (Table 4.05). PT3.3 was identified in every environment except Morogoro 2017 and explained 13.8% of variation for the trait on average. The physical distance of PT3.3

was from 31,434,552 to 36,084,796 bases. The additive effect was 0.8% on average, and the peak LOD score was 9.5 (Table 4.05). PT6.1 was identified in Arusha in 2017 and explained 19.0% of phenotypic variation. The physical distance of the QTL spanned from 14,488,021 to 24,610,619 bases, the additive effect was 0.7%, and the peak LOD score was 6.8 (Table 4.05). PT7.1 was found by Casañas et al. (2013) to be responsible for 19% of the variation in protein concentration in the seed, while in the current study, it explained 11.5% of protein concentration variation (Table 4.05). PT7.1 was identified in Morogoro in 2016, had a peak LOD score of 6.4, and an additive effect of 0.7%. The physical distance of this QTL was from 1,996,890 to 3,209,979 bases (Table 4.05). PT8.1 explained 8.4% of phenotypic variation and was identified in Morogoro in 2017. The QTL was located from 53,079,387 to 61,247,715 bases, had an LOD score of 2.9, and an additive effect of 0.5% (Table 4.05).

Dry Seed Weight

Eleven QTL for 100 seed weight were identified on chromosomes Pv02, Pv03, Pv05, Pv06, and Pv11 (Fig. 4.06, Table 4.05). TZ-37 contributed all alleles for seed weight. SW2.7, SW2.8, and SW2.9 were identified in Arusha in 2016. SW2.7 had a peak LOD of 4.6, the additive effect was 1.9 g, and the QTL explained 9.8% of variation for the trait. The QTL spanned a region from 9,551131 to 9,593,930 bases. SW2.8 had a peak LOD score of 5.8, explained 12.0% of protein concentration variation, had a physical distance of 12,896,409 to 13,110,313 bases, and had an additive effect of 2.2 g (Table 4.05). SW2.9 explained 5.3% of phenotypic variation and had an additive effect of 1.5 g. The peak LOD score was 5.3, and the physical location of the QTL was from 14,919,797 to 21,254,430 bases. SW3.2 was identified in Morogoro in 2017 and spanned a region of 40,330,058 to 42,320,680 bases. The QTL had a peak LOD score of 10.1, an additive effect of 1.9 g, and explained 15.8% of variation for

protein concentration (Table 4.05). SW5.1 explained 8.8% of phenotypic variation and was identified in Arusha in 2016 and Morogoro in 2017. The QTL was located from 3,533,038 to 4,336,493 bases, had an LOD score of 6.7, and an additive effect of 1.4 g (Table 4.05). SW5.2 was identified in Morogoro in 2016 and explained 11.9% of phenotypic variation. The physical distance of the QTL spanned from 35,770,054 to 37,273,196 bases, the additive effect was 2.3 g, and the peak LOD score was 4.6 (Table 4.05). SW5.3 was identified in Morogoro in 2016, had a peak LOD score of 4.6, and explained 4.3% of 100 seed weight variation. The QTL had a physical distance of 36,812,730 to 37,653,109 bases and had an additive effect of 1.4 g (Table 4.05). SW6.17 was identified in Arusha in 2016 and 2017, and it explained 6.7% of phenotypic variation, on average. The location of the QTL was between 24,607,532 and 24,945,156 bases, the peak LOD score was 5.3, and the additive effect was 1.4 g (Table 4.05). SW6.18 was identified in Arusha in 2017 and explained 4.1% of phenotypic variation. The physical distance of the QTL spanned from 27,581,319 to 29,079,040 bases, the additive effect was 1.2 g, and the peak LOD score was 3.1 (Table 4.05). SW11.1 was identified in Arusha in 2016 and the physical location was from 137,201 to 246,489 bases. The QTL explained 8.4% of 100 seed weight variation, had an additive effect of 1.3 g, and a peak LOD of 4.5 (Table 4.05). SW11.2 was identified in Morogoro in 2016 and explained 9.3% of phenotypic variation. SW6.18 had an additive effect of 1.3 g and a peak LOD of 4.5. The QTL had a physical distance of 6,077,563 to 7,613,114 bases. SW6.17 co-localized with CT6.1 as well as HC6.3 and PT6.1 (Table 4.05).

DISCUSSION

Phenotypic Analyses

Growing location influenced cooking time, 100 seed weight, and seed coat percentage. The mean cooking time was at least 15 minutes longer for lines grown in Morogoro in both years compared to lines grown in Arusha. Cooking time effects based on where genotypes were grown have been reported before and explanations for what environmental aspects affect cooking time vary including how fertilizer was managed, how deep seed was planted, and temperature and rainfall differences at each location (Erskine et al., 1985, Proctor and Watts, 1987, Singh et al., 1990, and Coşkuner and Karababa, 2003). Though cooking times were different by location, the difference in cooking time was similar in this work. The difference in RIL mean cooking time was 1.4 times and 1.5 times greater in Morogoro than Arusha in 2016 and 2017, respectively. This similarity in cooking time differences was also observed between the parental lines used with TZ-27 requiring 1.5 to 2.0 times longer to cook in Morogoro compared to Arusha across both years. These observations are important because they show that although cooking time can be affected by the environment, differences in cooking time between lines tend to be consistent regardless of environmental differences. This idea was articulated especially well by Iliadis (2003) where it was stated that "plant breeding to create new varieties with short cooking time has greater merit than efforts to identify soil types, which can produce [genotypes] with a short cooking time". Cooking time difference similarity was also observed by Proctor and Watts (1987), where navy bean variety seafarer cooked between 33.8 and 49.2 minutes, which was 1.1 and 1.3 times faster than Ex Rico 23 across three different locations.

A moderate inverse correlation was found between seed weight and cooking time indicating that larger seeds cook faster than smaller seeds. This result contrasts with previous work where larger seeds required a longer cooking time (Cichy et al., 2015, Black et al., 1998, Bressani et al, 1988). In the bulk of work that found small seeds cook faster, the provided explanation was that small seeds had less physical distance for water and heat to travel to permeate the seed. That explanation is called into question if larger seeds can have faster cooking time than small seeds. Other factors must be involved in order to explain cooking time differences such as seed volume or porosity. Work by Yuste-Lisbona et al. (2014) showed that seed weight was different when grown in different environments, but the measures of seed volume (length, width, and thickness) did not always change to the same degree as weight. For example, when line PMB0225 was grown under short-day conditions in 2009, 100 seed weight was 71.1 g, while weight was less in 2011 at 66.7 (Yuste-Lisbona et al., 2014). The seed width was significantly higher in the lower weight sample leading to a higher seed volume in the lower weight sample. Differences in porosity could also explain how seeds with more weight would cook faster. Wani et al. (2017), found a strong direct correlation between porosity (percentage of empty space in the entire seed) and cooking time. In that study, the two fastest cooking lines had the lowest average porosities even though one of the two lines (Master Bean) had the greatest 100 seed weight of the 4 lines tested (Wani et al., 2017). Additionally, some QTL for seed size and cooking time overlap partly (CT6.1 and SW6.17) or have short centimorgan distances between them indicating little recombination between adjacent QTL (CT5.1 and SW5.2, CT6.1 and SW6.18). This could result in a population with fast cooking lines that are also large seeded. Finding QTL for seed weight on Pv06 that overlaps with cooking time is especially important as multiple authors have found seed weight QTL on this chromosome

(Yuste-Lisbona et al., 2014, Blair et al., 2012, Cichy et al., 2009, Park et al., 2000). Past literature and current work suggest that though easy and quick to measure, seed weight is not a good predictor of cooking time as other factors such as porosity, seed volume, or the population used can also affect cooking time and should be accounted for in addition to seed weight.

Hydration capacity, was inversely correlated to cooking time in agreement with research by Cichy et al. (2015). However, the inverse correlation was only recorded for lines grown in Arusha. Lines grown in Morogoro did not have any correlation between cooking time and hydration capacity. One explanation for the difference in hydration capacity findings by location was that the temperature in Morogoro was higher than Arusha during the field season. Studies have shown that seed stored in high heat and humidity have an increased difficulty taking up water as well as longer cooking times (Hentges et al., 1990, Maurer et al., 2004, Coelho et al., 2007) so it is possible that seed harvested from areas with higher temperatures and humidity could have similar difficulty in water uptake. Based on this study and the results obtained for the two parental lines (Chapter 2), the amount of water taken up by the seed does not influence cooking time. However, experiments on the parental lines showed differences in enzymatic activity when soaked but no difference when unsoaked (Chapter 2). This result suggests that while the amount of water taken up by the seed is not important for cooking time, the presence of water is important for affecting enzymatic activity, which influences cooking time.

The percentage of seed coat could act as a predictor of cooking time. Seed coat percentage had moderate direct correlations with cooking time such that as the percentage of seed coat increased, cooking time increased as well. This complements work by Wang et al., (2003) and Avola and Patane et al. (2010) who found that thicker seed coats can result in prolonged

cooking times. These traits require further testing as the percentage of seed coat was not significantly correlated to cooking time in Arusha in 2016. Additional testing in different genetic backgrounds as well as different environments would be needed to provide further evidence that seed coat percentage could be a good predictor of cooking time in dry bean.

Cooked protein concentration correlations were small in 2016 and moderate in 2017, and all were inverse indicating that faster cooking lines have more protein than those that require longer cooking times. This finding is similar to research by Mubarak (2005) who noted that longer cooking times result in leaching of vital nutrients such as protein and vitamins, and this conclusion was also reached by Wiesinger et al. (2016). Other work also showed that protein concentration and cooking time were linked (Afzal et al., 2003, Shimelis and Rakshit, 2005). It should be noted that the current work tested protein concentrations of cooked seed. However, Wiesinger et al. (2016) did not observe any significant differences in protein concentrations (g protein 100 g seed^{-1}) between raw and cooked seed among 12 genotypes and 3 cooking classes. It is possible that the greater content of proteins means that faster cooking lines have a greater quantity of enzymes or transporters that can help soften the seed or readily allow heat into the seed respectively. Polygalacturonase activity has been shown to correlate to cooking time such that the greater the activity, the faster the seed cooks (Martínez-Manrique et al., 2011). Greater quantities of this enzyme could lead to greater activity, faster cooking time, and higher protein concentration. In addition, a greater amount of protein has been shown to result in lesser amounts of other macromolecules like starches and fat and higher amounts of fats and starch could lead to longer cooking times (Wang et al., 2010). Additional work in the TT population would need to be done analyzing raw seed for protein concentration to determine if higher amounts of protein in raw seed could act as a predictor of faster cooking times.

QTL Analyses

Genotyping by sequencing was performed to discover polymorphic markers between TZ-27 and TZ-37. This method was employed because prior methods revealed very little genetic differences between TZ-27 and TZ-37. The *Phaseolus vulgaris* BARCBean6K BeadChip provided only 550 polymorphic markers across the entirety of the genome (data not shown). The fewest markers were found on chromosome Pv06, which only had 10 polymorphic markers between the two parental lines (data not shown). In contrast, GBS revealed 2,427 SNPs to use for QTL analysis with an average of 220.6 SNPs per chromosome. By using this technique, 1,877 additional markers were discovered between TZ-27 and TZ-37 compared to the 6000k SNP chip. The genetic map that was developed from the TT population was 1137.14 cM in size. This size was similar to other maps developed for *P. vulgaris*. Freyre et al. (1998) had a map size of 1200 cM and Hoyos-Villegas et al. (2015) estimated a genome size of 1499 cM. The average coverage across the genome was one marker per 0.5 cM, which is one of the densest coverages of the *P. vulgaris* genome to date (Bassi et al., 2017, Hoyos-Villegas et al., 2015, Remans et al., 2008, Ochoa et al., 2006).

QTL for cooking time were found on chromosomes Pv01, Pv03, Pv04, Pv05, Pv06, Pv10, and Pv11. Six QTL were found in one environment, while four QTL (CT1.1, CT3.2, CT6.1, and CT10.1) were found in more than one environment. These 10 QTL together explained 83.5% of variation for the trait, however, no line in the population contained all 10 QTL for cooking time. Lines did exist that contained 9 of the QTL, and those lines cooked, on average, 20.5 min faster than lines with none of the cooking time QTL (Fig. 4.08). Of the 10 discovered QTL for cooking time, 4 were found in more than one environment. CT1.1 was found in Arusha in 2016 and 2016, while CT10.1 was found in both years at the Morogoro location. CT3.2 and

CT6.1 were identified in both locations in 2017. These QTL were examined for their effects on cooking time, and lines with all 4 QTL cooked 16 minutes faster than ones with none of these QTL (Fig. 4.07). Nine of the ten cooking time QTL resulted in a 20.5 min reduction in cooking time compared to lines with none of the QTL (Fig. 4.08). Other research found SNPs associated with cooking time on Pv02, Pv03, and Pv06 (Cichy et al., 2015), but those SNPs did not fall within the QTL discovered in this study. CT1.1 was found in another study by Garcia et al. (2012), making this QTL a possible candidate for marker assisted selection. In that study CT1.1 explained 21% of variation for cooking time whereas the current study showed that CT1.1 explained 5.4% of variation. Though work on the genetics behind cooking time is limited, it can be discerned using this work and literature on the topic that Pv01, Pv03, Pv06, and Pv10 are important areas determining cooking time in dry bean.

The QTL that explained the most variation for cooking time in this study was CT3.2, which was found at both locations in 2017. A possible candidate gene within this QTL is feruloyl esterase (Table S4.01). This enzyme breaks the bond between ferulate and polysaccharides such as pectin, which could lead to a reduction in structural integrity of the cell wall, a key component to reducing cooking time. Other candidate genes include pectate lyase (CT1.4 and CT6.1), polygalacturonase (CT1.4, and CT10.1), and an inhibitor of pectin methylesterase (PMEi) (CT10.1). These enzymes are associated with cell wall degradation and have been shown to effect cooking time in bean (Garcia et al., 1998, Martínez-Manrique et al., 2011). Enzymatic activity tests on the parental lines showed that pectin methylesterase activity was significantly lessened in the fast cooking TZ-37 parent compared to TZ-27 (Chapter 2). Overexpression of a pectin methylesterase inhibitor in TZ-37, reduced expression in TZ-27 or a less functional inhibitor in TZ-27 could explain these differences in enzymatic activity. PMEi

was first discovered in kiwi in 1990 and has since been found in many other plant species (Giovane et al., 2004). PMEi binds reversibly with pectin methylesterase to prevent its action (Giovane et al., 2004). Briefly, pectin methylesterase exposes carboxylic acid groups on pectin that can then bind with Ca forming cross-links between pectin molecules leading to decreased cell separation and longer cooking times (Jolie et al., 2010). By breaking down pectin and weakening adhesion of adjacent cells at the middle lamella, polygalacturonase could contribute to cooking time differences. Specifically, one study found that an increase in polygalacturonase activity was associated with decreased cooking times (Martínez-Manrique et al., 2011).

Of the 10 QTL for hydration capacity across chromosomes Pv01, Pv03, Pv05, and Pv06, two overlapped with QTL for cooking time. This finding might explain how some studies find a relationship between hydration capacity and cooking time, while others do not (Kinyanjui et al., 2015). Based on this study, hydration capacity does not affect cooking time even though QTL overlap with cooking time. Genes of interest within hydration capacity QTL include those for transport and barriers for fluid movement. A porin expressing gene was found in HC1.1. This was not a surprising finding as porins are responsible for the movement of molecules across membranes. HC1.1 explained 7.9% of variation for this trait. Two QTL on chromosome Pv06 explained the most variation for hydration capacity (17.0-18.5%). K and Mn transporters, and an ion channel were discovered among the 10 hydration capacity QTL (data not shown). It is possible that water can pass through these channels as well based on the small size of water molecules thus increasing hydration capacity.

Of the discovered QTL for seed coat percentage, the ones that explained the greatest variation for the trait were found on chromosomes Pv06 and Pv10. SCP6.2 explained 17.4% of variation

for seed coat percentage and contained a gene for wax esters biosynthesis. Previous work has established that bean seeds can imbibe water through the testa and micropyle (Swanson et al., 1985). However, water movement through the testa is inhibited in seed that contain a waxy exterior (Bushey et al., 2002). Differences in production of wax esters could explain why some lines have a greater seed coat percentage than others. SCP10.1 was a small QTL only containing 9 genes but should be investigated further as it explained 34.3% of variation for the trait in Morogoro in 2016.

Cooked protein concentration QTL were found on chromosomes Pv03, Pv06, Pv07, and Pv08. PT7.1 explained 11.5% of the variation for protein concentration and was also found by Casañas et al. (2013). Kamfwa et al. (2015) also found a QTL for percent N in seed on Pv07. In studying 206 different accessions, Katuuramu et al. (2018) also found protein concentration QTL on Pv07 as well as on Pv03 and Pv06. No QTL from the previous two studies overlapped with QTL from the current study, but it does highlight the importance of these chromosomes in controlling protein content as they continue to be discovered by independent researchers. It should be noted that PT6.1 was in close proximity to a QTL identified by Katuuramu et al. (2018) separated by only 2.3 Mb. Genes for an amino acid transporter were found within the QTL and could explain differences in protein concentration as amino acids are the building blocks of proteins. Ribosomal genes and genes related to ubiquitination were found throughout the QTL discovered for protein concentration and are likely causative agents in the amount of protein present. Other genes discovered in protein QTL included pectin methylesterase inhibitors, wax esters biosynthesis, and polygalacturonase, which were previously explained. A lack of or additional expression of these genes could have some effect on the overall protein concentration but could also affect other traits in this study as well.

Of the 10 QTL identified for 100 seed weight, SW6.17 stood out as it co-localized with QTL controlling three other traits (cooking time, protein concentration, and hydration capacity). Selection in this area of the genome would likely have the most impact if one wanted to manipulate multiple traits at one time. This is especially interesting as independent research by multiple authors have also shown the importance of chromosome Pv06 in controlling seed weight (Yuste-Lisbona et al., 2014, Blair et al., 2012, Cichy et al., 2009, Park et al., 2000). This area of the genome could explain the curious result of large seed cooking faster than small seed in the TT population, which contrasts with the findings of others where small seed tends to cook faster (Cichy et al., 2015, Black et al., 1998, Bressani et al, 1988). It is possible that a recombination occurred breaking the tight linkage in that area of seed weight and cooking time in TZ-37 resulting in seed with greater weight that cooked faster. This combination of traits was then brought into the TT RIL population through TZ-37.

CONCLUSION

Four traits that have been linked to cooking time in the literature were studied. Of those, hydration capacity was only correlated with cooking time in Arusha, and there was no significant difference between the parental lines for this trait. The other traits were more promising candidates to explore further with seed weight inversely correlating with cooking time in every environment, and seed coat percentage (direct) and protein concentration (inverse) correlating with cooking time in 3 of 4 environments.

A dense linkage map was developed with markers present every 0.5 cM on average. Thirty of the 47 QTL discovered for these traits were less than 1 Mb in size. These small physical distances allow for a greater ease of fine mapping these regions to narrow down the specific

area effecting each trait. A few candidate genes were discovered that point to the underlying cause of differences in cooking time in this work. Pectate lyase, polygalacturonase, and an PMEi were found, and each directly or indirectly effects cell adhesion through structural changes to pectin. Earlier studies of the parental lines (TZ-27 and TZ-37), suggested that cooking time differences were caused by some enzymatic process that occurred during the 12-hour soaking period before cooking (Chapter 2). Results from that work, provided evidence for the enzymatic hypothesis in that differential activity of pectin methylesterase, pectate lyase, polygalacturonase, or a combination of those three enzymes act during soaking to allow some lines to cook faster than others. Testing of the TT population for differences in enzymatic activity would need to be conducted to provide further support to this hypothesis.

Cooking time is an important trait to consider as a breeding objective. It influences whether people purchase beans, and a faster cooking bean means reduced labor and decreased fuel costs. These benefits would be especially useful in developing countries where burning firewood has negative health consequences. Though cooking time is affected by environment, a trend can be observed that fast cooking genotypes cook quicker than slow cooking genotypes regardless of where they are grown suggesting stability across environments. The identified QTL for cooking time explained much of the variation for the trait, and genotypes with 9 of the 10 QTL for fast cooking time, cooked 20.5 minutes faster than genotypes without any fast cooking QTL. In addition, the 4 QTL that were found in more than one environment reduced cooking time by 16 minutes compared to lines without those 4 QTL. This highlights the important genetic contribution to cooking time, making it an excellent candidate for marker assisted selection (MAS). Phenotyping a population for cooking time can take months even with a population of around 160 genotypes. Using MAS to select for faster cooking lines in

earlier generations, can result in fewer lines to phenotype for cooking time and more rapid progress through a breeding program so that fast cooking genotypes can be identified sooner. This would be extremely beneficial in developing countries where a faster cooking bean, would mean less time inhaling the combustion products of a wood burning fire as well as less time gathering firewood leaving more time for other things such as education. In addition, a faster cooking bean would likely be consumed more often leading to increased health benefits associated with bean consumption. Shellie-Dessert and Hosfield (1990), reasoned that shortening cooking time by 15 minutes would lead to a decrease of 150,000 metric tonnes of firewood being burned annually in Rwanda. It can be assumed that the amount of wood burned would be decreased even further when considering all countries that use firewood as cooking fuel. Discovery of 10 cooking time QTL that save 20.5 minutes of cooking time is an important step in increasing bean consumption and simultaneously decreasing the amount of firewood required to cook beans.

APPENDICES

APPENDIX A

CHAPTER 4 FIGURES AND TABLES

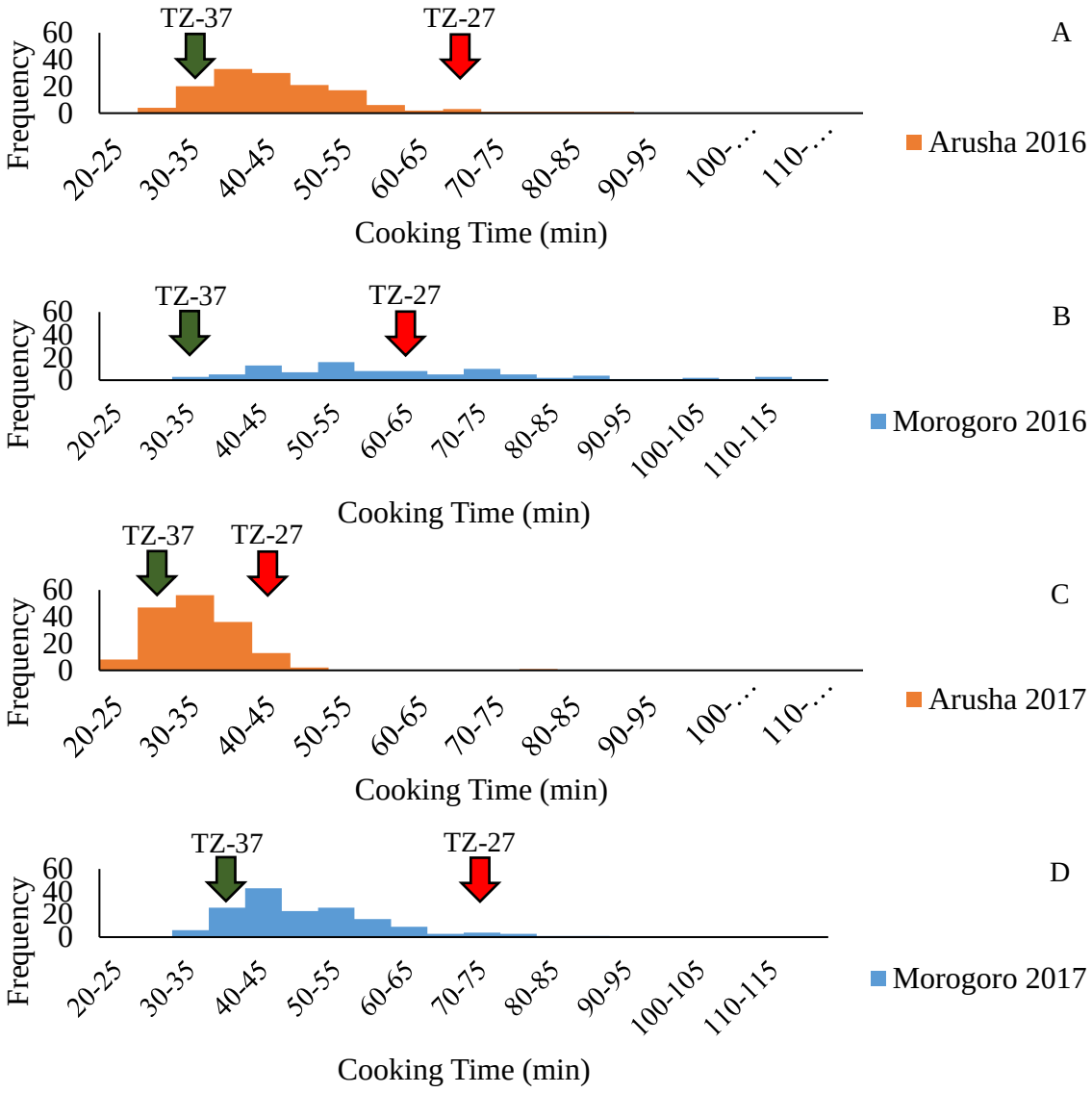


Figure 4.01. Cooking time histograms for beans grown in Arusha and Morogoro in 2016 and 2017. Distribution of cooking times for beans grown in Arusha (A and C) and Morogoro (B and D) in Tanzania in 2016 (A and B) and 2017 (C and D). Frequencies were determined by using the average of 2 replications for each line. Arrows indicate parental values for this trait.

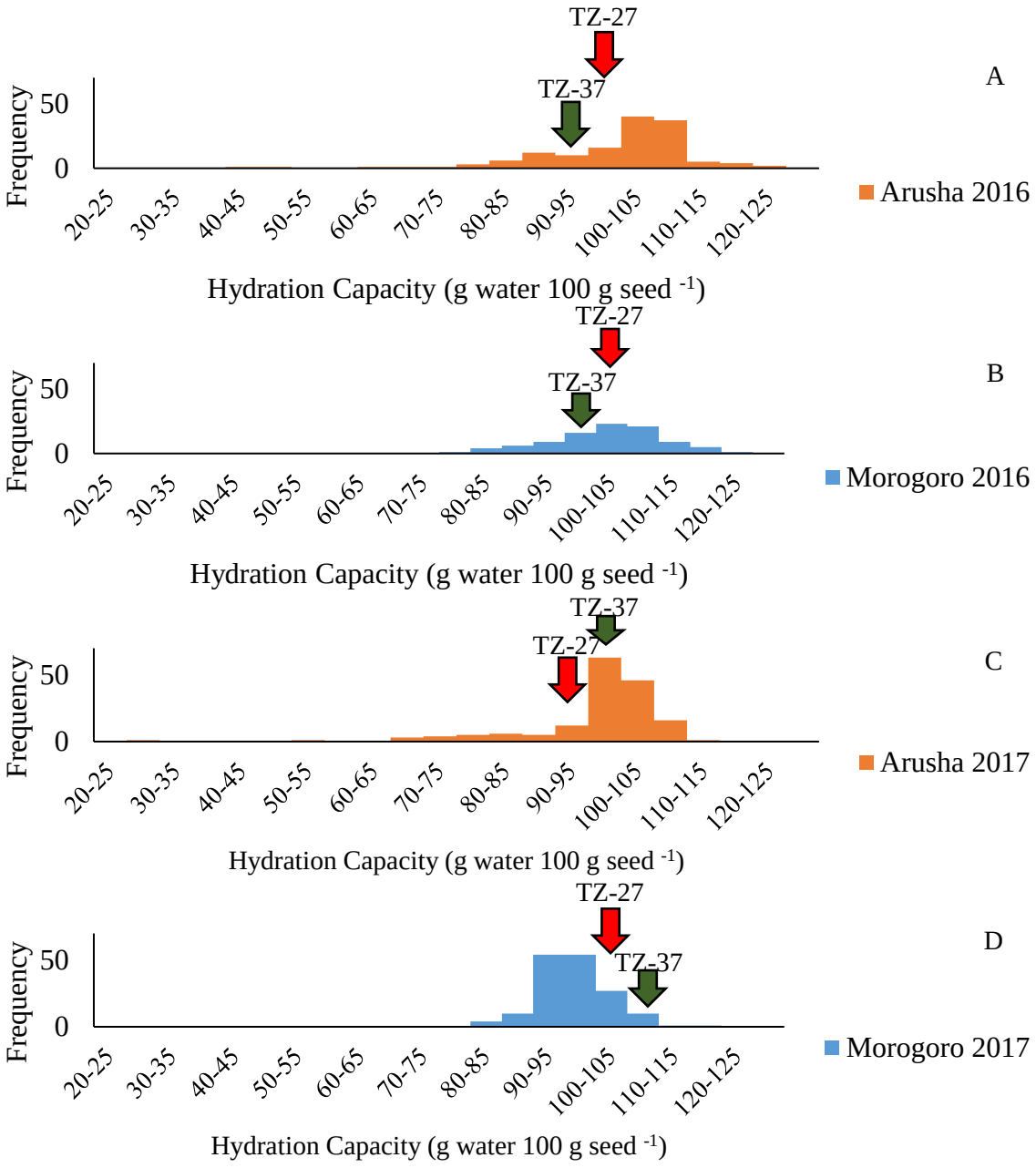
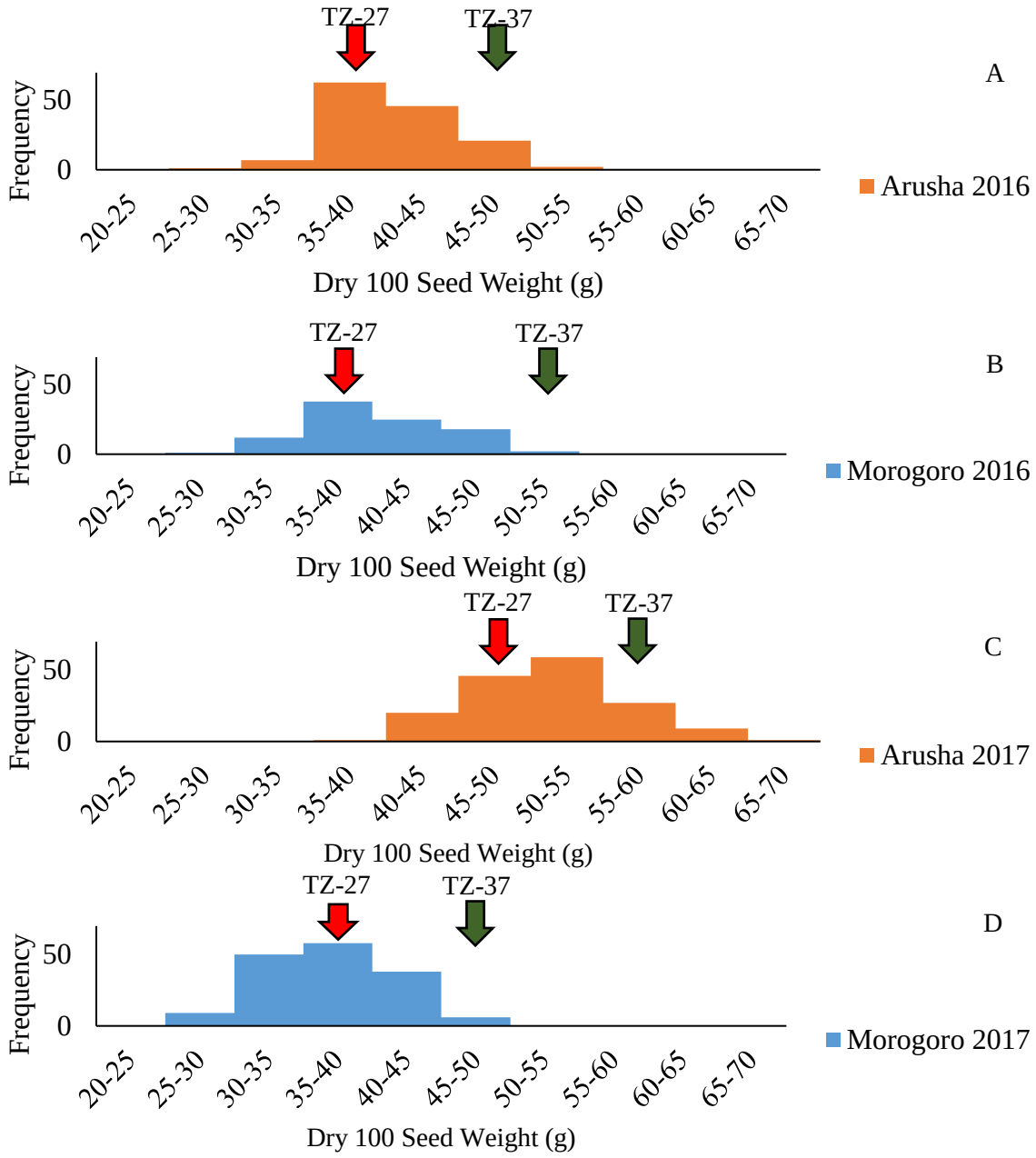


Figure 4.02. Hydration capacity histograms for beans grown in Arusha and Morogoro in 2016 and 2017. Distribution of hydration capacity for beans grown in Arusha (A and C) and Morogoro (B and D) in Tanzania in 2016 (A and B) and 2017 (C and D). Frequencies were determined by using the average of 2 replications for each line. Arrows indicate parental values for this trait.



c

Figure 4.03. Seed weight histograms for beans grown in Arusha and Morogoro in 2016 and 2017. Distribution of 100 seed weight for beans grown in Arusha (A and C) and Morogoro (B and D) in Tanzania in 2016 (A and B) and 2017 (C and D). Frequencies were determined by using the average of 2 replications for each line. Arrows indicate parental values for this trait.

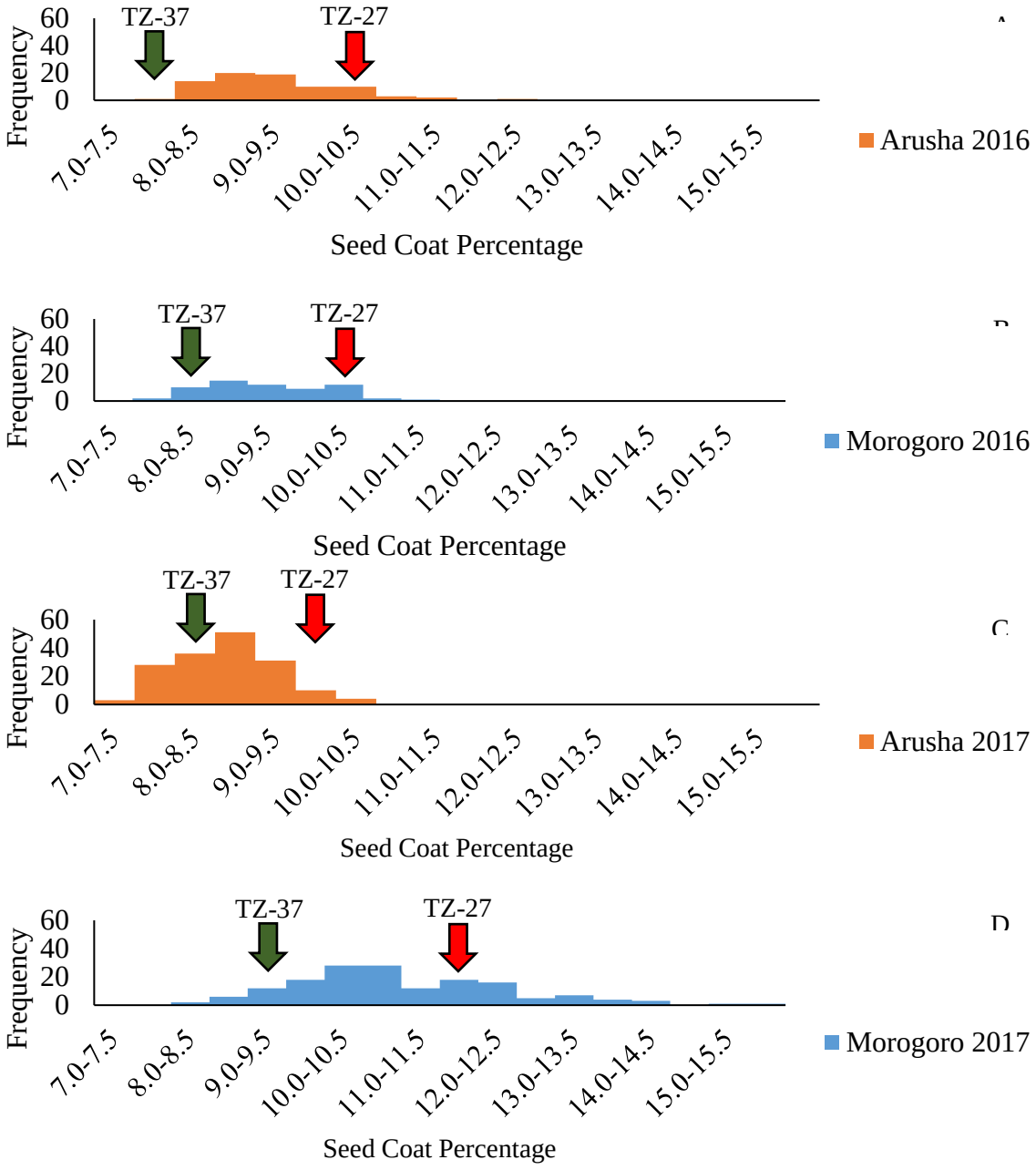


Figure 4.04. Seed coat percentage histograms for beans grown in Arusha and Morogoro in 2016 and 2017. Distribution of seed coat percentage for beans grown in Arusha (A and C) and Morogoro (B and D) in Tanzania in 2016 (A and B) and 2017 (C and D). Frequencies were determined by using the average of 2 replications for each line. Arrows indicate parental values for this trait.

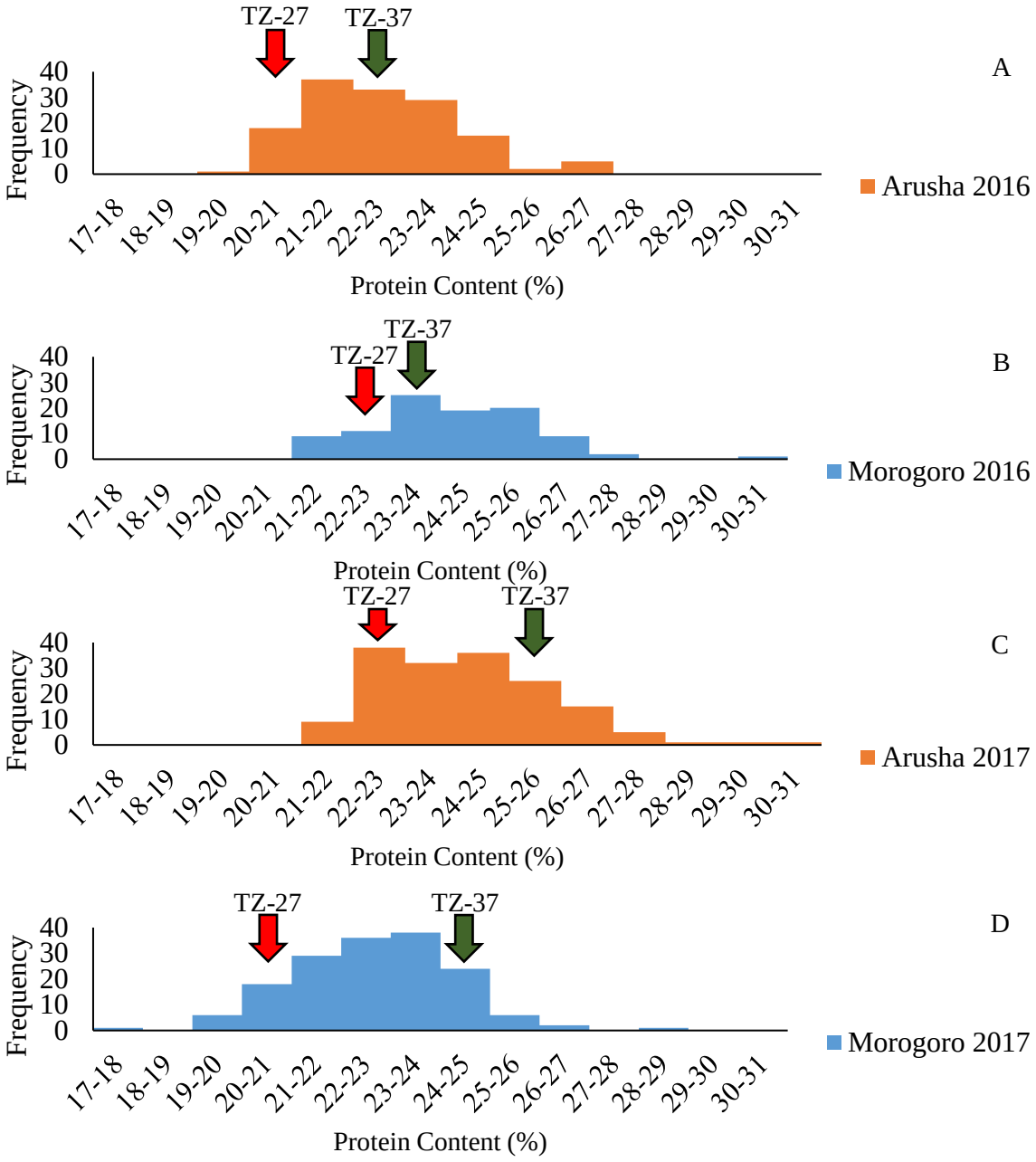


Figure 4.05. Protein concentration histograms for beans grown in Arusha and Morogoro in 2016 and 2017. Distribution of protein concentration for beans grown in Arusha (A and C) and Morogoro (B and D) in Tanzania in 2016 (A and B) and 2017 (C and D). Frequencies were determined by using the average of 2 replications for each line. Arrows indicate parental values for this trait.

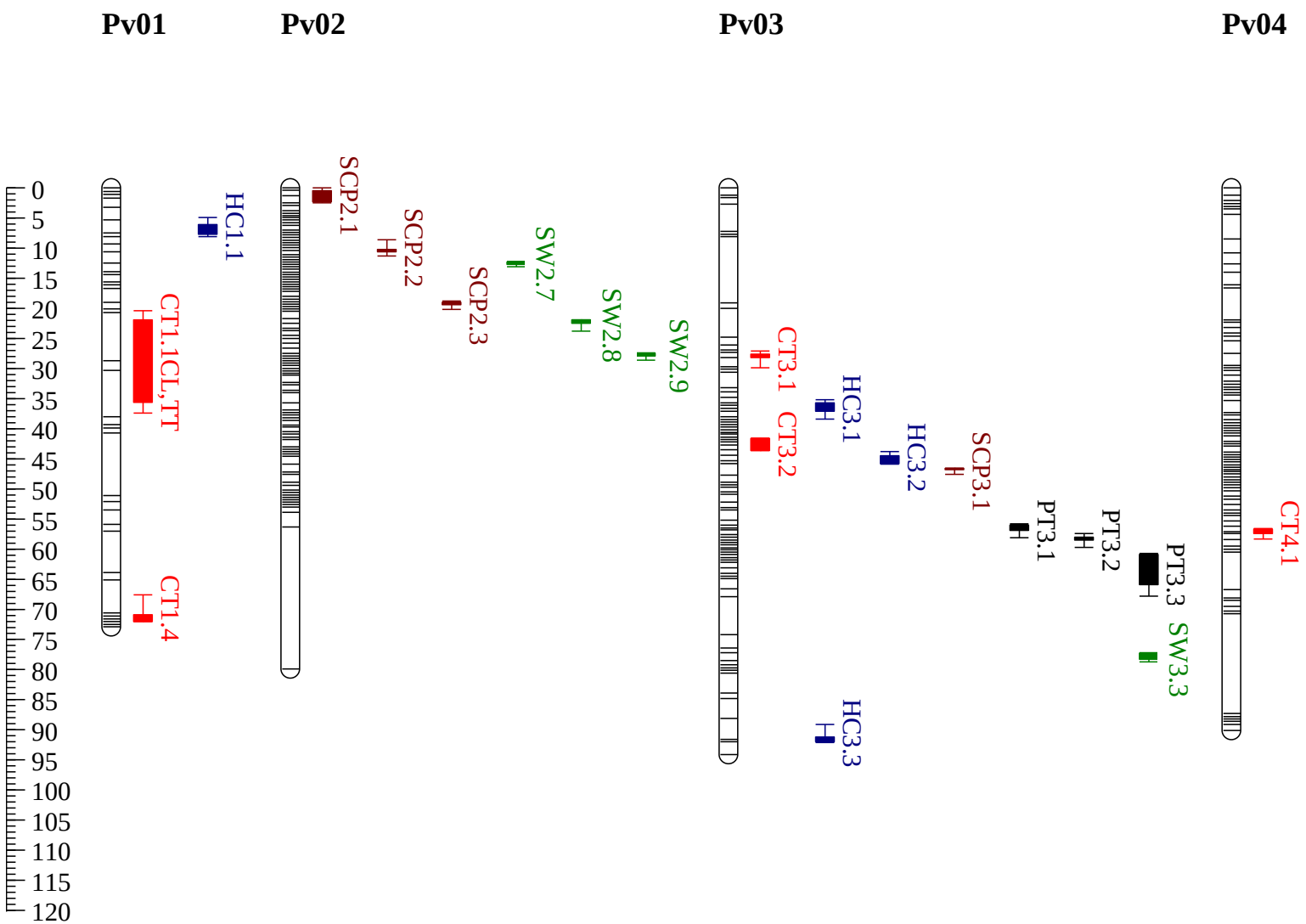
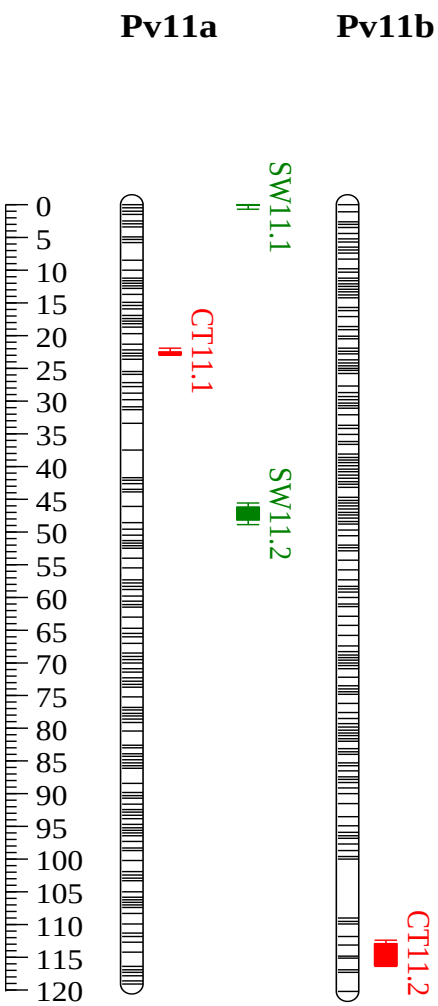


Figure 4.06. QTL map for cooking time (CT), hydration capacity (HC), seed coat percentage (SCP), protein concentration (PT), and 100 seed weight (SW) in the TZ-27/TZ-37 (TT) RIL population. Letters after the QTL name indicate the population that QTL was found in. The ruler on the left indicates size in cM. Linkage group 11 begins at 0 cM at the top of Pv11a and proceeds through to the bottom of Pv11b, which ends at 240 cM.

Figure 4.06 (cont'd)

Figure 4.06 (cont'd)



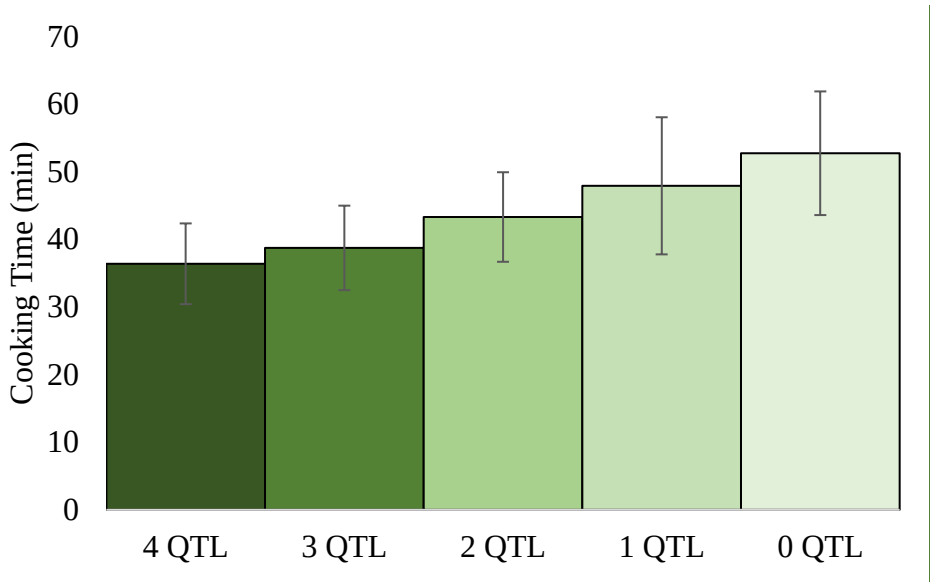


Figure 4.07. Effect of QTL that were found in multiple environments for cooking time. Cooking time values for QTL with 0 – 4 QTL for fast cooking times were averaged across all years and locations tested. Error bars represent standard deviations of two replicates. Out of 161 lines tested, 4 had 4 QTL, 15 had 3 QTL, 11 had 2 QTL, 16 had 1 QTL, and 17 had 0 QTL.

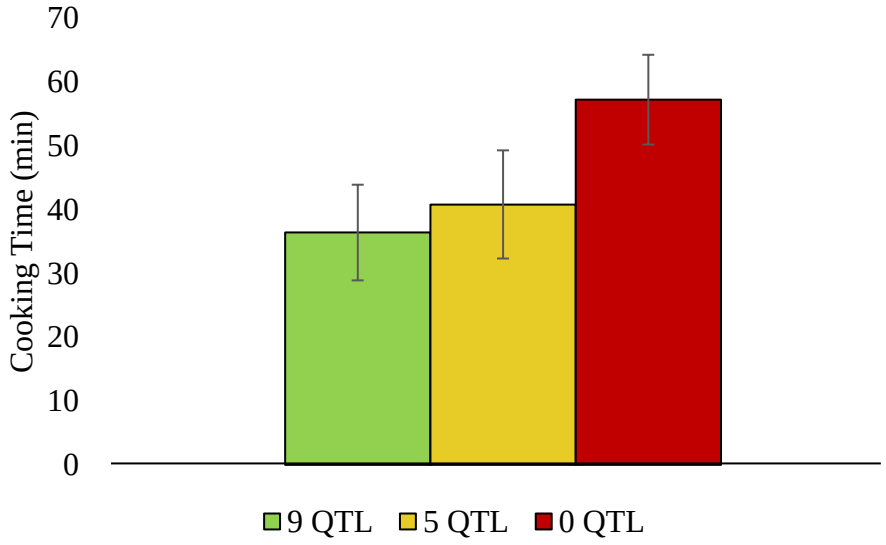


Figure 4.08. Effect of the number of cooking time QTL for cooking time. Cooking time values for lines with 0, 5, and 9 QTL for cooking time were averaged across all years and locations studied. Error bars represent standard deviations of two replicates. Of 161 lines tested, 4 had 9 QTL, 6 had 5 QTL, and 6 had 0 QTL.

Trait	Source of Variation	Degrees of Freedom	Type III SS	Mean Square	F Statistic	p Value
Cooking Time	Genotype	162	86388	533.3	12.6	<0.0001
	Year	1	31943	31943	756.9	<0.0001
	Location	1	60965	60965	1444.6	<0.0001
	Genotype*Year	150	24280	161.9	3.8	<0.0001
	Genotype*Location	162	30855	190.5	4.5	<0.0001
	Genotype*Location*Year	82	15469	188.6	4.5	<0.0001
100 Seed Weight (Dry)	Genotype	162	17790	109.8	10.9	<0.0001
	Year	1	2642.9	2642.9	263.3	<0.0001
	Location	1	10917	10917	1087.9	<0.0001
	Genotype*Year	150	2242.3	15.0	1.5	0.0007
	Genotype*Location	162	2481.4	15.3	1.5	0.0002
	Genotype*Location*Year	83	1098.7	13.4	1.3	0.03
Hydration Capacity	Genotype	162	52521	324.2	5.3	<0.0001
	Year	1	3478.4	3478.4	56.8	<0.0001
	Location	1	124.9	124.9	2.0	0.2
	Genotype*Year	150	16991	113.3	1.9	<0.0001
	Genotype*Location	162	22921	141.5	2.3	<0.0001
	Genotype*Location*Year	83	10115	123.4	2.0	<0.0001
Seed Coat Percentage	Genotype	162	608.3	3.8	15.4	<0.0001
	Year	1	37.6	37.6	154.5	<0.0001
	Location	1	159.8	159.4	655.4	<0.0001
	Genotype*Year	105	75.1	0.7	2.9	<0.0001
	Genotype*Location	162	219.4	1.4	5.6	<0.0001
	Genotype*Location*Year	34	27.0	0.8	3.6	<0.0001
Protein Concentration	Genotype	162	1645.2	10.2	5.6	<0.0001
	Year	1	0.04	0.04	0.02	0.9
	Location	1	1.3	1.3	0.7	0.4
	Genotype*Year	150	436.0	2.9	1.6	<0.0001
	Genotype*Location	162	397.1	2.5	1.3	0.008
	Genotype*Location*Year	83	166.7	2.0	1.1	0.2

Table 4.01. Analysis of variance for cooking time and other traits from 163 genotypes grown in Arusha and Morogoro in Tanzania during 2016 and 2017. The ANOVA analysis was carried out using SAS 9.4 statistical software package.

Traits	Parents	Arusha			Morogoro			RILs	Arusha		Morogoro		
	Years	TZ-27	TZ-37	p value	TZ-27	TZ-37	p value	Mean	Range	p value	Mean	Range	p value
Cooking Time	2016	66.9	35.6	***	62.9	33.3	**	44.0	23.4 – 94.5	***	62.8	28.4 – 134.6	***
	2017	41.0	27.7	***	70.4	35.3	***	33.1	21.4 – 79.1	***	48.7	30.0 – 100.7	***
100 Seed Weight (Dry)	2016	36.8	50.0	***	38.2	51.5	**	40.6	28.2 – 56.4	***	40.5	25.4 – 55.7	***
	2017	47.5	57.5	***	27.5	41.3	***	51.4	35.4 – 66.4	***	37.1	26.4 – 50.2	***
Hydration Capacity	2016	97.9	93.1	NS	105.1	97.4	NS	99.5	36.8 – 133.1	***	102.1	66.4 – 159.0	**
	2017	91.1	98.3	NS	100.4	106.2	NS	96.4	23.9 – 111.3	***	96.4	72.8 – 131.1	***
Seed Coat Percentage	2016	10.1	7.9	***	10.4	8.2	***	9.3	7.5 – 12.3	***	9.3	7.7 – 11.8	***
	2017	9.6	8.4	**	11.6	9.2	**	8.6	7.1 – 10.7	***	11.0	8.1 – 16.6	***
Protein Concentration	2016	20.8	22.6	NS	22.5	24.0	NS	22.5	17.4 – 27.3	**	24.3	20.1 – 30.3	**
	2017	22.1	25.8	***	20.8	24.6	**	24.2	20.7 – 31.1	***	22.7	16.9 – 28.8	***

Table 4.02. Means and ranges of 2 replications of five traits measured in the TT RIL population and the parental lines, TZ-27 and TZ-37.

A\B	100 Seed Weight	80% Cooking Time	Hydration Capacity	Seed Coat Percentage	Protein Concentration
100 Seed Weight		-0.6***	-0.4***	-0.4***	-0.04
80% Cooking Time	-0.3***		0.1	0.5***	-0.2*
Hydration Capacity	-0.3***	-0.3***		0.1	0.3**
Seed Coat Percentage	-0.5***	0.04	0.1		-0.1
Protein Concentration	0.1	-0.1	0.1	0.00	

C\D	100 Seed Weight	80% Cooking Time	Hydration Capacity	Seed Coat Percentage	Protein Concentration
100 Seed Weight		-0.6***	-0.2***	-0.6***	0.4***
80% Cooking Time	-0.4***		-0.01	0.5***	-0.4***
Hydration Capacity	-0.2**	-0.5***		-0.1	0.2**
Seed Coat Percentage	-0.6***	0.4***	-0.03		-0.3***
Protein Concentration	-0.4***	-0.4***	0.1	-0.2***	

Table 4.03. Pearson correlations of traits measured for the TT RIL population at Arusha (A and C) **and Morogoro** (B and D) **in Tanzania during the 2016** (A and B) **and 2017** (C and D) **growing seasons.** Correlations significant at * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.0001$. No asterisk represented no statistical significance.

Chromosome #	# of Markers	Chromosome Size (cM)	Marker Density (Average Distance between Markers [in cM])
1	59	72.9	1.2
2	364	79.9	0.2
3	170	94.1	0.6
4	233	90.1	0.4
5	201	69.5	0.4
6	263	105.1	0.4
7	81	74.5	0.9
8	302	118.6	0.4
9	129	99.5	0.8
10	149	92.5	0.6
11	476	240.4	0.5
Total	2427	1137.1	0.5

Table 4.04. Linkage map information. Over 2,427 markers were discovered resulting in an overall genome size of 1137.1 cM. Marker density varied by linkage group with the average coverage across the entire genome being 1 marker every 0.5 cM.

QTL Name	LG	Trait	Year	Location	LOD Threshold	LOD Max	R^2 (%)	Additive	Physical Location	Flanking Markers	QTL range (cM)	Number of SNPs within QTL
CT1.1$^{CL, TT}$	Pv01	Cooking Time	2016	Arusha	2.76	2.8	5.4	-2.8	4687531 - 4776925	Chr01_4687531, Chr01_4776925	26.3 - 34.6	4
CT1.1$^{CL, TT}$	Pv01	Cooking Time	2017	Arusha	2.55	2.7	4.1	-1.5	4687531 - 4776925	Chr01_4687531, Chr01_4776925	30.2 - 37.4	4
CT1.4^{TT}	Pv01	Cooking Time	2017	Morogoro	2.69	3.1	4.1	-2.25	50446775 - 51415447	Chr01_50446775, Chr01_51415447	67.6 – 72.0	10
CT3.1^{TT}	Pv03	Cooking Time	2017	Morogoro	2.69	6.3	10.1	-3.5	5345202 - 5684988	Chr03_5345202, Chr03_5684988	27.1 – 29.9	5
CT3.2^{TT}	Pv03	Cooking Time	2017	Morogoro	2.69	10.2	15.3	-4.2	14820087 - 16876828	Chr03_14820087, Chr03_16876828	41.6 – 43.6	10
CT3.2^{TT}	Pv03	Cooking Time	2017	Arusha	2.55	8.0	20.5	-14.3	14931976 - 16827835	Chr03_14931976, Chr03_16827835	42 – 43.2	7
CT4.1^{TT}	Pv04	Cooking Time	2017	Arusha	2.55	2.9	3.5	-1.4	40823247 - 41649034	Chr04_40823247, Chr04_41649034	56.6 – 58.3	6
CT5.1^{TT}	Pv05	Cooking Time	2017	Arusha	2.55	3.3	3.9	-1.3	6659800 - 6983523	Chr05_6659800, Chr05_6983523	33.4- 35.0	14
CT6.1^{TT}	Pv06	Cooking Time	2017	Arusha	2.55	9.7	14.5	-2.6	24610619 - 25311110	Chr06_24610619, Chr06_25311110	85.6 – 89.3	9
CT6.1^{TT}	Pv06	Cooking Time	2017	Morogoro	2.69	6.9	9.7	-3.4	24945213 - 25311110	Chr06_24945213, Chr06_25311110	87.0 – 89.3	4
CT10.1^{TT}	Pv10	Cooking Time	2017	Morogoro	2.69	4.2	6.3	-2.8	5660338 - 6984937	Chr10_5660338, Chr10_6984937	20.9 – 25.0	7
CT10.1^{TT}	Pv10	Cooking Time	2016	Morogoro	2.91	3.5	7.8	-5.9	5660338 - 6984937	Chr10_5660338, Chr10_6984937	20.9 – 25.0	7
CT11.1^{TT}	Pv11	Cooking Time	2016	Arusha	2.76	6.3	15.0	-6.2	3077505 - 3153110	Chr11_3077505, Chr11_3153110	21.9 – 23.0	9

Table 4.05. QTL information for cooking time (CT), hydration capacity (HC), seed coat percentage (SCP), protein concentration (PT) and 100 seed weight (SW). The largest LOD, r^2, and additive score within the QTL were reported. Positive additive values indicated the allele was contributed by TZ-27, and negative values indicated TZ-37 contributed the allele. The threshold LOD was generated by running 1000 permutations and represents the minimum LOD score significant at $p < 0.05$. Flanking markers are named for the physical placement on the named linkage group. TT represents the TZ-27 x TZ-37 RIL population. Other superscript letters represent a QTL that was discovered in another unrelated population.

Table 4.05 (cont'd)

CT11.2[TT]	Pv11	Cooking Time	2017	Arusha	2.55	3.5	5.1	-1.6	50757624 - 51161990	Chr11_50757624, Chr11_51161990	232.5 – 236.5	7
HC1.1[TT]	Pv01	Hydration Capacity	2017	Morogoro	2.81	7.3	7.9	1.6	1344330 - 2835419	Chr01_1344330, Chr01_2835419	4.9 – 8.1	6
HC3.1[TT]	Pv03	Hydration Capacity	2016	Morogoro	2.86	3.9	15.3	-5.4	7222712 - 9239838	Chr03_7222712, Chr03_9239838	35.2 – 38.4	12
HC3.2[TT]	Pv03	Hydration Capacity	2017	Morogoro	2.86	4.1	9.7	-1.8	16827844 - 17859277	Chr03_16827844, Chr03_17859277	43.8 – 45.9	7
HC3.3[TT]	Pv03	Hydration Capacity	2017	Arusha	2.59	2.9	7.0	3.5	46933182 - 49610607	Chr03_46933182, Chr03_49610607	89.1 – 92.1	5
HC5.1[TT]	Pv05	Hydration Capacity	2017	Arusha	2.59	3.6	8.1	3.8	1494629 - 1875228	Chr05_1494629, Chr05_1875228	0.0 – 1.5	13
HC5.2[TT]	Pv05	Hydration Capacity	2016	Arusha	2.73	3.1	8.9	4.0	6831009 - 13825799	Chr05_6831009, Chr05_13825799	34.4 – 36	17
HC6.1[TT]	Pv06	Hydration Capacity	2017	Arusha	2.59	7.7	18.5	-5.5	4786327 - 4908107	Chr06_4786327, Chr06_4908107	18.3 – 21.3	8
HC6.2[TT]	Pv06	Hydration Capacity	2017	Arusha	2.59	7.0	17.0	-5.2	7046027 - 7337422	Chr06_7046027, Chr06_7337422	32.6 – 35.7	7
HC6.3[TT]	Pv06	Hydration Capacity	2017	Morogoro	2.86	5.4	13.1	-1.9	24610619 - 24964521	Chr06_24610619, Chr06_24964521	85.4 – 87.1	7
HC6.4[TT]	Pv06	Hydration Capacity	2017	Morogoro	2.86	3.7	9.8	-1.7	25311110 - 27373518	Chr06_25311110, Chr06_27373518	93.9 – 97.1	6
SCP2.1[TT]	Pv02	Seed Coat Percentage	2016	Arusha	2.91	4.5	9.2	-0.5	5504885 - 5776592	Chr02_5504885, Chr02_5776592	0.0 – 2.5	4
SCP2.2[TT]	Pv02	Seed Coat Percentage	2016	Arusha	2.91	3.2	6.9	-0.5	9136872 - 9459188	Chr02_9136872, Chr02_9459188	8.6 – 11.3	18
SCP2.3[TT]	Pv02	Seed Coat Percentage	2017	Arusha	2.89	6.4	8.0	-0.2	12204074 - 12776363	Chr02_12204074, Chr02_12776363	18.8 – 20.2	19
SCP3.1[TT]	Pv03	Seed Coat Percentage	2016	Morogoro	3.00	4.5	12.8	-0.6	17258583 - 17859277	Chr03_17258583, Chr03_17859277	46.6 – 47.6	2
SCP6.1[TT]	Pv06	Seed Coat Percentage	2016	Arusha	2.91	7.5	18.9	-0.4	1469365 - 2737435	Chr06_1469365, Chr06_2737435	6.4 – 8.8	24
SCP6.2[TT]	Pv06	Seed Coat Percentage	2016	Arusha	2.91	6.8	17.4	-0.4	3805946 - 4721385	Chr06_3805946, Chr06_4721385	16.2 – 17.2	4
SCP6.3[TT]	Pv06	Seed Coat Percentage	2017	Arusha	2.89	7.2	9.0	-0.2	7847018 - 7945276	Chr06_7847018, Chr06_7945276	45 – 46.3	7

Table 4.05 (cont'd)

SCP8.1[TT]	Pv08	Seed Coat Percentage	2016	Arusha	2.91	3.1	6.0	-0.2	46269208 - 46441851	Chr08_46269208, Chr08_46441851	64.1 – 65.7	11
SCP10.1[TT]	Pv10	Seed Coat Percentage	2016	Morogoro	3.00	10.1	34.3	-0.5	35246620 - 35646609	Chr10_35246620, Chr10_35646609	81.6 – 83.4	2
PT3.1[TT]	Pv03	Protein Concentration	2016	Morogoro	2.90	8.4	17.6	-0.9	22619427 - 29362352	Chr03_22619427, Chr03_29362352	55.8 – 56.8	11
PT3.2[TT]	Pv03	Protein Concentration	2016	Arusha	2.83	7.3	13.4	-0.7	29365504 - 29527739	Chr03_29365504, Chr03_29527739	57.4 – 58.5	4
PT3.3[TT]	Pv03	Protein Concentration	2016	Arusha	2.83	6.6	12.2	-0.6	31431552 - 31801066	Chr03_31431552, Chr03_31801066	60.7 – 61.4	5
PT3.3[TT]	Pv03	Protein Concentration	2017	Arusha	3.03	3.5	9.0	-0.5	31434663 - 36084796	Chr03_31434663, Chr03_36084796	61.3 – 65.5	14
PT3.3[TT]	Pv03	Protein Concentration	2016	Morogoro	2.90	9.5	20.2	-0.9	32118690 - 36084796	Chr03_32118690, Chr03_36084796	62.4 – 67.8	8
PT6.1[TT]	Pv06	Protein Concentration	2017	Arusha	3.03	6.8	19.0	-0.7	14488021 - 24610619	Chr06_14488021, Chr06_24610619	82.6 – 84.1	5
PT7.1[XC, TT]	Pv07	Protein Concentration	2016	Morogoro	2.90	6.4	11.5	0.7	1996890 - 3209979	Chr07_1996890, Chr07_3209979	7.2 – 11.0	3
PT7.2[TT]	Pv07	Protein Concentration	2016	Arusha	2.83	2.9	3.8	0.4	5773055 - 6626914	Chr07_5773055, Chr07_6626914	21.7 – 22.5	4
PT8.1[TT]	Pv08	Protein Concentration	2017	Morogoro	2.77	2.9	8.4	-0.5	53079387 - 61247715	Chr08_53079387, Chr08_61247715	82.1 – 92.9	3
SW2.7[TT]	Pv02	100 Dry Seed Weight	2016	Arusha	2.74	4.6	9.8	-1.9	9551131 – 9593930	Chr02_9551131 – Chr02_9593930	12.2 – 12.7	5
SW2.8[TT]	Pv02	100 Dry Seed Weight	2016	Arusha	2.74	5.8	12.0	-2.2	12896409 - 13110313	Chr02_12896409, Chr02_13110313	21.9 – 23.8	8
SW2.9[TT]	Pv02	100 Dry Seed Weight	2016	Arusha	2.74	5.3	5.3	-1.5	14919797 - 21254430	Chr02_14919797, Chr02_21254430	27.4 – 28.6	9
SW3.2[TT]	Pv03	100 Dry Seed Weight	2017	Morogoro	2.95	10.1	15.8	-1.9	40330058 - 42320680	Chr03_40330058, Chr03_42320680	77.2 – 78.7	4
SW5.1[TT]	Pv05	100 Dry Seed Weight	2016	Arusha	2.74	4.0	7.6	-1.3	3533038 - 3958861	Chr05_3533038, Chr05_3958861	13.1 – 15.9	5
SW5.1[TT]	Pv05	100 Dry Seed Weight	2017	Morogoro	2.95	6.7	9.9	-1.4	3533038 - 4336493	Chr05_3533038, Chr05_4336493	14.8 – 18.0	13
SW5.2[TT]	Pv05	100 Dry Seed Weight	2016	Morogoro	2.80	4.6	11.9	-2.3	35770054 - 37273196	Chr05_35770054, Chr05_37273196	43.9 – 48.1	3
SW5.3[TT]	Pv05	100 Dry Seed Weight	2016	Morogoro	2.80	1.6	4.3	-1.4	36812730 - 37653109	Chr05_36812730, Chr05_37653109	48.7 – 59.6	5

Table 4.05 (cont'd)

SW6.17^{TT}	Pv06	100 Dry Seed Weight	2017	Arusha	2.90	3.8	5.1	-1.3	24607532 - 24945156	Chr06_24607532, Chr06_24945156	83.9 – 86.1	5
SW6.17^{TT}	Pv06	100 Dry Seed Weight	2016	Arusha	2.74	5.3	8.6	-1.4	24607532 - 24964521	Chr06_24607532, Chr06_24964521	84.0 – 87.0	8
SW6.18^{TT}	Pv06	100 Dry Seed Weight	2017	Arusha	2.90	3.1	4.1	-1.2	27581319 - 29079040	Chr06_27581319, Chr06_29079040	99.1 – 104.1	4
SW11.1^{TT}	Pv11	100 Dry Seed Weight	2016	Arusha	2.74	4.5	8.4	-1.3	137201 - 246489	Chr11_137201, Chr11_246489	0.0 – 0.7	8
SW11.2^{TT}	Pv11	100 Dry Seed Weight	2016	Morogoro	2.80	3.5	9.3	-1.6	6077563 - 7613114	Chr11_6077563, Chr11_7613114	45.6 – 48.9	4

APPENDIX B

CHAPTER 4 SUPPLEMENTAL TABLE

QTL	Trait	Number of genes in QTL region	Candidate Genes within QTL
CT1.1[CL, TT]	Cooking Time	2	Phvul.001G046200.1 – Esterified Suberin Biosynthesis*
CT1.4 [TT]	Cooking Time	147	Phvul.001G256800.1 – Pectate Lyase Phvul.001G256900.2 – Pectate Lyase Phvul.001G265400.2 – Glucosyl/Glucoronosyl Transferases Phvul.001G266200.1 – Polygalacturonase Phvul.001G267300.1 – Magnesium Transporter
CT3.1 [TT]	Cooking Time	8	Phvul.003G045701.1 – Myb-like DNA-binding domain Phvul.003G045702.1 – Myb-like DNA-binding domain Phvul.003G045703.1 – Myb-like DNA-binding domain Phvul.003G046000.1 – Myb-like DNA-binding domain Phvul.003G046200.1 – Myb-like DNA-binding domain Phvul.003G046300.1 – Myb-like DNA-binding domain
CT3.2 [TT]	Cooking Time	36	Phvul.003G106100.1 – Ulp1 Peptidase Phvul.003G096200.2 – Feruloyl Esterase
CT4.1 [TT]	Cooking Time	41	Phvul.004G120300.1 – Ubiquitin-conjugating enzyme Phvul.004G121700.1 – Ubiquitin family Phvul.004G121800.1 – Ubiquitin family Phvul.004G123100.1 – Peroxidase Phvul.004G123100.1 - Peroxidase

Table S4.01. Gene information for cooking time QTL. Candidate genes for each QTL are named based off their identification in Phytozome (version 11). Genes with an asterisk represent a location near the QTL but not within the QTL.

Table S4.01 (cont'd)

CT5.1 [TT]	Cooking Time	18	Phvul.005G051900.3 – Suberin monomers biosynthesis
CT6.1 [TT]	Cooking Time	50	Phvul.006G142000.1 – Sugar (and other) Transporter Phvul.006G142500.1 – Calcium-binding protein 39-related Phvul.006G142700.2 – Na+/dicarboxylate, Na+/tricarboxylate and phosphate transporters Phvul.006G143200.1 – Wax esters biosynthesis II Phvul.006G147500.2 – Pectate lyase Phvul.006G147500.2 – Pectate lyase
CT10.1 [TT]	Cooking Time	72	Phvul.010G038900.1 – Polygalacturonase Phvul.010G039000.1 – Polygalacturonase/pectinase Phvul.010G041500.1 – Plant invertase/pectin methylesterase inhibitor Phvul.010G042200.1 – UDP-glucoronosyl and UDP-glucosyl transferase Phvul.010G042300.1 – UDP- glucoronosyl and UDP-glucosyl transferase Phvul.010G042600.1 – UDP- glucoronosyl and UDP-glucosyl transferase
CT11.1 [TT]	Cooking Time	10	Phvul.011G033400.1 – 50S ribosome-binding GTPase
CT11.2 [TT]	Cooking Time	35	Phvul.011G196200.1 – Salt stress response/antifungal Phvul.011G196200.1 – Salt stress response/antifungal

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