SELECTION OF DRY BEAN GENOTYPES ADAPTED FOR DROUGHT TOLERANCE IN THE NORTHERN GREAT PLAINS

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Angela María Linares-Ramírez

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

DOCTOR OF PHILOSOPHY

SUPERVISORY COMMITTEE:

Dr. Juan M. Osorno
Chair

Dr. Timothy G. Porch

Dr. Edward Deckard

Dr. Luis Del Rio Mendoza

Approved:

11/16/13
Date

Dr. Richard Horsley
Department Chair

ABSTRACT

Drought stress is a production constraint that growers face in the Northern Great Plains. This research was conducted to (1) assess the differences in seed yield due to drought within each one of the three most important dry bean market classes grown in the region (pinto, navy, and black), (2) to estimate the genetic gain and progress over time due to the use of improved dry bean cultivars from 1981 to 2011, (3) and to evaluate the 'Buster'/SER 22 recombinant inbred line population under drought conditions and identify loci associated with drought tolerance that could be used in Marker Assisted Selection (MAS) in the future. Results showed that, on average, drought stress (rain-fed and /or dryland conditions) can reduce seed yield by 35% in North Dakota. Seed yield in black cultivars can be reduced as much as 36% in dryland when compared to irrigated conditions, followed by navy and pinto with 31.5 and 32% of seed yield reduction, respectively. Data over 26 years showed yield gains of 15, 14, and 11kg ha^{-1} yr^{-1} for pinto, black, and navy beans under dryland conditions, respectively, while yield reductions were observed under irrigated conditions. The results will help to design strategic plans toward the genetic improvement of dry beans in the region. On the other hand, quantitative trait loci (QTL) were detected for seed yield, 100-seed weight, days to maturity, days to flower, and leaf temperature. The genetic map had a length of 778.4 cM and was based on 378 single nucleotide polymorphism (SNP) markers. Transgressive segregation was also observed for all phenotypes under study. Some of the QTL identified could be useful for selection purposes under optimal, irrigated, and drought conditions.

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PREFACE

New challenges are present every day in the agricultural world and drought is one of them. Drought, due to either insufficient or unpredictable rainfall, has been identified worldwide as a dry bean (*Phaseolus vulgaris* L.) production problem exceeded in magnitude only by dry bean diseases. In North Dakota, for example, it is becoming more important given that dry bean production is being pushed from the fertile, non-irrigated Red River Valley, located in the eastern part of the state, towards the central and western regions of the state, where drought stress occurs more frequently.

This dissertation includes the result of the research work concerning drought stress in dry beans, which was carried out over the period from August 2008 to September of 2013 in the Department of Plant Sciences at North Dakota State University. Five years ago, it all started out with me spending at least a few minutes each day with anxiety, stress, and doubt about whether I will ever finish my thesis, what I was doing with my life, and whether I made the right decision to come to Fargo. I yet don't recognize most of the answers to many questions, but after all it has soured into a great journey.

Certainly, I would not have reached this point if it was not for the valuable help and contributions made by others, especially my advisor Dr. Juan M. Osorno, Dr. Carlos A. Urrea, and Dr. Timothy E. Porch, who believed that something good was about to come from this research. Last but not least many thanks, to Gonzalo Rojas-Cifuentes, Albert "Jody" VanderWal, Rian Lee, and Dr. Sujan Mamidi, who took in the patience and devotion to guide me and help me to address fundamental questions, while opening my mind to fresh thoughts.

This dissertation has been divided into three main sections. The first section includes a complete recap of the production and economic importance of dry beans, drought stress in the

Great Plains, drought mechanisms and dry bean response to drought, as well as other issues linked to breeding for drought stress. The second section reviews the ongoing variety trials conducted at the Carrington Research and Extension Center every year. The primary aim of this stdy is to provide agronomic information to the farmers about the new cultivar releases and cultivars widely grown in the area. For this study, the main objective was to assess the differences in seed yield due to drought stress within each one of the three most important dry bean market classes grown in the region (pinto, navy, and black), and the second objective was to estimate the genetic gain and progress over time due to the use of improved dry bean cultivars from 1981 to 2011.

The third section focuses on the evaluation of a recombinant inbred line population developed from a cross between 'Buster', a drought-susceptible pinto cultivar, and SER 22, a drought-tolerant small red breeding line from the International Center for Tropical Agriculture (CIAT) under drought and irrigated conditions. The identification of loci associated with drought tolerance that could be used in Marker Assisted Selection in the near future is then explained.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

LITERATURE REVIEW

Production and Economic Importance of Dry Beans

Dry bean (*Phaseolus vulgaris* L.) is the most important food legume in the world and represents 50% of the grain legumes consumed worldwide. In 2012, dry beans were produced on 29.2 million ha in 120 countries, and the total production was 23.3 million MT (FAOSTAT, 2013). U.S. dry bean production supplies both the home market (canning and dry), as easily as the export markets in various states. The U.S. economy gains about $2.0 billion per year from the dry beans (USDA-ERS, 2012). Average seed yield varies significantly worldwide, from 638 kg ha^{-1} in countries like Uganda, to 1,944 kg ha^{-1} in countries such as the U.S., Brazil, Mexico, and China (FAOSTAT, 2013).

Furthermore, bean consumption is increasing in mature economies because of the rising importance of ethnic foods and the perceived health benefits related to their consumption. This includes higher levels of calcium, phosphorus, potassium, thiamin, and niacin. Dry beans help reduce blood cholesterol levels and their low amounts of sodium and fat would help to protect against heart disease (Andersen et al., 1984; Andersen and Gustafson, 1989; McClean et al., 2004), while also containing potential preventive and curative effects t of other diseases. With the exclusion of meat products, dry beans are the highest source of protein available. Beans also have more fiber than any other unprocessed food. Even considering that beans are a beneficial source of fiber and have other beneficial nutritional components, the average consumption in the U.S. was 3 kg per person, with a high of 3.3 kg in 2010 and a low of 2.7 kg in 2009 (U.S. Dry Bean Council, 2012). In developing countries, bean consumption can be as large as 54.5 kg per year, especially in Africa and Latin America where beans are one of the most significant factors in the diet (Broughton et al., 2003; Lynch, 2007).

Although drought events occur with increasing regularity resulting in yield reductions worldwide, the panorama in the U.S. dry bean production was an increase of 12% of production during the last decade (Vandermark et al., 2013; USDA-NASS, 2012). However, this scenario seems to be the exception more than the rule, with the frequency of drought and high temperature stress increases and dry bean production being pushed to more marginal environments. Approximately 80% of the total dry bean production occur in the central and northern Great Plains of the U.S.: Colorado, Minnesota, Nebraska, North Dakota, and Wyoming (USDA-NASS, 2013). North Dakota ranks first in U.S. dry bean production, accounting for 40% of the total production, and together, Michigan, Minnesota, and Nebraska account for the other half of U.S. production (USDA-NASS, 2013).

The last five-year average production in North Dakota was 483,384 MT, the average planted area was 273,000 ha, and the harvested area was 260,000 ha (USDA-NASS, 2013). Statewide average yield from 2007 to 2012 was 1,655 kg ha^{-1}. Pintos accounted for 55% of the total production, being the major market class grown in the region, followed by navy beans accounting for 23%, blacks 16, pinks 3.2, great northern 0.2, and other minor classes making up the remainder (USDA-ERS, 2013). North Dakota along with Minnesota account for approximately 50% of the total U.S. production.

Drought Stress in the Great Plains

Genetic improvement of dry bean for drought tolerance is becoming more important due to the shift of dry bean acres from more humid and fertile areas into more marginal zones where low water availability and extreme temperatures are limiting factors. This is occurring not only in the U.S., but also in other regions (Beebe et al., 2013; Beebe et al., 2008; Porch et al., 2007; Wortmann et al., 1998). In North Dakota, for example, dry bean production is being pushed from

the fertile, non-irrigated Red River Valley, located in the eastern part of the state, towards the central and western regions of the state, where irrigation is needed for productivity. Currently, most commercial cultivars are showing low tolerance to these harsher environments. At the same time, declining ground water and diminished surface water supplies have exacerbated yield losses due to drought across the inter-mountain west and Great Plains over the past eight years. In 2006, for example, more than 60% of the country was abnormally dry or had drought conditions, including the Dakotas, Minnesota, Montana, and Wisconsin (MacPherson, 2006). In 2012, 80% of the contiguous U.S. were under extreme drought. In addition, the highest record for temperature was recorded that year in Arizona, Oklahoma, Missouri, Arkansas, Kansas, Nebraska, and Illinois (NOAA, 2012). Particularly, the central U.S. region beat the near record of low precipitation levels the same year (NOAA, 2012). In 2012, around 1,692 counties across 36 states in the U.S. were legally declared primary natural disaster areas due to severe drought. Hundreds of additional counties bordering the primary disaster areas were designated as "contiguous" disaster areas eligible for federal aid (Baker, 2012).

Crops like corn and soybeans were noted to be failing or yielding less in the 2012 production season due to the drought's presence in farming areas in Illinois, Mississippi, Indiana, Missouri, Arkansas, Tennessee, and Kentucky (USDA-NASS, 2012). Consequently, food prices were expected to rise dramatically because of the resulting supply shortfall. In contrast, North Dakota had a record seed yield (kg ha^{-1}) even though it was a dry year. Seed yield increase was mainly due to the low pressure of diseases and the good performance of varieties planted in the area (USDA-NASS, 2012). However, declining groundwater levels in irrigated areas of the Great Plains, for example, have already required a shift to more limited irrigation or a return to dryland crop production in some areas (Census of Agriculture, 1992, 1997, and 2002). At the same time,

when water is available, irrigated farming can provide increased income stability when compared to dryland farming. However, irrigated agriculture also can be more expensive due to the production cost of the water itself. For example, water allocations for both ground and surface water are already in place in areas of Nebraska and Colorado, where beans are produced (Urrea et al., 2009). Because of these kind of situations, the development of dry bean cultivars that require less water or have a better water use efficiency (WUE) will enhance management options for maintaining profitability for farmers. According to Blum (2005), other alternatives to mitigate drought stress can be considered such as the development of more efficient irrigation management strategies, the increase of the use of no-till planting and residue management, and modifications in cropping systems, among others.

Drought Mechanisms and Dry Bean Response to Drought Stress

May and Milthrope (1962) defined drought as a meteorological and environmental event which is caused by the absence of rainfall for a period of time long enough to cause a reduction of soil moisture and damage to plants. Plant damage is usually caused by a disturbance of physiological and metabolic processes.

Drought resistance, the adaptation by which plants survive in regions subject to drought, has been classified into three components: drought escape, drought avoidance, and drought tolerance (Levitt, 1972; Fischer and Maurer, 1978). Cultivars adapted to drought-stressed environments may have different levels of all three components (Hall and Shulze, 1980). Jones et al. (1981) noted that adaptations contributing to drought escape are a characteristic of plants growing in areas of wet and dry seasons. Drought-escaping plants complete their life cycle, or at least their reproductive cycle, before the supply of water in the soil is limited. Two features of drought-escaping plants that appear to be significant are rapid development (growth) and the

development of plasticity. High metabolic activity and rapid growth are hypothesized to confer a fitness advantage because this enables a plant to complete its life cycle rapidly. If rainfall is limited, drought-escaping plants produce a small amount of vegetative growth, few flowers, and few seeds; an abundance of vegetative growth, flowers, and seeds are produced when rainfall is plentiful (Mulroy and Rundel, 1977). To achieve the latter, some plant species frequently have indeterminate growth habits. Indeterminate plants bear flowers (and fruit) in the leaf axils. Shoots continue to grow and the plants remain relatively unbranched and vining. Indeterminate growth habit is an important survival mechanism because it enables large amounts of seed produced in the wet season to carry the species through prolonged drought periods.

However, drought escape, a mechanism associated with earliness, has been often negative correlated with yield in years or seasons of adequate rainfall (May and Milthrope, 1962). For example, comparative studies indicate that drought-escaping winter annuals such as *Camissonia claviformis* have an unusually high stomatal conductance and photosynthetic capacity relative to other C3 plants (Mooney et al., 1976). In addition, there is a commonly observed life history trade-off between leaf life span and photosynthetic rate (Reich et al., 1999). Thus, if drought escape is adaptive, selection should favor plants with high stomatal conductance, high photosynthetic rate, and low WUE because this will allow for increased growth and accelerated development.

Jones et al. (1981) subdivided adaptations contributing to drought tolerance into dehydration postponement – which is the ability of a plant to survive periods of water deficit while maintaining high tissue water potential. This occurs by means of physiological or morphological modifications that reduce transpiration or maintain absorption of water by the roots. Levitt (1972), for convenience, simply referred to this as drought avoidance. However,

Jones et al. (1981) pointed out that plants with this mechanism do not avoid drought, but avoid tissue dehydration. In this case, plants maintain low metabolic activity and growth rate to reduce the demand for resources throughout the period of drought (McKay et al., 2003). If dehydration avoidance is adaptive under low moisture, natural selection should favor conservative water use through stomatal closure (Geber and Dawson, 1997; Arntz and Delph, 2001). Because stomatal closure typically reduces transpiration more than photosynthesis (Ehleringer 1993; Maherali et al., 2003), it will increase the ratio of photosynthesis to transpirational water loss, also known as WUE (Givnish 1986; Schulze et al., 1987). There is evidence of direct and indirect selection for increased WUE under drought in both annual and perennial species (Heschel et al., 2002, 2004; Ludwig et al., 2004), but relatively few studies have tested whether decreased stomatal conductance is itself adaptive under drought. Both drought escape and dehydration avoidance predict that physiological phenotypes function interactively with plant development to influence fitness under drought (Ackerly et al., 2000; Arntz and Delph, 2001).

Dehydration tolerance (referred to as drought tolerance by Levitt, 1972), is characterized as the ability of a plant to endure water deficits at low water potential. This occurs by means of physiological or metabolic processes that maintain turgor pressure or tolerate desiccations. This mechanism may be influenced by the processes under consideration of stress during the grow cycle.

Drought Stress in Dry Beans

Short, intermittent periods of drought reduce both bean quality and bean yields (Wallace, 1980). The extent and duration of both intermittent and terminal drought stress in dry bean are directly associated with seed yield (Singh, 1995) and their effects are amplified by interactions with high temperature, disease, and soil type, among others (Ramirez-Vallejo and Kelly, 1998).

Several studies have identified the importance of root architecture and its efficiency as critical factors of drought response in dry bean. Root growth has been associated with tolerance to drought stress (yield) under field conditions (Sponchiado et al., 1989), with drought-tolerant genotypes of dry bean reaching 1.3 meters depth on average while susceptible genotypes only grew to 0.8 meters depth. Crop growth, canopy temperatures, and soil moisture extraction were also associated with root growth and drought tolerance (Sponchiado et al., 1989). Effective and efficient root systems allow plants to reach water and nutrients at deeper soil levels and over larger areas (Chaves et al., 2003; Lynch, 2007). In semi-arid regions, plant breeders have been able to increase crop productivity by selecting plants that have greater root depth (Chaves et al., 2003). Scientists have demonstrated that bean plants with an erect architecture often have deep tap roots that are better, able to utilize available water in the soil profile (Ho et al., 2005, Sponchiado et al., 1989). Kelly et al. (1987) reported that Mesoamerican bean lines having a Type II growth habit had more stable seed yields than Type III bean lines in rain-fed production regions in Michigan. It may be possible, therefore, to indirectly select for increased drought tolerance by breeding for improved architectural phenotypes, keeping in mind that the difference is mostly explained by disease pressure being higher in Type III bean plants (Kelly et al. 1987).

According to Ramirez et al. (2007), robust drought tolerance is based on mechanisms that confer tolerance in the presence of drought stress. The efficiency with which genotypes access and use available water, respond to water deficits, and in turn produce harvestable yield, is an empirical definition of water use efficiency. Muñoz-Perea et al. (2007) suggested WUE as a key trait to consider when selecting drought-tolerant genotypes. If there is not a direct association with crop yield in mechanistic methods, plant breeders usually prefer more labor intensive, but direct empirical (yield derived) methods of determining drought tolerance (Specht et al., 1986).

Mechanistic and empirical evaluations have been implemented by scientists and collaborators in Puerto Rico and in Nebraska, where drought tolerance research has focused on the analysis of the physiological response to drought stress at the reproductive stage, determining water use in dry bean in a semi-arid environment, and breeding for drought tolerance through both local and shuttles breeding efforts (Porch et al., 2012; McClean et al., 2011).

There are many phenotypes and indices to estimate the impact of drought adaptation (see McClean et al., 2011, and Beebe et al., 2013 for a complete list). For example, the crop water stress index (CWSI) was estimated by Ramirez et al. (2007) as a function of direct canopy and air temperature, and was found to be well-correlated with yield components and with the water available in the root zone, indicating that this index is an indicator of the plant-soil-water status. In addition, WUE, transpiration efficiency (TE), harvest index (HI), and geometric mean (GM) indices were found to be effective for the evaluation of dry bean genotypes under stress and non-stress conditions (Ramirez Builes et al., 2011). Canopy temperature, stomatal conductance, relative water content, and yield components are some phenotypes that have been evaluated in dry beans under drought stress. Results from studies using these indices indicate that under greenhouse conditions SEN 3 and SER 21 show better WUE. Under field conditions, 'Morales' (Beaver and Miklas, 1999) and SER 16 had larger reductions in TE and WUE under drought stress. Ramirez et al. (2011) recommended SEN 3 and SER 21 as germplasm lines that can be used for further WUE research and for the improvement of WUE as a trait of interest.

Even though there are many phenotypes to evaluate drought most scientists agree to use seed yield as the more empirical trait to be measured. The main reason to pick seed yield as the primary trait to be measured is because drought is a very complex phenotype, which is affected by many genetic factors/mechanisms. When doing research, many factors are important to

consider, among them, the phenotypes to be evaluated, the experimental units, as well as, the phenotypic data collection. Data should be collected using appropriate experimental designs in which carefully selected checks provide reliable estimates of the performance of the lines under consideration. For indirect selection purposes, seed yield is also necessary to be able to make correlations among phenotypes. For example, traditional agronomic phenotypes such as phenology, shoot biomass, days to flowering, days to maturity, and yield components in the past have been associated with tolerance to drought and heat. Abiotic stress indices, such as the geometric mean and percent yield reduction, will also be important in assessing genotype response to stress versus non-stress conditions in order to ensure broad adaptation and yield stability (Ramírez-Vallejo and Kelly, 1998; Porch et al., 2009; Urrea et al., 2009). Incorporating measurements of canopy temperature depression and stomatal phenotypes such as the number, size, and conductance of stomata (Hetherington and Woodward, 2003; Beebe et al., 2010), along with general phenology, would be useful to link the underlying physiological responses to abiotic stress and to the plasticity necessary for adaptation to a changing environment (Nicotra et al., 2010). However, more connections between several detailed morphological, physiological, metabolic, and genetic processes, the environment, and its interactions are only just in the pipeline. Until the fundamental mechanisms of drought resistance characteristics are known more precisely, plant breeding programs will have to utilize screening techniques that are based on plant response rather than on specific plant characteristics to improve drought tolerance.

Genetics of Drought Tolerance

Genetic improvement of drought tolerance depends on effective methods for selection of superior genotypes and on the understanding of the genetics of the trait. In dry bean, seed yield was found to be the most reliable measure of drought tolerance (Ramírez-Vallejo and Kelly,

1998; White et al., 1994a). Early genetic studies found that drought tolerance, measured as seed yield, was an additive trait with significant interaction with the environment (White et al., 1994b). A wide range of narrow-sense and broad-sense heritabilities (0.09 to 0.80) have been reported under drought stress depending on environmental conditions and the market class of the bean lines evaluated (Schneider et al., 1997; Singh, 1995). For example, Schneider et al. (1997) reported narrow-sense heritabilities of 0.55 ± 0.16 and 0.59 ± 0.16 for seed yield under stress and non-stress respectively, while 0.20 ± 0.15 and 0.19 ± 0.15 were reported for the same author using a different population under stress and non-stress, respectively. A value of 0.82 ± 0.16 and 0.79 ± 0.16 was reported for 100-seed weight under stress and non-stress, respectively.

Likewise, studies made in Colombia and Mexico by White et al. (1994b) showed that under rain-fed conditions, narrow-sense heritability estimates from regressions of F_3 plants ranged from 0.09 ± 0.18 to 0.75 ± 0.25 for seed yield, from 0.26 ± 0.09 to 0.34 ± 0.09 for days to maturity and from 0.57 ± 0.04 to 0.80 ± 0.04 for 100-seed weight.

Consequently, due to the inconsistency among estimates, conventional selection would be more successful for improving yield performance, for example, for both stress and non-stress. Genetic improvement of dry bean for drought tolerance has been slow because of unreliable techniques to measure plant response to drought, phenological plasticity, and the inability to create repeatable screening environments (Ramírez-Vallejo and Kelly, 1998). Through the identification of single phenotypes that compose a plant's response to drought stress, the genetics of these phenotypes and the molecular markers associated with these phenotypes can be developed more thoroughly. Due to the complexity of screening for drought-stress tolerance, an alternative breeding approach is indirect selection through the use of molecular markers (Schneider et al., 1997). In the first MAS study of drought tolerance in bean, MAS was

confirmed to be more effective than phenotypic selection when heritability was low (Schneider et al., 1997). Thus, the physiological and genetic response to drought stress needs to be dissected into its components to allow for the development of MAS approaches through the identification of markers associated with these drought-related traits.

Relationship between Seed Yield and Yield Components

The common bean is very sensitive to soil water conditions, so both seed yield and quality can suffer greatly from even brief periods of water shortage (Halterlein, 1983). Yield compensation during a recovery from the water deficit may occur such that seed yield will not be reduced.

Sammons et al. (1979) suggested that for a seed-yielding species, drought should be measured under field conditions for a full growing season, so that reproductive responses to water stress can be observed. As a result, the most promising route for plant improvement under drought stress probably involves selection under water-limiting conditions (Painter, 1966; Urrea et al., 2009; Urrea and Porch, 2010; Ramírez-Vallejo and Kelly, 1998; Terán and Singh. 2002).

The degree of seed yield reduction by water deficit or enhancement through irrigation will depend on the degree, duration, and timing of the deficit, and on the proportion of the biological yield that comprises the economic yield of the crop (Begg and Turner, 1976). Hence, crops whose yield comprise the bulk of the aboveground portion of the crops, such as leafy vegetables and pasture, are often more sensitive to drought stress than crops whose yield is the reproductive portion of the plant only. Korte et al. (1983) working in soybean reported (*Glycine max* L.) that the magnitude of seed yield increase when the crop is irrigated depends upon the phenotypic timing of the temporal sequence with which the components of seed yield are established and fixed. Although the effects of drought stress during the pre-flowering growth

stage of dry beans, there is a general agreement that when water deficits occur during flowering and pod development, seed yields are reduced (Dubetz and Mahalle, 1969; Maurer et al., 1969; Robins and Domingo, 1956; Porch et al., 2009, 2012). Dry beans seem to suffer from drought stress just before flowering through pod filling, where drought apparently causes the most severe damage. However, plants can at least partly overcome the effects of drought stress during the vegetative stages of growth (Halterlein, 1983).

Begg and Turner (1976) noted that one of the most important consequences of the sensitivity of cell enlargement to small water deficits is a marked reduction in leaf area. Leaf growth (cell enlargement) is generally more sensitive to drought stress than to stomatal resistance and carbon dioxide assimilation. However, the rate of respiration will gradually decrease when compared to the photosynthesis rate (Kramer, 1969). In general, a reduction in leaf area will reduce the crops' growth rate, particularly during the early stages of growth when there is an incomplete light reception. Also, a reduction in leaf area is a permanent damaging effect and the cause of a determinate crop, there is no scope for compensation via an increase in the number of leaves (Begg and Turner, 1976). Data presented by Brun et al. (1972) showed that the fraction of evapotranspiration accounting for transpiration was closely correlated to the amount of leaf area covering a given soil area, with transpiration at approximately 50% of total evapotranspiration at leaf area index (LAI) of 2, and as much as 95% at LAI of 4.

Genetic Sources for Improving Drought Tolerance

Exotic germplasm evaluation has played an important role in the incorporation of new phenotypes into the U.S. germplasm. Several breeding programs across and outside the country have discovered and/or developed different germplasm sources for drought tolerance mostly in the last two decades. Studies conducted at CIAT, reported BAT 477 as a Mesoamerican drought-

resistant line with a deep rooting ability and greater absorption efficiency (Guimares et al., 1996). In Honduras, Tio Canela 75 (Rosas et al., 1997), a small red bean used in most drought studies at CIAT as a resistance check (Beebe et al., 2008), was released from the Zamorano Pan-American Agricultural School.

A few years later, Rao (2001) suggested that the accession G21212, a Mesoamerican landrace, can be used as a drought-tolerant source because it had a greater mobilization of photosynthates to the seed under drought stress. In the same year, Singh et al. (2001) released SEA 3 and SEA 15 as drought-tolerant dry bean germplasm. More recent field evaluations of advanced lines at CIAT resulted in the identification of three lines (SER 16, SEA 5, and SER 5) that were superior in their adaptation to drought stress conditions (Rao et al., 2006). Beebe et al. (2008) concentrated efforts on obtaining drought-resistant genotypes in commercial classes of dry beans, in addition to focusing on disease resistance, and yield response of drought tolerance lines to edaphic environments, especially those with low soil P availability.

In the U.S., evaluations for drought tolerance have identified genotypes belonging to the pink and red market class as better yield performers when compared to pintos and great northern (Singh, 2007). Muñoz-Perea et al. (2007), reported higher mean seed yield in the cultivars from the red market class than cultivars from the pinto market class. Initial reports about drought tolerance mentioned that the Durango race, especially pinks, pintos, and reds, are the most tolerant (Nienhuis and Singh, 1988), as well as, the combination of the Mesoamerican and the Durango races for drought tolerance purposes. Among pinto cultivars, ‘Othello’ (Burke et al., 1995) appeared to be the most resistant to drought stress. While in Nebraska, Urrea et al. (2009) reported yield reductions up to 69% for SEN 20, 55% for Matterhorn (Kelly et al., 1999), whereas Bill-Z (Wood et al., 1989), a pinto bean, had 50% in yield reduction. All of these results

clearly show the need for drought tolerant cultivars adapted to the Great Plains. Efforts have been made through cooperative shuttle breeding program, similar to the one created by Norman Borlaug, starting in 2005 for dry beans, and is still ongoing under the coordination of T.G. Porch (USDA, TARS-Puerto Rico) and C.A. Urrea (UNL- PHREC). The program focuses on drought stress occurring during reproductive development, which can cause excessive abortion of buds, flowers and pods, resulting in a dramatic yield reduction because the physiological effects of abiotic stress tend to be more pronounced during reproductive versus vegetative development. Breeding lines are selected at both locations in early generations and the selected lines are then advanced and tested in yield trials through sequential testing at both locations. The goal is to identify lines adapted to multiple drought niches across climatic zones, and after seven years it is beginning to produce some positive results. Recently, two black bean germplasm with multiple-stress-tolerance was released from that program and named TARS-MST1 and SB-DT1 (Porch et al., 2012).

As seen through this review, bean breeders and geneticists have been able to make some progress in developing germplasm and cultivars that are tolerant to abiotic stressors like drought. More research needs to be completed to ensure competitive production levels in regions with regular dry cycles in their agricultural production, and to mitigate hunger in those countries where the dry beans are the main staple food.

References

Ackerly, D.D., S.A. Dudley, S.E. Sultan, J. Schmitt, J.S. Coleman, C.R. Linder, D.R. Sandquist, M.A. Geber, A.S. Evans, T.E. Dawson, and M. J. Lechowicz. 2000. The evolution of plant ecophysiological phenotypes: recent advances and future directions. Bioscience 50:979–995.

Andersen, J.W., L. Story, B. Sieling, W.J.L. Chen, and M.S. Petro. 1984. Hypocholesterolemic effects of oat-bran or bean intake for hypercholesterolemic men. Am. J. Clin. Nutr. 40:1146-1155.

Andersen, J.W., and N.J. Gustafson. 1989. Hypocholesterolemic effect of oat and bean products, Michigan Dry Bean Digest. 13:2-5.

Arntz, A.M., and L.F. Delph. 2001. Pattern and process: evidence for the evolution of photosynthetic phenotypes in natural populations. Oecologia 127:455–467.

Baker, P. 2012. Drought Puts Food at Risk, U.S. Warns. The New York Times. http://www.nytimes.com/2012/07/19/us/drought-puts-food-at-risk-us-warns.html?_r=0 (accessed 18 July 2012).

Beaver, J.S., and P.N. Miklas. 1999. Registration of 'Morales' small white bean. Crop Sci. 39:1257.

Beebe, S.E., I.M. Rao, C. Cajiao, and M. Grajales. 2008. Selection for drought resistance in common bean also improves yield in phosphorus limited and favourable environments. Crop Sci. 48, 582–592.

Beebe, S.E., I.M. Rao, M.W. Blair, J.A. Acosta-Gallegos. 2010. Phenotyping common beans for adaptation to drought. In: J.M. Ribaut and P. Monneveux, editors, 'Drought phenotyping in crops: from theory to practice. Generation Challenge Program Special Issue on Phenotyping'. p. 311–334.

Beebe, S.E., I.M. Rao, M.W. Blair, and J.A. Acosta-Gallegos. 2013. Phenotyping common beans for adaptation to drought. Front. Physiol. 4:35.

Begg, J.E., and N.C. Turner. 1976. Crop water deficits. Adv. Agron. 28:161-217. Academic Press, Inc. New York, USA.

Blum, A. 2005. Drought resistance, water-use efficiency, and yield potential-are they compatible, dissonant, or mutually exclusive? Aust. J. of Agric. Res. 56: 1159-1168.

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

Brun, L.J., E.T. Kanemasu, and L.W. Powers. 1972. Evapotranspiration from soybean and sorghum fields. Agron. J. 64:145-148.

Burke, D.M. Silbernagel, J. Kraft, and H. Koehler. 1995. Registration of 'Othello' pinto bean. Crop Sci. 35:943

Census of Agriculture. 1992. Farm and ranch irrigation survey. USDA, Wash. D.C.

Census of Agriculture. 1997. Farm and ranch irrigation survey. USDA, Wash. D.C.

Census of Agriculture. 2002. Farm and ranch irrigation survey. USDA, Wash. D.C.

Chaves, M.M., J.P. Maroco, and J.S. Pereira. 2003. Understanding plant responses to drought – from genes to whole plant. Funct. Plant Biol. 30:239-264.

Dubetz, S., and P.S. Mahalle. 1969. Effect of drought stress on bush beans (*Phaseolus vulgaris* L.). J. Amer. Soc. Hort. Sci. 94:479-481.

Dudley, S.A. 1996. Differing selection on plant physiological phenotypes in response to environmental water availability: a test of adaptive hypotheses. Evolution 50:92–102.

Ehleringer, J. R. 1993. Carbon and water relations in desert plants: an isotopic perspective. In J. R. Ehleringer, A. E.Hall, and G. D. Farquhar, editors, Stable isotopes and plant carbon water relations. Academic Press, New York. p. 155-172.

FAOSTAT. 2013. FAO statistical databases and data sets. Food and agriculture organization of the United Nations. http://faostat.fao.org/. (accessed 8 July 2013).

Fischer, R.A., and R. Maurer. 1978. Drought resistance in spring wheat cultivars: I. Grain yield responses. Aust. J. Agric. Res. 29:897–912.

Geber, M.A., and T.E. Dawson. 1997. Genetic variation in stomatal and biochemical limitations to photosynthesis in the annual plant, Polygonum arenastrum. Oecologia 109:535–546.

Givnish, T.J. 1986. Economics of gas exchange. In: T.J. Givnish, ed. On the economy of plant form and function. Cambridge Univ. Press, Cambridge, U.K. p. 11–24.

Guimaraes, C.M., O. Brunini, and L.F. Stone. 1996. Adaptaçao do feijoeiro (*Phaseolus vulgaris* L.) a seca. I. Densidade e efficiencia radicular. Pesq. Agropec. Bras. Brasilia 31:393–399. [In Portuguese].

Hall, A.E., and E.D. Schulze. 1980. Drought effects on transpiration and leaf water status of cowpea in controlled environments. Aust. J. Plant Physiol. 7:141-148.

Halterlein, A.J. 1983. Bean (water requirements, roots systems, irrigation, drought behavior, kidney beans). In: I.D. Teare and M.M. Peet, editors, Crop-water relations. Wiley, Inc. New York. p. 157-185.

Heschel, M.S., and C. Riginos. 2005. Mechanisms of selection for drought stress tolerance and avoidance in *Impatiens capensis* (Balsaminaceae). Am. J. Bot. 92:37–44.

Heschel, M.S., K. Donohue, N. Hausmann, and J. Schmitt. 2002. Population differentiation and natural selection for water-use efficiency in Impatiens capensis (Balsaminaceae). Int. J. Plant Sci. 163:907–912.

Heschel, M. S., S.E. Sultan, S. Glover, and D. Sloan. 2004. Population differentiation and plastic responses to drought stress in the generalist annual *Polygonum persicaria*. Int. J. Plant Sci. 165:817–824.

Hetherington, A.M., and F.I. Woodward. 2003. The role of the stomata in sensing and driving environmental change. Nature 424:901-908.

Ho, M.D., J.C. Rosas, K.M. Brown, and J.P. Lynch. 2005. Root architectural tradeoffs for water and phosphorus acquisition. Funct. Plant Biol. 32:737–748.

Ishag, H.M.1982. The influence of irrigation frequency on growth and yield of groundnuts (*Arachis hypogea* L.) under arid conditions. J. Agric. Sci. 99:305-310.

Jones, M.M., N.C. Turner, and C.B. Osmond. 1981. Mechanism of drought resistance. In: L.G. Paleg, and D. Aspinall, editors, The physiology and biochemistry of drought resistance in plants. Academic Press, Sydney, Australia. p 15-37.

Kelly, JD, M.W. Adams, A.W. Saettler, G.L. Hosfield, and A. Ghaderi. 1987. Yield stability of determinate and indeterminate dry bean cultivars. Theor. Appl. Genet. 74:516-521.

Kelly, J.D., G.L. Hosfield, G.V. Varner, M.A. Uebersax, and J. Taylor. 1999. Registration of 'Matterhorn' great northern bean. Crop Sci. 39:589-599.

Korte, L.L., J. E. Specht, J.H. Williams, and R.C. Sorensen. 1983. Irrigation of soybean (*Glycine max* L.) genotypes during reproductive ontogeny: II. Yield component responses. Crop Sci. 23:528-533.

Kramer, P.J. 1969. Plant and soil water relationships, a modern synthesis. McGraw Hill Pub. Co. p. 482.

Levitt, J. 1972. Responses of plants to environmental stress. Academic Press, New York.

Ludwig, F., D.M. Rosenthal, J.A. Johnston, N. Kane, B.L. Gross, C. Lexer, S.A. Dudley, L.H. Rieseberg, and L.A. Donovan. 2004. Selection on leaf ecophysiological phenotypes in a desert hybrid *Helianthus* species and early generation hybrids. Evolution 58: 2682–2692.

Lynch, J.P. 2007. Roots of the second green revolution. Aust. J. Bot. 55:493-512.

MacPherson, J. 2006. More than 60 percent of U.S. now has abnormally dry or drought conditions. Assoc. Press Release. http://www.mindfully.org/Air/2006/US-Drought-Conditions29jul06.htm (accessed 19 June 2012).

Maherali, H., H.B. Johnson, and R.B. Jackson. 2003. Stomatal sensitivity to vapor pressure difference over a subambient to elevated CO_2 gradient in a C_3/C_4 grassland. Plant Cell Environ. 26:1297–1306.

Maurer, A.R., D.P. Ormrod, and N.J. Scott. 1969. Effect of soil water regimes on growth and composition of snap beans. Can. J. Sci. 49:271-278.

May, L.H., and F.L. Milthrope. 1962. Drought resistance of crops plants. Field Abstr. 15:93-98.

McClean, P.E., J. Burridge, S. Beebe, I.M. Rao and T.G. Porch. 2011. Crop improvement in the era of climate change: an integrated, multi-disciplinary approach for common bean (*Phaseolus vulgaris*). Funct. Plant Biol. 38:927–933.

McClean, P., J. Kami, and P. Guelps. 2004. Genomics and Genetics in Common Bean. In: R.F. Wilson, H.T. Stalker, and E.C. Brummer, editors, Legume Crops Genomics. AOCS Press, Champaign, IL. p. 60-82.

McKay, J. K., J. H. Richards, and T. Mitchell-Olds. 2003. Genetics of drought adaptation in Arabidopsis thaliana. I. Pleiotropy contributes to genetic correlations among ecological phenotypes. Mol. Ecol. 12:1137–1151.

Mooney, H.A., J. Ehleringer, and J.A. Berry. 1976. High photosynthetic capacity of a winter annual in Death Valley. Science 194:322–324.

Mulroy, T.W., and P.W. Rundel. 1977. Annual plants: Adaptation to soil moisture stress. Agron. J. 58: 109-114.

Muñoz-Perea, C.G., R.G. Allen, D.T. Westermann, J.L. Wright, and S.P. Singh. 2007. Water use efficiency among dry bean landraces and cultivars on drought-stresses and non-stressed environments. Euphytica 155:393-402.

Nicotra, A.B. O.K. Atkin, S.P. Bonser, A.M. Davidson, E.J. Finnegan, U. Mathesius, P. Poot, M.D. Purugganan, C.L. Richards, F. Valladares, M. van Kleunen. 2010. Plant phenotypic plasticity in a changing climate. Trends in Plant Science 15:684-692.

Nienhuis, J., and S.P. Singh. 1988. Genetics of seed yield and its components in common bean *(Phaseolus vulgaris* L.) of Middle-American origin. I. General combining ability. Plant Breed. 101:143-154.

NOAA. 2012. State of the Climate National Oceanic and Atmospheric Administration National Climatic Data Center. July 2012: hottest month on record for contiguous United States. http://www.ncdc.noaa.gov/sotc/index.php. (accessed 9 Aug 2012).

Painter, L.I. 1966. Method of sub ejecting growing plants to a continuous soil moisture stress. Agron. J. 58:459.

Porch, T.G., C.A. Urrea, J.S. Beaver , S. Valentin, P.A. Peña, and J.R. Smith. 2012. Registration of TARS-MST1 and SB-DT1 Multiple-Stress-tolerant Black Bean Germplasm. J. Plant Reg. 6:75-80.

Porch, T.G., R. Bernsten, J.C. Rosas, and M. Jahn. 2007. Climate change and the potential economic benefits of heat tolerant bean cultivars for farmers in Atlántida, Honduras J. of Agric. Univ. of Puerto Rico 91:133-148.

Porch, T.G., V.H. Ramirez, and E.W. Harmsen. 2009. Evaluation of common bean for drought tolerance in Juana Diaz, Puerto Rico. Agron. J.195:328-334.

Ramirez, V.H., E.W. Harmsen, and T.G. Porch. 2007. Crop drought stress index and yield components for new common bean (*Phaseolus vulgaris* L.) genotypes in greenhouse and field environments. Poster session presented at: Sociedad Puertorriqueña de Ciencias Agrícolas Reunión Científica Anual 2007. Cataño, PR. p.35

Ramirez Builes, V.H., T.G. Porch and E.W. Harmsen. 2011. Genotypic differences in water use efficiency of common bean under drought stress. Agron. J. 103:1206-1215.

Ramírez-Vallejo, P., and J.D. Kelly. 1998. Phenotypes related to drought resistance in common bean. Euphytica 99:127-136.

Rao, I.M. 2001. Role of physiology in improving crop adaptation to abiotic stresses in the tropics: The case of common bean and tropical forages. In: M. Pessarakli, ed. Handbook of plant and crop physiology. Marcel Dekker, New York. p. 583–613.

Rao, I.M., S. Beebe, J. Polania, M.A. Grajales, R. Garcia. 2006. Differences in drought resistance of advanced lines developed for the last 3 decades, in Annual Report 2006. Project IP-1: Bean Improvement for the Tropics (Cali, Colombia: CIAT). p. 2–6.

Reich, P.B., D.S. Ellsworth, M.B. Walters, J.M. Vose, C. Gresham, J.C. Volin, and W.D. Bowman. 1999. Generality of leaf trait relationships: a test across six biomes. Ecology 80: 1955–1969.

Robins, J.W., and C.E. Domingo. 1956. Moisture deficits in relation to the growth and development of dry beans. Agron. J. 48:67-70.

Rosas, J.C., O.I. Varela and J.S. Beaver. 1997. Registration of 'Tio Canela 75' Small Red Bean. Crop Sci. 37:1391.

Sammons, D.J., D.P. Peters, and T. Hymowtz. 1979. Screening soybeans for drought resistance. II: Drought box procedure. Crop Sci. 19: 719-722.

Schneider, K.A., M.E. Brother, and J.D. Kelly. 1997. Marker-assisted selection to improve drought resistance in common bean. Crop Sci. 37:51-60.

Schulze, E.D., R.H. Robichaux, J. Grace, P.W. Rundel, and J.R. Ehleringer. 1987. Plant water balance. Bioscience 37:30–37.

Singh, S.P. 1995. Selection for water-stress tolerance in interracial populations of common bean. Crop Sci. 35:118-124.

Singh, S.P., H. Terán, and J.A. Gutierrez. 2001. Registration of SEA 5 and SEA 13 drought tolerant dry bean germplasm. Crop Sci. 41:276–277.

Singh, S.P. 2007. Drought resistant in race Durango dry bean landraces and cultivars. Agron. J. 99:1219-1225.

Specht, J.E., J.H. Williams, and C.J. Weidenbenner. 1986. Differential responses of soybean genotypes subjected to a seasonal soil water gradient. Crop Sci. 26: 922-934.

Sponchiado, B.N., J.W. White, J.A. Castillo, and P.G. Jones. 1989. Root growth of four common bean cultivars in relation to drought tolerance in environments with contrasting soil types. Exp. Agric. 25:249-257.

Terán, H., and S.P. Singh. 2002. Comparison of sources and lines selected for drought resistance in common bean. Crop Sci. 42:64-70.

U.S. Dry Bean Council. 2012. Health and Nutrition. United States Dry Bean Council, Pierre, SD. http://www.usdrybeans.com (accessed 3 Aug 2012).

USDA-ERS. 2013. Dry edible beans. In Vegetable and melons outlook/VGS-330/December 16, 2011. www.ers.usda.gov/Briefing/DryBeans/PDFs/DBnOutlook.pdf . USDA- ERS, Washington, DC. (accessed 16 July 2013).

USDA-NASS. 2012. North Dakota Agricultural statistics 2011. North Dakota Agricultural Statistics Service, North Dakota field office, U.S. Department of Agriculture, National Agricultural Statistics Service. http://www.nass.usda.gov/Statistics by State/ North Dakota/Publications/Annual Statistical Bulletin/index.asp. (accessed 17 July 2012).

USDA-NASS. 2013. North Dakota Agricultural statistics 2012. North Dakota Agricultural Statistics Service, North Dakota field office, U.S. Department of Agriculture, National Agricultural Statistics Service. http://www.nass.usda.gov/Statistics by State/ North Dakota/Publications/Annual Statistical Bulletin/index.asp. (accessed 29 September 2013).

Urrea, C.A., C. D. Yonts, D. J. Lyon, and A. E. Koehler. 2009. Selection for drought tolerance in dry bean derived from the Mesoamerican gene pool in Western Nebraska. Crop Sci.49:2005-2010.

Urrea, C.A., and T.G. Porch, 2010. Phenotypic evaluation of a subset of the *Phaseolus vulgaris* Core collection, the *P. acutifolius* germplasm collection and cultivars for drought tolerance in Nebraska and Puerto Rico. Annu. Rept. Bean Improv. Coop. 53:164-165.

Wallace, D.H. 1980. The genetics of photosynthesis and crop productivity with emphasis on beans. In: Proc 14th Int Congr. Genet., vol 1, book 2. MIR Publ, Moscow. p. 306–317.

White, J.W., J.A. Castillo, J.R. Ehleringer, C.A. Garcia, S.P. Singh. 1994a. Relations of carbon isotope discrimination and other physiological phenotypes to yield in common bean (*Phaseolus vulgaris*) under rainfed conditions. J. Agric. Sci. 122:275-284.

White, J.W., M. Ochoa, P. Ibarra, S.P. Singh. 1994b. Inheritance of seed yield, maturity and seed weight of common bean (*Phaseolus vulgaris*) under semi-arid rainfed conditions. J. Agric. Sci. 122:265-273.

Wood, D.R., M. Ballarin, M.A. Brick, H.F. Schwartz, and C.H. Pearson. 1989. Registration of Bill-Z pinto bean. Crop Sci. 29:488.

Wortmann, C.S., R.A, Kirkby, C.A Eledu, D.J. Allen. 1998. Atlas of common bean (*Phaseolus vulgaris* L.) production in Africa. CIAT publication no. 297. Centro Internacional de Agricultura Tropical (CIAT). Cali, Colombia. 131 p.

PAPER I. GENETIC PROGRESS IN DRY BEAN SEED YIELDS UNDER DRYLAND AND IRRIGATED CONDITIONS IN THE NORTHERN GREAT PLAINS OVER THE PAST 26 YEARS

Angela M. Linares-Ramírez[1], Blaine Schatz[2], Kenneth F. Grafton[1], and Juan M. Osorno[1]

[1]Dept. of Plant Sciences, North Dakota State University, Fargo, ND 58108-6050

[2] Carrington Res. and Ext. Center, North Dakota Ag. Expt. Station, Carrington, ND 58421.

Abstract

One of the main limitations of dry bean (*Phaseolus vulgaris* L.) production and other crops worldwide is water availability. In North Dakota, the availability of water is becoming more significant given the fact that dry bean production is being pushed from the fertile, non-irrigated Red River Valley located in the eastern part of the state, towards the central and western regions of the state, where drought stress occurs more frequently. This research was conducted 1) to assess the differences in seed yield due to water availability within each one of the three most important dry bean market classes grown in the region (pinto, navy, and black), and (2) to estimate the genetic gain and progress over time due to the use of improved dry bean cultivars from 1981 to 2011. Results showed that, on average, water limitation can reduce seed yield by 31.5% under dryland conditions. Seed yield in black cultivars can be reduced as much as 36% in dryland when compared to irrigated conditions, followed by navy and pinto with 35 and 32% of seed yield reduction, respectively. Yield gains up to 18, 14, and 11 kg $ha^{-1}yr^{-1}$ were obtained for pinto, black, and navy beans under dryland conditions, respectively, while yield reductions were observed under irrigated conditions. A significant yield gap was observed for the three market classes between the dryland and irrigated growing conditions. Results suggest that dry bean cultivars have not yet reached a yield plateau for pinto, black, and navy market classes. However, there is a need to keep improving management practices that could allow for a better

expression of dry bean yield potential. Genetic improvement towards disease resistance, especially in regards to white mold and foliar diseases, should be considered a priority.

Introduction

Breeding for seed yield improvement is the primary goal of all dry bean breeding programs worldwide. According to Beaver and Osorno (2009), in recent years, scientists working in the U.S. and in developing countries have made improvements in dry bean seed yield using conventional plant breeding techniques. However, Vandemark et al. (2013) report that with the use of agronomic and biotechnology tools, major improvements have been achieved since molecular plant breeding has helped to generate, characterize, utilize, and exploit the genetic diversity available in dry beans (*Phaseolus vulgaris* L.).

U.S. dry bean breeding programs are challenged by environmental conditions such as temperature, rainfall, humidity, soil physical and chemical structure, and the incidence of diseases, among others (Brick and Grafton, 1999). Because of land extension and the diversity of climatic zones, dry bean breeders have concentrated breeding efforts for specific agricultural productions areas rather than for all of the U.S.

The fertile soils of the Red River Valley, the most important agricultural area in North Dakota and drained by the Red River of the North, are formed from lacustrine deposits of silt from Lake Agassiz (Sansome, 1983). Geographical events in the Ice Age era defined the formation of the soils that now are mainly used for agricultural production. The heavy clay, poorly-drained soils in the Red River Valley tend to conserve soil moisture, which is a portion of the state.

Parts of North Dakota were once under a glacier, which alternately advanced and deteriorated with variations in climate (Perkins, 2002). As the ice sheet disintegrated, it created

at its front an immense proglacial lake formed from its melt waters. This lake was named Lake which, where drained, became the Red River Valley. Due to the unique climatic characteristics of the region, breeding efforts in North Dakota for dry beans started in the early 1980s at NDSU when growers tried to identify alternative crops to grow in the region. Since then, the genetic material developed at NDSU has allowed for the release of several high-yielding pinto, black, and navy cultivars, as well as a red cultivar, all of which have excellent adaptation to the harsh conditions of North Dakota with includes disease pressure, frost, and flooding (Osorno et al., 2008; Osorno et al., 2010).

Since 1991, North Dakota ranks first in U.S. dry bean production, accounting for 40% of dry bean production nationwide. Together, Michigan, Minnesota, and Nebraska account for the other half of U.S. production (USDA-NASS, 2012). The average production in North Dakota was 483,384 MT, the average planted area was 273,000 ha, and the harvested area was 260,000 ha (USDA-NASS, 2012). Statewide average yield from 2005 to 2011 was 1,655 kg ha^{-1}. Pintos accounted for 55 % of the total production, being the major market class grown in the region, followed by navy beans accounting for 23%, blacks 16%, pinks 3.2%, great northern 0.2%, and the remaining percentage in other minor classes (USDA-ERS, 2011).

Dry bean production is an important market for North Dakota's economy. Just last year (2012), dry bean varieties released from NDSU within each one of the three most important market classes grown in the region (pinto, navy, and black) contributed approximately $110 million to the state's economy (Northarvest Bean Grower Magazine, 2013). Conventional plant breeding techniques have been successful in extending the range of adaptation of dry beans, but an estimation of how successful they have been in terms of genetic improvement is unknown.

For that, estimation based on yield gains can help express the genetic improvement of cultivars adapted to the region.

Several authors have discussed the difference between on-farm yields and potential yield (or on-station yield) (Bingham, 1967; MacKey, 1979; Evans and Fisher, 1999). On-farm yield takes into consideration all factors affecting crop production, including cultivar's genetic potential, the environment, management practices, and their interactions, while the potential yield (on-station yield) primarily focuses on the maximum expression of the genetic potential under optimum or near optimum environmental and management conditions. On-farm yield, or yields taken from production statistics in farmer's fields, are recorded through surveys and published by the USDA-National Agricultural Statistics Service (USDA-NASS, 2013). In general, potential yield (on-station yield) studies are performed by neutral sources of information like research and extension service units.

Then, if the on-farm yield is considered as the data obtained from farmer's fields, and the potential yield (on-station data) data is obtained under optimal production conditions, the difference between them is known as the yield gap (Fisher and Maurer, 1978; Godfray et al., 2010; Vandermark et al., 2013). In most cases, the yield gap occurs due to biotic and abiotic stresses such as water regime, soil conditions, and disease pressure, among others. Over the past 30 years, potential yield trials (on-station yield) have been conducted at the Carrington Research and Extension Center, ND, under dryland and irrigated conditions. The main objective of this trial is to provide agronomic information about new cultivars available and cultivars widely grown in the region.

Another objective of this study is to assess the differences in seed yield due to growing conditions (dryland vs. irrigated) within each one of the three most important market classes

grown in the region (pinto, navy, and black) and to estimate the genetic gain and progress over time of each market class due to the use of improved dry bean cultivars from 1981 to 2011.

Materials and Methods

Environments and locations

Dry bean variety trials have been conducted under dryland (rain-fed) and irrigated conditions since 1981 at the Carrington Research and Extension Center, ND (47°27.0' N, -99°7.6' W, 484 m elevation). Carrington (Foster County) represents the Central North Dakota region, which is part of the new expansion region for dry bean production and an important region for certified seed production of dry beans. Soil at the Carrington site is a complex Letcher fine sandy loam and Hecla fine sandy loam (USDA-NRCS, 2012). The average precipitation during the growing season (May through September) for this area is 327 mm and the average maximum temperature during the growing season is 18°C and the maximum 27°C (NDAWN, 2013)

Genetic material

An average of 30 dry bean cultivars released over the past 30 years (1981 to 2011) from both public and private breeding programs across the U.S. are included every year in the variety trial. On average, 9 pinto, 7 navy, and 3 black bean cultivars were included each year within the variety trial data used for this study. However, the genotypes changed from year to year and it is for this reason that only the means from each market class are used in this study.

Experimental design

Evaluation of water availability using non-stressed (irrigated) and drought-stressed (dryland) blocks was performed in the different fields in close proximity (<500 m) within the Carrington Research and Extension Center, according to their scheduled crop rotation. Within each block, the selected genotypes were assigned to experimental units using a randomized

complete block design (RCBD) with four replications every year. Each plot consisted of 4.6 m two-row plots spaced 0.6 m apart. At maturity, plants were pulled by a custom rod Pickett® cutter/weeder, windrowed, and, about five days later, threshed with an experimental plot thresher (Almaco® SPC 20). Seed yield (kg ha^{-1}) data were collected on a plot basis at harvest maturity. A sprinkler irrigation system was used for the irrigated trial. The dryland block can be considered as a rain-fed plot since rainfall was the only source of water throughout the entire growing season.

Phenology (e.g., days to flowering, days to maturity), yield components (e.g., seed weight), and other data of interest such as disease incidence and hail damage were collected throughout the growing season for most of the year. Due to the lack of consistency amongst years in the collection of data, however, the only variable selected for this study was seed yield (kg ha^{-1}).

Climatic variables

Environmental data such as temperature (°C), total annual rainfall (mm), and rainfall during the growing season (May to September) were obtained from data recorded by an automated weather station near the study site and reported by the North Dakota Agricultural Weather Network (NDAWN). However, NDAWN provides data starting only in 1990, so for the purposes of this study only data from 1990 to 2011 were used (www.ndawn.ndsu.nodak.edu) (Table 1.1).

Table 1.1. Total amount of yearly rainfall, and total amount of rainfall from May to September (growing season) at the Carrington Research and Extension Center, ND, from 1990- 2011 (22 years).

Year	Total Rainfall	Rainfall May to September
	mm	mm
1990	373.4	350.8
1991	508.7	456.9
1992	216.4	189.3
1993	597.9	572.5
1994	395.1	295.1
1995	400.1	326.6
1996	339.1	302.5
1997	250.4	184.1
1998	287.0	176.3
1999	439.0	419.1
2000	457.2	314.7
2001	406.4	355.6
2002	314.9	266.2
2003	401.3	344.2
2004	395.7	350.3
2005	324.6	279.4
2006	297.7	223.0
2007	441.9	407.5
2008	449.6	341.4
2009	351.8	219.2
2010	406.4	346.5
2011	533.4	486.4
Average	390.4	327.6
SD[†]	91.3	100.6

[†]SD: Standard deviation.

Parameters estimated

To quantify drought severity or water availability in experiments side by side, drought intensity index (DII) was calculated as suggested by Fischer and Maurer (1978) for the Carrington variety trial across year. DII is an estimation based on the mean seed yield of genotypes across market classes under two growing conditions. The equation for drought intensity index is

$$\text{DII} = 1 - \frac{X_d}{X_i}$$

where X_d is the mean seed yield across genotypes of the three market classes under dryland conditions or drought stress (DS), and X_i is the mean seed yield across genotypes of the three market classes under non-stress (NS) or irrigated conditions. The higher the value of DII, the greater the stress. DII values range from 0 to 1, where values above 0.7 are indicative of severe stress (Ramírez-Vallejo and Kelly, 1998), values between 0.3 and 0.6 are considered moderate stress (Urrea et al., 2009), and values below 0.3 indicate minor stress.

Because of the difficulty of selecting for both improved performance under DS and high seed yield potential under NS conditions, it is advisable to utilize multiple indices when making selections (Schneider et al., 1997; Abebe et al., 1998; Urrea et al., 2009). Therefore, the drought susceptibility index (DSI), geometric mean (GM), and percentage of seed yield reduction (PYR) can be used as additional indices to evaluate the response to drought and yield stability by genotypes. The drought susceptibility index is an estimation that helps to determine and select drought-tolerant germplasm for further evaluation and inclusion in breeding programs (Ramírez-Vallejo and Kelly, 1998; Porch et al., 2009; McClean et al., 2011). The drought susceptibility index is based on the change in seed yield under NS and DS environments for a genotype:

$$\text{DSI} = \left(1 - \frac{Y_d}{Y_i}\right) \div DII$$

where Y_d is mean seed yield of the same genotype under DS, and Y_i is mean seed yield for the same genotype under NS (Fischer and Maurer, 1978). A small difference (low DSI value) suggests greater drought tolerance of the genotype.

However, DSI does not differentiate between genotypes that perform well under both environments (e.g., tolerant to drought stress and high yielding under non-stress) and those that perform poorly under both environments (e.g., poor adaptation) (Schneider et al., 1997; White

and Singh, 1991; Abebe et al., 1998; Urrea et al., 2009). Furthermore, Rosielle and Hamblin (1981) suggest that selections based on DSI alone will lead to reduced productivity. Another index, percent yield reduction, is the estimation of the actual seed yield reduction due to limited water supply. The percentage of seed yield reduction in this study was calculated as:

$$\text{PYR} = \left[\frac{\text{mean seed yield of a genotype under NS} - \text{mean seed yield of the same genotype under DS}}{\text{mean seed yield of the genotype under NS}}\right] \times 100.$$

Higher values for percent of yield reduction suggest that the genotypes within a market class do not have good stability across different treatments, while low values indicate better stability.

The geometric mean is an index based on the performance under both DS and NS conditions:

$$\text{GM} = \sqrt{Y_s \times Y_i}$$

where Y_s is the mean seed yield of a genotype under DS conditions and Y_i is the mean seed yield of a genotype under NS conditions (Schneider et al., 1997; Urrea et al., 2009; Porch et al., 2009). All tests were considered significant at $p < 0.05$.

Seed yield gains in dry bean for market class

Linear regression analysis was performed on average seed yield production data from North Dakota for the pinto, black, and navy market classes (each market class considered separately) retrieved from USDA-NASS (2013), and considered as the on-farm yield data. At the same time, the historical data from the Carrington variety trial was used as the estimation of the potential yield (on-station yield). On average, 9 pinto, 7 navy, and 3 black bean cultivars were included each year within the variety trial data used for this study. However, the genotypes changed from year to year and therefore only means from each market class are used in this study. The on-farm and potential yield data was plotted to estimate the yield gap and to estimate yield gains within and among market classes.

Statistical analysis

Data was analyzed by market class using the mean of each genotype planted in both conditions (dryland and irrigated) per year, from 1981 to 2011. Since genotypes were not always repeated every year, analysis of genotype was not performed. A total of four years (1983, 1984, 2001, and 2002) that had dryland seed yield values higher than the irrigated trial (due mostly to disease pressure) were eliminated from the analysis because it is not representative of the historical data.

Data was analyzed using the SAS GLM procedure (SAS Institute, 2011), where market class and condition (dryland vs. irrigated) were considered fixed effects, and years were considered as random effects. As natural precipitation was part of the irrigated and dryland treatments, the soil water regime experienced by plants varied among years. Therefore, each year was analyzed separately. Years were considered homogeneous when the ratio of the effective error variances for seed yield was less than tenfold (Bartlett, 1947). If the variance for year was homogeneous, and when appropriate, the data was combined across the years. In the combined analyses, years were considered a random effect, and conditions and market classes as fixed effects.

Means were separated using an F-protected Least Significant Difference at $p \leq 0.05$ level of significance. Different regression models among the means were used in order to verify if seed yield, rainfall, and conditions by market class are associated. Only significant regressions were discussed ($p > 0.05$).

Results and Discussion

The historical data (26 years) from an ongoing variety trial at the Carrington Research and Extension Center was combined across years and analyzed. Low seed yield values observed

under the irrigated trial in 1983, 1984, 2001, and 2002 in comparison to the dryland trials may be mostly due to disease pressure. For example, white mold is the most prevalent dry bean disease affecting production in the North Dakota area, which is caused by the fungus *Sclerotinia sclerotiorum* Lib. de Bary. Irrigated production fields have favorable conditions for the development of white mold. Plants tends to develop more biomass, creating a canopy structure between rows that helps to keep in the soil moisture, but blocks the air flow, and together with cold night temperatures creates an ideal micro-environment for disease development. Seed yield losses from this disease are, on average as high as 20%, and in some cases, yield losses may go up to 65% (Schwartz et al., 2006). Other diseases of high impact in North Dakota are halo blight (caused by *Pseudomonas syringae* pv. *phaseolicola*), bacterial brown spot (caused by *Pseudomonas syringae* pv. *syringae*), rust (*Uromyces appendiculatus*), anthracnose (*Colletotrichum lindemuthianum*), and common bacterial blight (caused by *Xanthomonas campestris* pv. *phaseoli* Syn. *Xanthomonas axonopodis* pv. *phaseoli*). Other factors to consider are changes in soil fertility, extreme high and low temperatures, relative humidity, and the distribution of precipitation during the growing season, among others.

Results by market class

Highly significant differences ($p < 0.01$) were found between growing conditions (irrigated or dryland), conditions within years, and market classes (Table 1.2). The greatest source of variation observed pertained to conditions within years. The market-class-by-condition interaction was not significant, being the smallest component of variance.

Table 1.2. Combined analyses of variance for seed yield in variety trials conducted at Carringnton Research and Extension Center, ND, across 26 years.

Source of variation	df[†]	SS	MS	F value	Pr > F
Year	25	237533275.5	9897219.8	25.17	<0.0001
Condition	1	158665424.8	158665424.8	402.48	<0.0001
Market Class	2	8595379.9	4297690.0	10.90	<0.0001
Market Class x Condition	2	616320.1	308160.0	0.78	0.4580
Error	126	333900710.9	394215.7		

[†]df = degrees of freedom.

Results showed that across years and market classes, seed yield (2,682 kg ha^{-1}) was significantly different ($p > 0.05$) under irrigated conditions when compared to dryland growing conditions (1,837 kg ha^{-1}) (Figure 1.1). On average, 325t o 635mm of water (including rainfall) was provided the irrigated treatment. Several authors have reported that seed yield in dry beans varies depending on a cultivar's genetic potential, management practices, the environment and their interactions (Beaver and Osorno, 2009; Beebe et al., 2008). Data from this trial suggests that yield potential can be reduced up to 844 kg ha^{-1} (31.5%) across all three market classes depending on growing conditions (Figure 1.1).

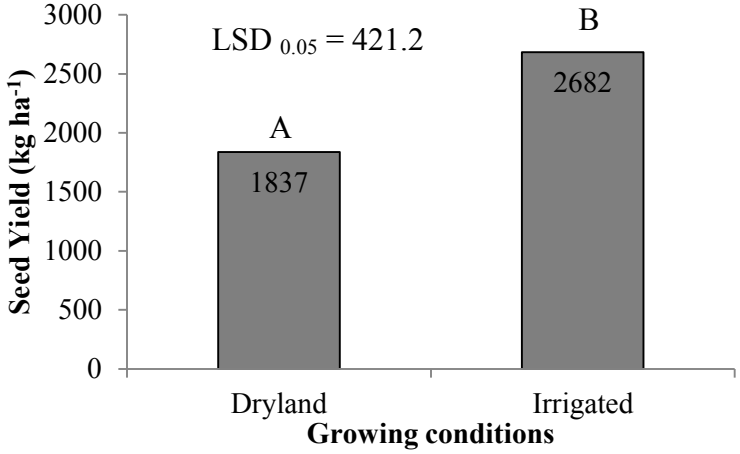

Figure 1.1. Mean seed yield of dry bean variety trials conducted at the Carrington Research and Extension Center, ND across market classes over 26 years and two growing conditions (dryland and irrigated).

When looking within each market class, results showed that across years pinto beans had the highest seed yield (2,384 kg ha^{-1}), but seed yield was not significantly different than blacks (2,241 kg ha^{-1}) (Table 1.3). However, seed yield of pinto beans was significantly different than navy beans (2,153 kg ha^{-1}). There was no difference between black and navy market classes (Table 1.3).

Table 1.3. Mean seed yield of dry bean variety trials by market class conducted at the Carrington Research and Extension Center, ND, over 26 years and two growing conditions (dryland and irrigated).

Market class	Seed yield†	Average of cultivars included in the trial per year
	kg ha^{-1}	
Pinto	2,384 a	9
Black	2,241 ab	7
Navy	2,153 b	3
CV (%)	18	
$LSD_{0.05}$	162	

†Means followed by the same letter are not significantly different at p=0.05.

After calculating different parameters such as drought intensity index (DII), drought susceptibility index (DSI), percent yield reduction (PYR), and the geometric mean (GM) results suggest that the trials had moderate drought conditions across most years (0.31) with a susceptibility index of 0.98 (Table 1.4). As shown in Table 1.5, pintos experienced the lowest percentage of seed yield reduction, followed by navy and blacks (29.5, 32.4, and 32.7%, respectively), as well as the highest geometric mean, followed by black and navy (2349.8, 2198.2, and 2112.8 kg ha^{-1}, respectively).

Table 1.4. Drought intensity index (DII) and drought susceptibility index (DSI) due to drought stress calculated for the whole trial over a period of 26 years.

	Drought parameters	
	DII	DSI
Carrington Variety Trial	0.31	0.98

Table 1.5. Percent yield reduction (PYR) and geometric mean (GM) due to drought stress calculated for each market class over a period of 26 years.

Market Class	PYR[†]	GM
	%	kg ha^{-1}
Black	32.7 ab	2198.2 ab
Navy	32.4 b	2112.8 b
Pinto	29.5 a	2349.8 a
Average	31.5	2220.3

[†]Means followed by the same letter are not significantly different at p=0.05.

Data shows that among the three market classes, pinto beans had a better yield stability than the other market classes (Table 1.5). Pinto beans belong to the race Durango, which has previously been reported as tolerant to drought due to the environmental conditions of its geographic origin, the semiarid and arid northern highlands of Mexico (Brick and Grafton, 1999). Pintos, great northern, and the North American reds and pinks that belong to this race have better adaptation to the Great Plains, and have been a good source of early maturity, drought tolerance, high harvest index, positive general combining ability (GCA) for seed yield (Nienhuis and Singh, 1988), and tolerance to some viral diseases, as well as anthracnose (Morales and Singh, 1991). Other important characteristics of Durango beans are the leaf morphology (i.e., dark and small) and the distribution of the internodes in the lower part of the plant. Internodes in this area are short, providing good ground cover, which can help to reduce evapotranspiration and also to conserve soil moisture. These characteristics increase tissue - water-retention capacity and facilitate seed filling over a longer period of time (Muñoz-Perea et al., 2006; Singh, 2007).

Results show that black and navy beans have less yield stability. One possible explanation is the genetic background of these two market classes. Navy and black beans belong to the race Mesoamerica, which is reported to be more sensitive to drought compared to the

Durango race (Muñoz-Perea et al., 2006; Singh, 2007). In general, black and navy beans are small- seeded types with mostly bush habits, originating in lowland Central America and Mexico. This region is distinguished by its flat plains that are often wet, due to the runoff from the large mountain range (American Cordillera) that goes through the center of the Central American isthmus (Singh, 1991).

Results also show that in some years, even with adequate rainfall, genotypes within market classes did not perform as expected in dryland conditions. This can be explained in part by the distribution of rainfall across the growing season. In North Dakota, rainfall distribution is highly variable, and it is not unusual to have heavy rain during the spring followed by dry periods (or vice versa). This rainfall pattern often results in drought stresses during the critical periods of flowering and pod filling (NDAWN, 2013). Also, dry bean production is more successful in areas where rainfall is light during the latter part of the growing season. For example, during the 2006 growing season, Carrington had 223 mm rainfall with an average seed yield of 2,117.12 kg ha^{-1} (Figure 1.2). Similar patterns were also observed from 1995 to 2010. Opposite results (low seed yield) were observed in 1993 and 2011, when average rainfall was 572.5 and 486.41 mm, respectively. However, from 1990 to 1992 irregular patterns were observed due to a period of drought that began in the 1980s. For example, in 1991 average rainfall was 456.9 mm and average seed yield was 893 kg ha^{-1}, for 1992 average rainfall was 189.3 mm and average seed yield was 1,463.86 kg ha^{-1}. The observed pattern suggests a severely dry year with an average rainfall of 186.3 mm but still with higher seed yields. However, average seed yield in the growing season of 1993 had relatively high values for rainfall (597.9 mm), but seed yield remained close to average (2,278 kg ha^{-1}).

Overall, the data suggest that rainfall has remained constant across years at Carrington (R^2 = 0.0448) (Figure 1.2). However, in the growing season of 2012 (not included in this study) on-farm yield statistics showed a seed yield record event of 1,904 kg ha^{-1} (1,700 lb ac^{-1}), even though it was a dry growing season (263 mm) when compared to the average rainfall of 327.6 mm (USDA-NASS, 2013). One possible reason behind the higher seed yield may have to do with unfavorable climatic conditions for disease development.

Can rainfall explain the variation in seed yield?

After trying different regression analyses (exponential, quadratic, logarithmic and polynomial) using the average seed yield across market classes and the growing season rainfall data from 1990-2001, the best fit was a linear regression model (Figure 1.3). However, linear regression cannot explain the relationship between seed yield and rainfall (R^2 = 0.0044) (Figure1.4), as well as the possibility that water may not yet be a limiting factor in Carrington, ND.

A decrease in seed yield may be due to an increase in disease pressure caused by excess moisture around the plants or to other factors previously discussed, like fertility, the cultivar's genetic potential, day and night temperatures, and the distribution of precipitation during the growing season, etc.

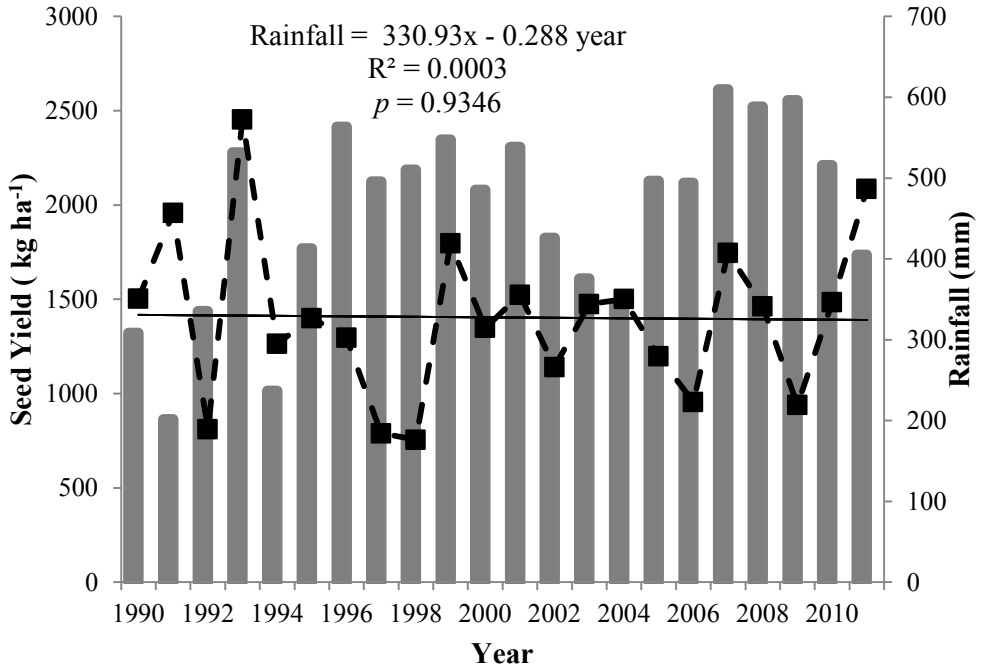

Figure 1.2. Mean seed yield (bars) and growing season rainfall (dashed line) across market classes under dryland conditions at the Carrington Research and Extension Center, ND from 1990 to 2011.

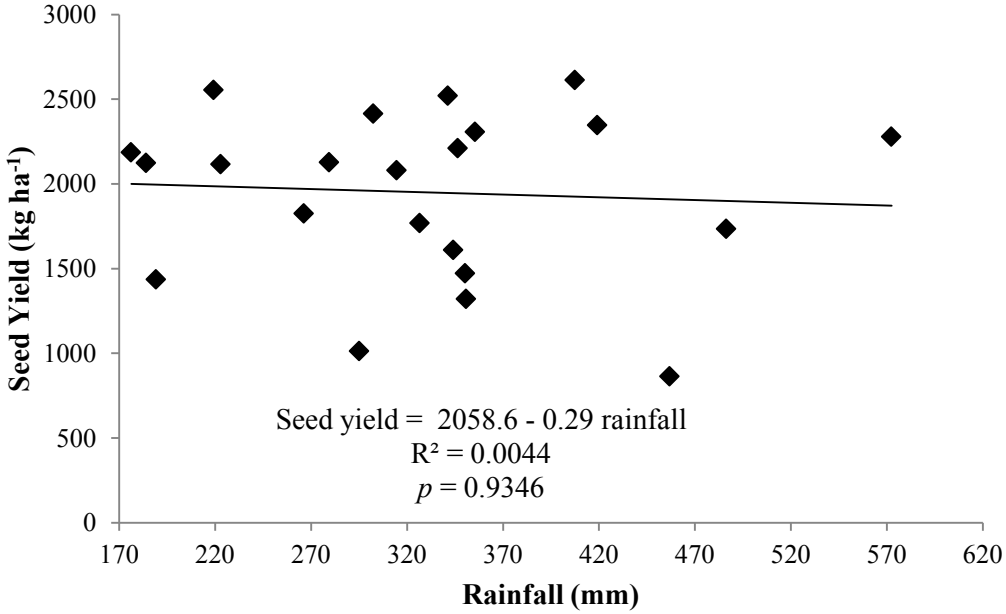

Figure 1.3. Relation between seed yield (kg ha^{-1}) and rainfall (mm) in dryland conditions across three market classes over 26 years in Carrington, ND.

Seed yield gains

The historical data from the Carrington variety trial were used as an estimate of potential yield (on-station yield), while the actual seed yield data obtained at the commercial level were used to estimate the on-farm yield. Initially, two on-farm estimates were taken into consideration for North Dakota: the average seed yield for pinto, black, and navy market classes (combined) and the North Dakota seed yield for pinto, black, and navy market classes (each market class considered separately) (USDA-NASS, 2013). Therefore, on-farm yield across the main three market classes grown in North Dakota (pinto, black, and navy) showed a seed yield increase of 23.11 kg ha^{-1} yr^{-1} between 1981 to 2011 (Figure 1.5). A lower on-farm estimate (12 kg $ha^{-1}yr^{-1}$) was reported for bean production nationwide (Vandemark et al., 2013). However, the data used herein belong to a specific production region (one environment; Carrington), if we take in consideration the statistics behind these analyses, and the genotype-by-environment interaction, the yield gain estimates for one agricultural production zone or region are more accurate than when compared to multiple production zones or environments.

Potential yield (or on-station yield) from the Carrington variety trial under dryland conditions shows seed yield gains of 8.45 kg $ha^{-1}yr^{-1}$, while under irrigated conditions, reductions of seed yield were observed (41.06 kg $ha^{-1}yr^{-1}$). Reductions (negative slope) observed under irrigated conditions can be due to the fact that in the 1980s, agricultural production in North Dakota did not experience as much rainfall as in recent years. Consequently, seed yields were higher than now most likely due to reduced disease pressure. At the same time, the yield gap between on-farm yield and dryland conditions is minimized, which is a good sign of efficient crop management in production fields. Interpretation of these results is difficult since data from three different market classes were combined across years, and the genetic diversity among them

is significantly wider. Therefore, for a more accurate assessment, analysis of seed yield performance of each market class under the two growing conditions was performed and is presented herein.

Under dryland conditions, yield gains for pinto beans showed a positive trend for both estimates (on-farm and on-station yield) (Figure 1.5). However, the on-farm yield regression was significant ($p = 0.0026$), while the potential yield was not ($p = 0.40$). The yield gain for pinto beans based on the on-farm yield data was 27.54 kg $ha^{-1}yr^{-1}$ (Figure 1.5; Figure 1.6). Even though the potential yield (on-station yield) was not significant, there is a yield gap between the on-farm and potential yield, suggesting the cultivar's genetic potential was not fully expressed in the farmers' fields (Figure 1.5; Figure 1.6). In contrast, pinto beans under irrigated conditions showed a negative trend. However, potential yield regression analysis was not significant ($p = 0.40$) (Figure 1.7). Recently, Vandemark et al. (2013) reported yield gains for pinto beans, ranging between 12.2 and 15.2 kg $ha^{-1}yr^{-1}$ for on-farm and potential yield, respectively.

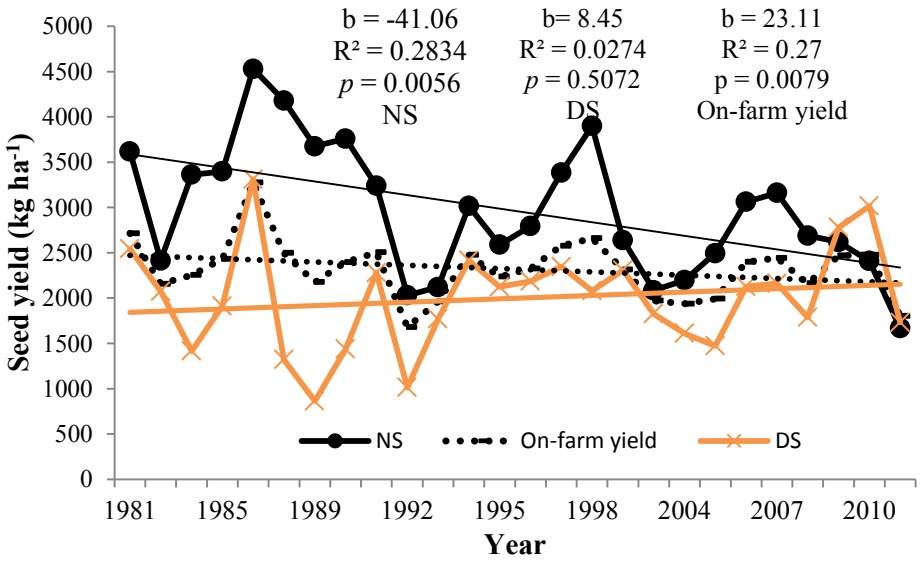

Figure 1.4. On-farm seed yield (kg ha^{-1}) of dry bean production in North Dakota averaged across pinto, black, and navy market classes between 1981 and 2011 and on-station yield data from the Carrington variety trial under dryland (DS) and irrigated conditions (NS).

In the case of the black bean market class, potential and on-farm yield trends were not significant ($p = 0.40$, $p = 0.30$, respectively) for cultivars grown under dryland conditions (Figure 1.7). Under irrigated conditions, the potential yield (or on-station yield) was significant ($p = 0.005$). However, the data show a negative trend for the potential yield (on-station yield) with yield loss of 58 kg $ha^{-1}yr^{-1}$ (Figure 1.8). On-farm yield data also suggests that black beans were introduced to the North Dakota area in the early 1990s, which may be the reason for the lack of information from 1981 to 1991.

For navy beans, on-farm yield showed a significant relationship to seed yield ($p = 0.0025$) when compared to the potential yield (or on-station yield) ($p = 0.60$). Yield gain in navy beans can be up to 32 kg $ha^{-1}yr^{-1}$ when grown under dryland conditions (Figure 1.9). In contrast, a negative trend ($p = 0.02$) for potential yield (on-station yield) was observed in navy beans grown under irrigated conditions (Figure 1.10). On-farm and potential yield (or on-station yield) ranged from 25 to -46 kg $ha^{-1}yr^{-1}$, respectively. While producers are reporting yields gains (25 kg $ha^{-1}yr^{-1}$), potential yield trials are showing yield losses up to 46 kg $ha^{-1}yr^{-1}$ (Figure 1.10). It's important to mention that this trial does not receive fungicide applications.

However, Vandemark et al. (2013) report yield gains for navy beans grown across the U.S. with fluctuations between 18.5 and 25.9 kg ha^{-1} yr^{-1}, which is greater than for pinto beans. Meanwhile, black beans showed a more varied range in yield gains between the on-farm and potential yield, 5.7 and 24.3 kg $ha^{-1}yr^{-1}$, respectively. However, dry bean cultivars have not yet reached a yield plateau in the three main market classes of North Dakota.

Yield gaps were observed among the three market classes under dryland growing conditions, suggesting that each cultivar's genetic potential was not fully expressed at the farm level.

For cultivars under irrigated conditions, the yield gap was higher in the early 1980s and lately seems to be less, but with a negative trend (Figure 1.6; Figure 1.8, and Figure 1.10). Data under irrigated conditions suggest that breeding efforts for disease resistance may be necessary since dry beans are affected more by bacterial and fungal diseases. However, crop management practices also should be evaluated. Results obtained from the irrigated trials show that irrigation can be a limiting factor in dry bean production in this region. These data suggest that the amount of water supplied by irrigation should be re-evaluated. As a consequence, the cost of production could decrease due to the cost of water, less fungicide and herbicide application, etc., in turn increasing cash farm returns.

In general, disease data were not collected in every year. Based on comments provided by the Carrington Research and Extension Center in the data file, however a high incidence of fungal and bacterial disease was observed in several growing seasons.

Sometimes the scientific community, farmers, consumers, etc. believe that using extreme amounts of water will increase agricultural profits. Instead, the idea is to have genotypes in the field that are water-use efficient. An ideal water-use-efficient genotype, from the agronomical point of view, is one that can perform well under dryland as well as under irrigated conditions.

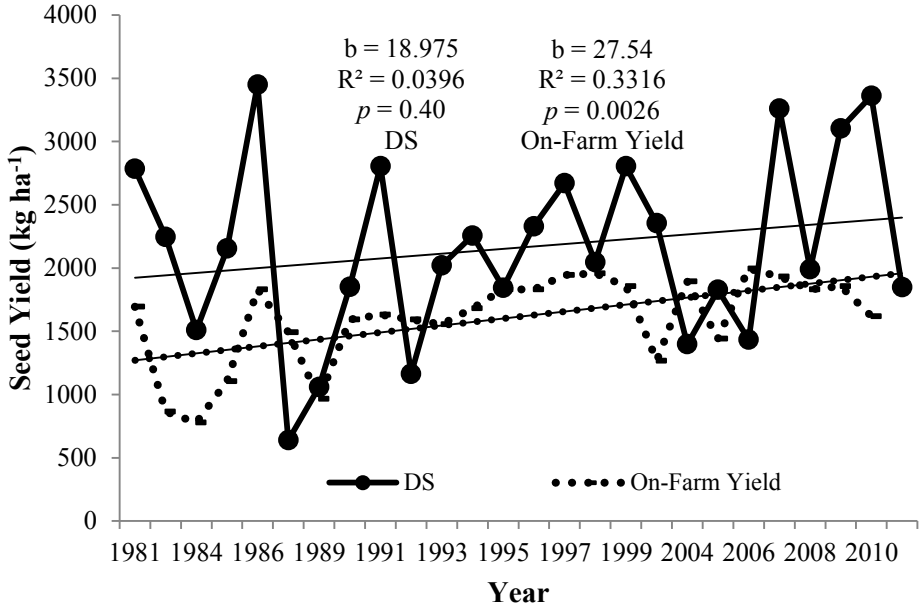

Figure 1.5. Pinto seed yield (kg ha^{-1}) under dryland (DS) conditions at the Carrington Research and Extension Center, ND, and on-farm yield for ND from 1981 to 2011.

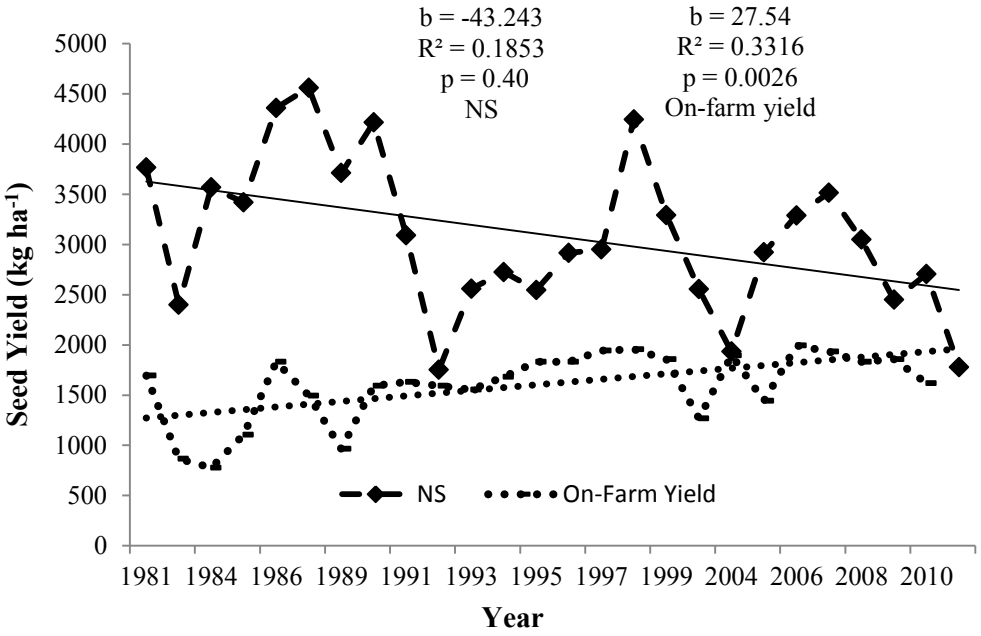

Figure 1.6. Pinto seed yield (kg ha^{-1}) under irrigated (NS) conditions at the Carrington Research and Extension Center, ND, and on-farm yield for ND from 1981 to 2011.

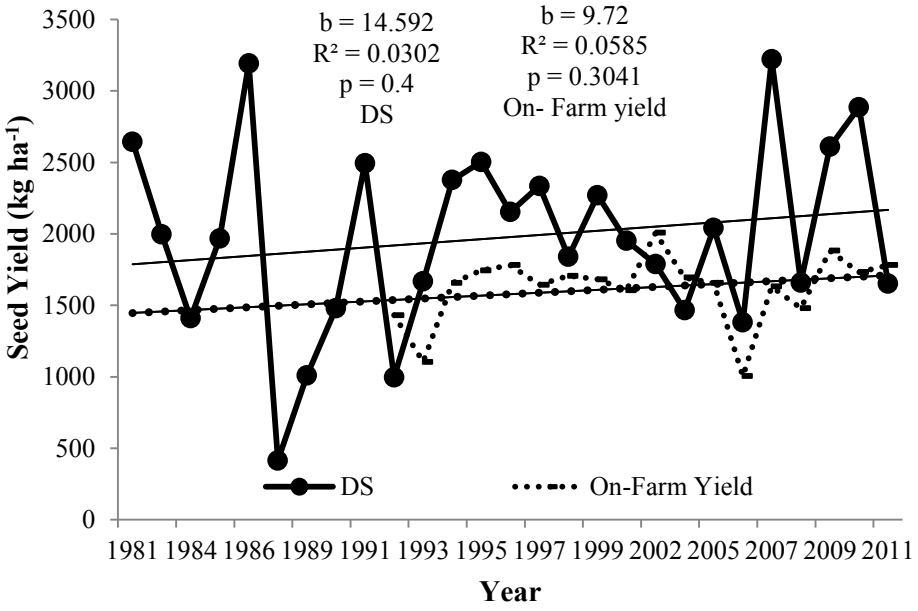

Figure 1.7. Black seed yield (kg ha^{-1}) under dryland (DS) conditions at the Carrington Research and Extension Center, ND, and on-farm yield for ND from 1981 to 2011.

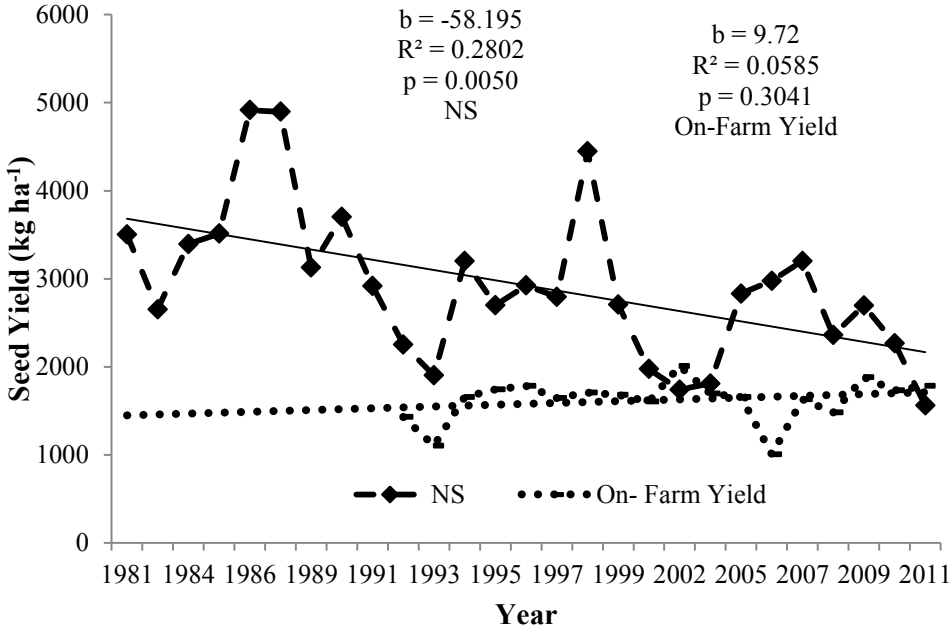

Figure 1.8. Black seed yield (kg ha^{-1}) under irrigated (NS) conditions at the Carrington Research and Extension Center, ND and on-farm yield for ND from 1981 to 2011.

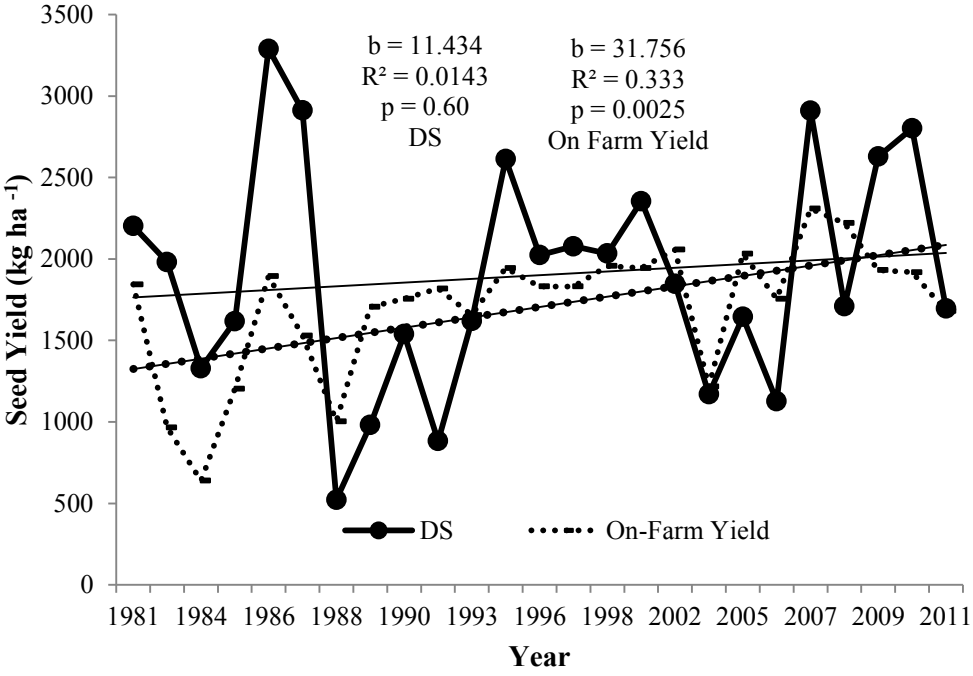

Figure 1.9. Navy seed yield (kg ha^{-1}) under dryland (DS) conditions at the Carrington Research and Extension Center, ND, and on-farm yield for ND from 1981 to 2011.

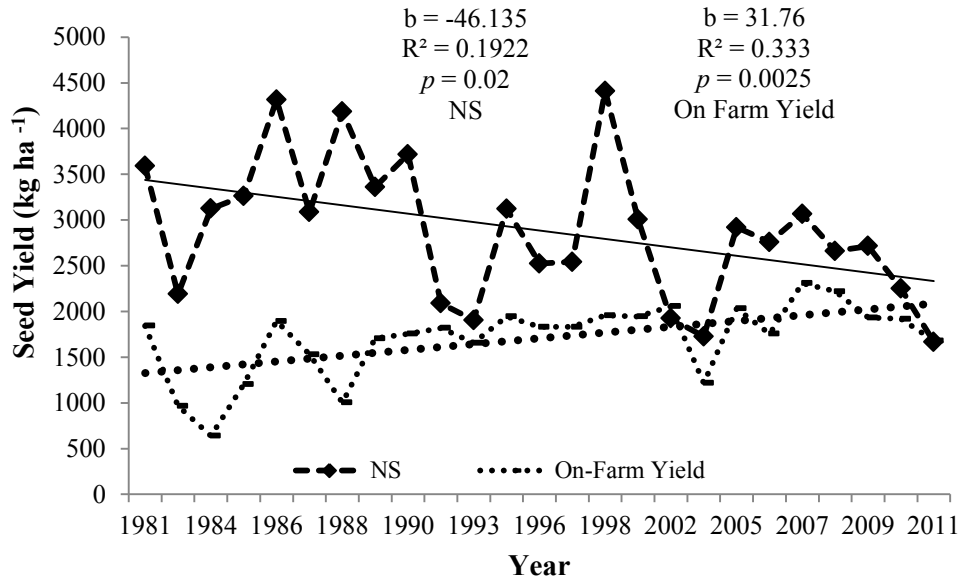

Figure 1.10. Navy seed yield (kg ha^{-1}) under irrigated (NS) conditions at the Carrington Research and Extension Center, ND, and on-farm yield for ND from 1981 to 2011.

Summary and Conclusions

The historic data obtained from the variety trial at the Carrington Research and Extension Center in North Dakota provides a unique and powerful overview of the dry bean genetic improvement in the Great Plains. The objective of this study was to assess the differences in seed yield due to growing conditions (dryland vs. irrigated) within each one of the three most important market classes grown in the region (pinto, navy, and black) and to estimate the genetic gain and progress over time of each market class due to the use of improved dry bean cultivars from 1981 to 2011.

Some important results and conclusions drawn from this research are:

- An average yield reduction of 844.7 kg ha^{-1} (31.5%) was observed in all market classes due to the dryland treatment.
- Black beans were the most affected by the irrigation treatment, with 877 kg ha^{-1} (32.7%) yield reduction, followed by navy and pinto beans with reductions of 832.6 and 824.5 kg ha^{-1} (32.4 and 29.5%, respectively).
- Pinto beans were more adapted to drought conditions, been less affected by the irrigated treatment, followed by navy and black beans.
- All three market classes experienced moderate drought conditions based on the DII, DSI, and PYR values. Geometric mean results suggest that pinto bean yields were more stable under both water treatments.
- Linear regression showed no significant relationship between seed yield and rainfall, suggesting that water may not yet be a limiting factor in Carrington, ND.
- On-farm yield across the main three market classes grown in North Dakota (pinto, black, and navy) show a seed yield increase of 23.11 kg $ha^{-1}yr^{-1}$ between 1981 and

2011. A lower on-farm estimate (12 kg $ha^{-1}yr^{-1}$) was reported for bean production across the U.S. (Vandemark et al., 2013). However, the authors estimated yield gains with different sources of data and the information obtained was similar. Based on that, differences in the estimation are due mainly to the genotype-by-environment interaction.

- Pinto bean yield gains between 1981 and 2011 showed a positive trend for on-farm yield under dryland conditions, with yield gains up to 27 kg $ha^{-1}yr^{-1}$.
- Potential and on-farm yield trends for black beans were not significant ($p = 0.40$, $p = 0.3$, respectively) for cultivars grown under dryland conditions. Under irrigated conditions, the potential yield trend (on-station yield) was significant ($p = 0.005$), with yield losses of 58 kg $ha^{-1}yr^{-1}$.
- For navy beans, on-farm yield was significant ($p = 0.0026$). However, the potential yield (on-station yield) ($p = 0.60$) was not. Yield gain in navy beans was 11 kg $ha^{-1}yr^{-1}$ when grown under dryland conditions. In contrast, a negative trend ($p = 0.02$) for potential yield (on-station yield) was observed in navy beans grown under irrigated conditions. On-farm and potential yield ranged from 25 to -46 kg $ha^{-1}yr^{-1}$, respectively.
- Yield gaps were observed among the three market classes under dryland growing conditions, suggesting that there is a need to keep improving management practices that could allow a better expression of the cultivar's genetic potential.
- Data suggest that dry bean cultivars have not reached a yield plateau in the three main market classes in North Dakota production. However, introgression of germplasm from other races like Jalisco should provide new sources of genetic

diversity to enhance yield into the foreseeable future in the NDSU breeding program.

- Analysis of the rainfall distribution over three different stages of the growing season per year (i.e., germination, blooming, and physiological maturity) is going to be performed in the near future to identify whether rainfall is affecting dry bean production at the germination stage, at blooming, or at physiological maturity.
    - This information would help to understand the possible causes behind the yield reduction, as well as defined strategies to genetic improvement.

References

Abebe, A., M.A. Brick, and R.A. Kirkby. 1998. Comparison of selection indices to productive dry bean lines under diverse environmental conditions. Field Crops Res. 58:15 23.

Bartlett, M.S. 1947. The use of transformations. Biometrics 3:39-52.

Beaver, J.S., and J.M. Osorno. 2009. Achievements and limitations of contemporary common bean breeding using conventional and molecular approaches. Euphytica 168:145-175.

Beebe, S.E., M.R. Idupulapati, C. Cajiao, and M. Grajales. 2008. Selection for drought resistance in common bean also improves yield in phosphorus limited and favorable environments. Crop Sci. 48: 582-592.

Bingham, J. 1967. Breeding cereals for improved yielding capacity. Ann. Appl. Biol. 59:312–315.

Brick, M., and K.F. Grafton. 1999. Improvement of medium-seeded race durango cultivars In: S.P. Singh, editors, Common bean improvement in the twenty-first century. Kluwer Academic Publish. Netherlands. p. 223-254.

Evans, L.T., and R.A. Fisher. 1999. Yield potential: its definition, measurement, and significance. Crop Sci. 39:1544-1551.

Fischer, R.A., and R. Maurer. 1978. Drought resistance in spring wheat cultivars: I. Grain yield responses. Aust. J. Agric. Res. 29:897–912.

Godfray, H.C.J., J.R. Beddington, I.R. Crute, L. Haddad, D. Lawrence, J.F Muir, J. Pretty, S. Robinson, S.M. Thomas, and C. Toulmin, C. 2010. Food Security: the challenge of feeding 9 billion people. Science 327:812-818.

MacKey, J. 1979. Genetic potentials for improved yield. In: S. Rajki, editors Proc. Workshop on Agricultural potentiality directed by nutritional needs. Akad. Kiado, Budapest. p. 121–143.

McClean, P.E., J. Burridge, S. Beebe, I.M. Rao and T.G. Porch. 2011. Crop improvement in the era of climate change: an integrated, multi-disciplinary approach for common bean (*Phaseolus vulgaris*) Funct. Plant Biol. 38:927–933.

Morales, F.J., and S.P. Singh. 1991. Genetics resistance to golden bean mosaic virus in *Phaseolus vulgaris* L. Euphytica 52:113-117.

Muñoz-Perea C.G., H. Terán, R.G. Allen, J.L. Wright, D.T. Westermann, and S.P. Singh. 2006. Selection for drought resistance in dry bean landraces and cultivars. Crop Sci. 46:2111-2120.

NDAWN. 2013. Weather data. North Dakota Agricultural Weather Network. North Dakota State University. http://www.ndawn.ndsu.nodak.edu/ (accessed 26 Oct 2013)**.**

Nienhuis, J., and S.P. Singh. 1988. Genetics of seed yield and its components in common bean *(Phaseolus vulgaris* L.) of Middle-American origin. I. General combining ability. Plant Breed. 101:143-154.

Northarvest Bean Grower Magazine, 2013Osorno, J.M., K.F. Grafton, G.A. Rojas-Cifuentes, R. Gelin, and A.J. VanderWal. 2008. Avalanche, a new navy bean for the Northern Plains. Annu. Rep. Bean Improv. Coop. 51:282–283.

Osorno, J. M., K.F. Grafton, G.A. Rojas-Cifuentes, R. Gelin, and A.J. VanderWal. 2010. Registration of 'Lariat' and 'Stampede' pinto beans. J. Plant Reg. 4:5-11.

Perkins, S. 2002. Once upon a lake: The life, times, and demise of the world's largest lake. Sci. News 162: 283–284.

Porch, T.G., V.H. Ramirez, and E.W. Harmsen. 2009. Evaluation of common bean for drought tolerance in Juana Diaz, Puerto Rico. Agron. J.195:328-334.

Ramírez-Vallejo, P., and J.D. Kelly. 1998. Traits related to drought resistance in common bean. Euphytica 99:127-136.

Rosielle, A.A., and J. Hamblin. 1981. Theoretical aspects of selection for yield in stress and non-stress environments. Crop Sci. 21:943-946.

Sansome, C.J. 1983. Minnesota underfoot: A field guide to the state's outstanding geologic features. Stillwater, MN: Voyageur Press. p. 174-181.

SAS Institute Inc. 2011. SAS/STAT Users' Guide. SAS Institute Inc., Cary, NC, USA.

Schneider, K.A., M.E. Brother, and J.D. Kelly. 1997. Marker-assisted selection to improve drought resistance in common bean. Crop Sci. 37:51-60.

Schwartz, H.F., K.Otto, H. Terán, M. Lema, and S.P. Singh. 2006. Inheritance of white mold resistance in *Phaseolus vulgaris* x *P. coccineus* crosses. Plant Dis 90:1167–1170.

Singh, S.P. 1991. Breeding for seed yield. In: A. van Schoonhoven and O. Voysest, editors,

Common beans. Research for crop improvement. CAB Int., CIAT, Colombia.

Singh, S.P. 2007. Drought resistant in race Durango dry bean landraces and cultivars. Agron. J. 99:1219-1225.

Urrea, C.A., C.D. Yonts, D.J. Lyon, and A.E. Koehler. 2009. Selection for drought tolerance in dry bean derived from the Mesoamerican gene pool in western Nebraska. Crop Sci. 49:2005-2010.

USDA-ERS. 2011. Dry edible beans. In: Vegetable and melons outlook/VGS-330/December 16, 2011. www.ers.usda.gov/Briefing/DryBeans/PDFs/DBnOutlook.pdf . USDA- ERS, Washington, DC (accessed 16 Dec. 2011).

USDA-NASS. 2012. North Dakota Agricultural statistics 2012. North Dakota Agricultural Statistics Service, North Dakota field office, U.S. Department of Agriculture, National Agricultural Statistics Service. http://www.nass.usda.gov/Statistics by State/ North Dakota/Publications/Annual Statistical Bulletin/index.asp (accessed 17 July 2012).

USDA-NASS. 2013. National Agricultural Statistics Service. Stadistics by subject. U.S. Department of Agriculture, National Agricultural Statistics Service. http://www.nass.usda.gov/Statistics_by_Subject/index.php?sector=CROPS (accessed 10 May 2013).

USDA-NRCS. 2012. Official Soil Series Descriptions. USDA-NRCS. http://soils.usda.gov/technical/classification/osd/ (accessed 18 Jan. 2012).

Vandemark, G.J., M.A. Brick, J.D. Kelly, J.M. Osorno, and C. Urrea. 2013. Yield gains in major U.S. field crops. In: Yield gains in edible grain legumes. In press.

White, J.W., and S. P. Singh. 1991. Breeding for adaptation to drought. In: A. van Schoonhoven and O. Voysest, editors. Common Beans, Research for Crop Improvement. CAB International, CIAT, Colombia. p. 501-560.

PAPER II. QTL IDENTIFICATION FOR DROUGHT TOLERANCE AND RELATED AGRONOMIC TRAITS IN THE BUSTER/SER 22 RECOMBINANT INBRED LINE POPULATION

A. M. Linares-Ramírez[1], C. A. Urrea[2], T.G. Porch[3], S. Mamidi[1], P.E. McClean[1], and J.M. Osorno[1]

[1]Dept. of Plant Sci., North Dakota State Univ., Fargo, [2]Univ. of Nebraska - Lincoln, Scottsbluff Res. & Ext. Center, NE, [3]USDA-ARS Tropical Agriculture Res. Sta., Mayagüez, PR.

Abstract

The identification of loci associated with drought tolerance could be an important strategy for implementing future Marker Assisted Selection (MAS). A population of 335 recombinant inbred lines (RIL) was derived from the cross between 'Buster', a drought-susceptible pinto cultivar, and SER 22, a drought-tolerant small red germplasm line developed at the International Center for Tropical Agriculture (CIAT). Evaluation in replicated irrigated and non-irrigated trials using a lattice design were performed at two locations (Mitchell, NE, and Juana Díaz, PR) over three years (2011-2013), for a total of twelve environments. The linkage map comprised a total of 378 single nucleotide polymorphism (SNP) markers for a total map distance of 778.4 cM divided across twelve linkage groups representing 47% of the estimated genome size of the 'Redhawk'/ 'Stampede' (1665.5 cM) consensus map. The number of markers per linkage group varied from 5 to 99. The average distance between markers was 2.06 cM. The largest interval between markers was 23.5 cM, located on Pv7. Gaps in the genetic map are present on Pv9, and Pv10. Transgressive segregation was observed for all traits under study. Three QTL for seed yield were found on two linkage groups using composite interval mapping. Additional QTL were identified for agronomic and physiological traits. With the results from this

study, plant breeders will have a better understanding of the genetics and possible mechanisms behind drought tolerance, as well as the possibility of the use of these QTL for genetic improvement.

Introduction

Drought, due to either insufficient or unpredictable rainfall, has been identified worldwide as a production problem in dry bean (*Phaseolus vulgaris* L.) and has been exceeded only in magnitude to dry bean diseases (Boyer, 1982).

Many traits that are important to agriculture are quantitative in nature, influenced by multiple genes and the environment. The efficient and robust identification and mapping of loci of interest are important goals for most breeding programs. Lately, the improvement and screening of quantitative traits using molecular tools has been an important goal for many breeding programs (Schneider et al., 1997a). With the pressure to increase production or to release a variety more rapidly, plant breeders are interested in identifying the most promising lines early in the selection process which contain quantitative trait loci (QTL) that contribute to an improved combination of desirable traits.

In dry beans, drought tolerance has become a trait in which scientists are putting a lot of effort because short intermittent periods of drought reduce both bean quality and seed yields (Nielson and Nelson, 1998; Wallace, 1980). The extent and duration of both intermittent and terminal drought stress in dry bean are directly associated with seed yield loss (Singh, 1995) and their effects are augmented by interactions with high temperature, disease, and soil type, among others (Ramirez-Vallejo and Kelly, 1998). Several studies have identified the importance of root architecture (deeper roots) and its efficiency as critical factors related to drought response in dry bean (Sponchiado et al., 1989; Acosta-Gallegos and Adams, 1991; Ho et al., 2005; Lynch, 2007).

Crop growth, canopy temperatures, and soil moisture extraction were also associated with root growth and drought tolerance (Chaves et al., 2003).

Conventional breeding methodologies for improving drought tolerance in crops are primarily based on germplasm screening, selection of drought tolerant germplasm, hybridization, and the use of direct or empirical methods to select for crop yield under different drought stress conditions tested over several years (Spech et al., 1986). This can be considered to be more labor intensive than the application of molecular tools for screening. For example, the ratio of biomass accumulation, expressed as crop grain yield, to water consumed, expressed as transpiration, evapotranspiration, or total water input to the system (Sinclair et al., 1984), is a definition of water use efficiency. According to Muñoz-Perea et al. (2007), water use efficiency (WUE) may be a key trait to consider when selecting for drought-tolerant genotypes. Mechanistic and empirical evaluations have been implemented in Puerto Rico and Nebraska, where drought tolerance studies have focused on the analysis of the physiological response to drought stress, and breeding for drought tolerance through both local and shuttle breeding efforts (Ramirez, 2007; Urrea and Porch, 2009).

Although bean breeders have developed improved germplasm with enhanced tolerance to several important abiotic stresses such as drought (Brick et al., 2008; Muñoz-Perea et al., 2006; Porch et al., 2012) and high temperature (Beaver and Miklas; 1999; Rosas et al., 2000; Rosas et al., 2003), few commercial cultivars have been released up to date. Genetic improvement of dry bean to drought stress is becoming increasingly important due to the shift of dry bean acreage from more humid and fertile areas to more marginal zones where low water availability and extreme temperatures are limiting factors. This shift is caused mostly by higher returns and/or ease of production of other commodities, and it is occurring not only in the U.S. but also in other

regions of the world as well (Wortmann et al., 1998; Porch et al., 2007; Beebe et al., 2008; Beebe et., 2012). As a result, development of drought-tolerant bean cultivars is an important strategy to minimize crop failure and improve food security in bean growing-regions. However, genetic improvement for drought tolerance in crops has been slow due to the lack of reliable techniques, as well as the inability to create repeatable screening environments.

Molecular tools can facilitate the identification of genomic regions controlling traits related to drought tolerance using QTL analysis. With the revolution of DNA markers in the 80s-from restriction fragment length polymorphism (RFLP) to microsatellites, a diverse number of mapping populations were developed. Most of them were created to evaluate disease resistance (Broughton et al., 2003), subsequently, drought and heat tolerance became a topic of concern and interest for the bean community and mapping populations were specifically created to evaluate drought, root structure, and function in plant nutrition (Blair et al., 2011).

Schneider et al. (1997b) studied the genetics of drought using multiple regression analysis and RAPD markers to detect drought tolerance in Michigan, USA, and Mexico in two RIL populations (Sierra/AC1028; Sierra/LEF-2RB). Four markers in one population and five in a second population of RIL were reported as important for drought tolerance. In a simulation of the application of MAS for drought tolerance QTLs, the authors found that MAS would have been effective in one population in Mexico, and in the other population in Michigan. We have not seen evidence of these markers being used or validated (Schneider et al. 1997b)

Beebe et al. (2006) also attempted to identify QTL for drought tolerance using the SEA 5/MD 23-24 RIL population. Results showed that one QTL was common in two drought seasons, and another QTL was specific to each of two seasons, and some were specific to non-stressed environments. Perhaps the more outstanding finding was that seed yield under drought and seed

yield under non-stress conditions are not mutually exclusive and can be combined. In fact, cultivars that are high-yielding under non-stress conditions have shown, despite a large seed yield reduction, higher than average seed yields under terminal drought-stressed conditions (Acosta-Díaz et al., 2004).

The goal of a better understanding of the genetics and the physiological mechanisms controlling drought is still a target for the dry bean community. Given the importance of plant breeding and genetics to understand the mechanisms of drought tolerance, this chapter aims to improve the understanding of the genetic responses to drought.

Materials and Methods

Genetic materials

A recombinant inbred line population was developed from a cross between 'Buster', a drought-susceptible pinto cultivar, and SER 22, a drought-tolerant small red germplasm line from CIAT, to identify drought tolerance QTL. The commercial cultivar 'Buster' belongs to the race Durango, and SER 22 belongs to the race Mesoamerica, both in the Middle American gene pool (Singh, 1991). Since SER 22 is the result of a wide cross, individual plant selection was made at the Univ. of Nebraska-Lincoln, Panhandle Res. & Ext. Center, Scottsbluff, NE, for the selection of a pure SER 22 selection to perform initial crosses. After the cross was completed in 2008, the single seed descent method was used to advance the lines from F_2 to F_5 (Table 2.1). This population is composed of 335 $F_{5:9}$ RILs that showed high levels of phenotypic variation that would facilitate mapping and phenotypic evaluation in the field.

Both parents differ greatly in phenological, morphological, and agronomical traits, such as plant structure, seed yield, and yield components under drought environments (Figure 2.1).

‘Buster’ is the result of a cross of (M5/M6) Aztec, where: M5 consists of a complex series of crosses including ‘Fiesta’, ‘Olathe’, ‘Aztec’, and JM126. M6 consists of BelNeb RR-1/Aztec (PVP No. 9900242; Seminis Vegetables Seeds, Inc. Filler, ID). According to its Plant Variety Protection Certificate, ‘Buster’ is unique and desirable as an upright pinto variety (Type IIIa) with good grain characteristics, seed size of 36.5 g 100^{-1}, an average of 94 days to maturity, resistance to rust (*Uromyces appendiculatus)*, bean common mosaic virus (member of the potyvirus group), and anthracnose (*Colletotrichum lindem uthianum*) Race 7, with high seed yield of 2,837 kg ha^{-1}, and good adaptation to North Dakota, but susceptible to anthracnose Race 73 (Osorno et al., 2010) and to drought (Singh, 2007).

On the other hand, SER 22 is a germplasm line released by CIAT; since there is not a publication from CIAT with the pedigree, Urrea et al., 2009 was used as reference. SER 22 is the result of a very wide cross, and its pedigree is as follows, SEA22//TLP 35/G21212//EAP 9504-30-B. SER 22 yields 3,347 kg ha^{-1} under non-stress conditions and 1,325 kg ha^{-1} under drought stress conditions for a yield reduction of 47% with a growth habit Type II. Under non-stress conditions, it tends to mature at an average of 82 days with a seed weight of 24.0 g 100^{-1}, while under stress conditions will mature at 77 days with a seed weight of 20.2g 100^{-1} under Mitchell, NE conditions (Urrea et al., 2009). SER 22 under the Juana Diaz, PR conditions seed yield reports been 859 and 536 kg ha^{-1} in reduced and drought stress, respectively (Porch et al., 2009) with yield reductions of 0.37, and stress tolerance index of 0.39.

Figure 2.1. Seed size sample of parental lines SER 22 (upper side) and 'Buster' (lower side).

Table 2.1. A summary of 'Buster'/SER 22 RIL population development at University of Nebraska, Scottsbluff Research and Extension Center.

Description	Generation	Selection	Year	Location	
Hybridization			2008	NE	Greenhouse
Advance	F_1	Bulked	2008	NE	Greenhouse
Advance	F_2	SPS[†]	2008	PR	Field
Advance	$F_{3:2}$	Bulked	2009	NE	Greenhouse
Advance	$F_{4:2}$	Bulked	2009	PR	Field
SPS[†];DNA extraction	F_5	SPS-DNA/Bulked	2010	PR	Field
Advance	$F_{6:5}$	Bulked	2010	NE	Field
Advance		Bulked	2011	PR	Field
Advance	$F_{7:5}$	Bulked	2011	NE	Field
Advance	$F_{8:5}$	Bulked	2012	PR	Field
Advance	$F_{9:5}$	Bulked	2012	NE	Field
Advance Subset			2013	PR	Field
Advance Subset			2013	NE	Field

[†]SPS: Single plant selection

Environments and locations

The RIL population, along with 'Buster', SER 22, and with commercial cultivars 'Beryl-R', 'Buckskin', 'Matterhorn', 'Morales', 'Raven', 'Verano', and 'UI 114' were used as checks

and evaluated at two locations: Univ. of Nebraska-Lincoln, Panhandle Res. & Ext. Center, Mitchell, NE, and the Experimental Station of the University of Puerto Rico in Juana Diaz. These locations varied in their moisture gradient, soils, average temperature, relativity humidity, and rainfall, among others. Both locations share drought and heat stress conferring broad adaptation and allowing screening for drought tolerance in multiple environments and seasons. The western half of Nebraska (USA), where Mitchell (41°56'.6''N, 103°41'.9''W, 1240 m elevation) is located, is characterized by a semi-arid temperate climate. The maximum average temperature during growing season can be up to 29.2 °C and the minimum 15.9 °C. The average precipitation is 174.5 mm. The soil at the Mitchell site is a silt loam (Typic Ustorthent) (Urrrea et al., 2009).

Puerto Rico is 18°N of the Equator, rather small in area (9,104 sq km), and far from any large land masses. It has, as may be expected, a tropical, uniform, and oceanic climate. Puerto Rico does not have definite seasons based on temperatures, as the contiguous states do, but based on climatic data a rainy season can be distinguished from May to November, and a dry season the other six months (NOAA, 2012). Juana Diaz (18°01'.81''N, 66°31'.713''W, 23 m elevation) is on the southern coast of the main island, where the annual average temperature is 26.2°C. The warmest month, on average, is July, with an average temperature of 27.7°C, and the coolest month is February, with an average temperature of 24.6°C. The average precipitation during the growing season is 99.06 mm. Soil at the Juana Díaz site is a Mollisol, with a mixture of clay loam, slightly acidic with high fertility (USDA-NRCS, 2013).

Both locations represent agricultural production regions where irrigation is a requirement. Mitchell, NE is a bean production area while Juana Diaz, PR is an agricultural zone where most seed companies have their nurseries but do not represent itself a dry bean production, is more a

horticultural (vegetables) irrigated production zone. However, drought stress can be a constraint during reproductive development of the plants. At Mitchell, NE drought can be very severe (DII: 0.69 to 0.80) while in Juana Diaz, PR tends to be less severe (Urrea et al, 2009; Porch et al., 2009).

Experimental design

Field trials for the 'Buster'/SER 22 RIL population were evaluated between 2011 and 2013 at Mitchell, NE, and Juana Diaz, PR using adjacent non-stressed (NS) and drought-stressed plots (DS) with furrow irrigation in Nebraska (Terán and Singh, 2002; Muñoz-Perea et al., 2006) and with drip irrigation in Puerto Rico for a total of 12 environments (two locations, three years, two stress levels) (Table 2.2). A rectangular lattice design 15 x 23, with two replications within each environment, was used. Within each sub-block, the selected genotypes were assigned to experimental units. Each plot consisted of one 4.6 m row spaced 0.6 m apart in Nebraska and 3m row spaced 0.6 m apart in Puerto Rico. Both the DS and NS plots were irrigated until flowering to ensure good plant establishment and normal vegetative growth. After flowering, water was only applied to NS plots. Standard agronomic practices were followed to ensure adequate crop growth and development.

Weather parameters such as daily rainfall, minimum and maximum temperature, and relative humidity were recorded with an automated weather station. Also, soil moisture probes were placed in the parental lines and commercial checks. Soil water content was measured to a depth of 0.23, 0.46, and 0.76 m.

Table 2.2. Environments, growing conditions, and locations used from 2011 to 2013.

Env. No.	Environment code†	Growing condition	Location	Year
1	NEDS11	Non-irrigated	Mitchell, NE	2011
2	NENS11	Irrigated	Mitchell, NE	2011
	NEC11			
3	PRDS12	Non-irrigated	Juana Díaz, PR	2012
4	PRNS12	Irrigated	Juana Díaz, PR	2012
	PRC12			
5	NEDS12	Non-irrigated	Mitchell, NE	2012
6	NENS12	Irrigated	Mitchell, NE	2012
	NEC12			
7	PRDS13	Non-irrigated	Juana Díaz, PR	2013
8	PRNS13	Irrigated	Juana Díaz, PR	2013
	PRC13			
9	NEDS1112	Non-irrigated	Mitchell, NE	2011 and 2012
10	NENS1112	Irrigated	Mitchell, NE	2011 and 2012
	NEC1112			
11	PRDS1213	Non-irrigated	Juana Díaz, PR	2012 and 2013
12	PRNS1213	Irrigated	Juana Díaz, PR	2012 and 2013
	PRC1213			
	NEPRDS111213	Non-irrigated	Mitchell, NE and Juana Díaz, PR	2011 to 2013
	NEPRNS111213	Irrigated	Mitchell, NE and Juana Díaz, PR	2011 to2013
	NEPRC111213			

†Environment code: NEDS11: Nebraska, Drought stress, Season 2011; NENS11: Nebraska, Non-stress, Season 2011; NEC11; Nebraska, Combined treatments, Season 2011; PRDS12: Puerto Rico, Drought stress, Season 2012; PRNS12: Puerto Rico, Non-stress, Season 2012; PRC12: Puerto Rico, Combined treatments, Season 2012; NEDS12: Nebraska, Drought stress, Season 2012; NENS12; Nebraska, Non-stress, Season 2012; NEC12; Nebraska, Combined treatments, Season 2012; PRDS13: Puerto Rico; Drought stress, Season 2013; PRNS13:Puerto Rico, Non-stress, Season 2013; PRC13: Puerto Rico, Combined treatments, Season 2013; NEDS1112: Nebraska, Drought stress; Seasons 2011 and 2012; NENS1112: Nebraska, Non-stress; Seasons 2011 and 2012; NEC1112: Nebraska, Combined treatments, Seasons 2011 and 2012; PRDS1213: Puerto Rico, Drought stress, Seasons 2012 and 2013; PRNS1213: Puerto Rico, Non-stress, Seasons 2012 and 2013; PRC1213: Puerto Rico, Combined treatments, Seasons 2012 and 2013; NEPRDS111213: Nebraska and Puerto Rico; Drought stress; Seasons 2011, 2012 and 2013; NEPRNS111213: Nebraska and Puerto Rico; Non-stress; Seasons 2011, 2012 and 2013; NEPRC111213: Nebraska and Puerto Rico; Combined treatments; Seasons 2011, 2012 and 2013.

Phenotyping in drought stressed and non-stressed environments

To evaluate plant response to drought, physiological, and agronomic data were collected under both DS and NS environments as described by Rao et al. (2008). At the flowering stage,

leaf temperature (^{0}C) data were recorded on a fully expanded young leaf with no foliar damage from 10:00 AM to 3:00 PM for an average of six days using a FLUKE Precision Infrared Thermometer Model 572. Two readings were collected in two different plants within each plot in NEDS11, NENS11, PRDS12, and PRNS12 environments. Also, stomatal conductance was recorded only in the commercial checks and parental lines at flowering stage, using the same criteria to collect the data for leaf temperature. Leaf temperature data was collected in the NEDS11 and NENS11 environments using a portable SC-1 Leaf Porometer (Decagon Devices Inc.). Five readings were recorded per plot.

Likewise, the following agronomic traits were evaluated: days to flower (d), and days to maturity (d) seed yield (kg ha^{-1}), 100-seed weight (g) in all environments. Days to flower (DTF) were taken when approximately 50% of the plants in a plot had at least one open flower, and days to maturity (DTM) was recorded as the number of days after sowing when 95% of the plants in a plot had all pods dry.

At harvest time, seed yield and seed yield components were measured on two plants randomly selected within each experimental plot. Plants were collected in a paper bag and transported to a shelter where the partitioning of stems and pods was done. Dry weight of stem biomass (g), pod biomass (g), number of pods per plant, number of seeds per pods, dry weight of wall biomass (g), and dry weight of pod biomass (g) were collected on NEDS11, NENS11, PRDS12, and PRNS12 environments. Remaining plants in the field plot were hand pulled, bulked, dried, weighted for biomass, and threshed with an experimental plot thresher (e.i. Almaco SPC 20). Seed yield and 100-seed weight data was collected.

Parameters estimated

To quantify drought severity, drought intensity index (DII) was calculated (Fischer and Maurer, 1978; Ramírez-Vallejo and Kelly, 1998), which is an estimation based on the mean seed yield across genotypes (RIL) under different conditions. The equation for drought severity index is

$$\text{DII} = 1 - \frac{X_d}{X_i}$$

where X_d is the mean yield averaged across RIL under drought stress (DS), and X_i is the mean seed yield averaged across RIL under non-stress (NS). The higher the value of DII the greater the stress. Values for DII range from 0 to 1, where values above 0.7 are indicative of severe or terminal stress (Ramírez-Vallejo and Kelly, 2008), values between 0.3 and 0.6 are considered moderate stress (Urrea et al., 2009), and values under 0.3 indicate minor stress.

Because of the difficulty of selecting for both, improved performance under drought stress and high seed yield potential under NS conditions, it is advisable to utilize multiple indices when making selections (Schneider et al., 1997a; Abebe et al., 1998; Urrea et al., 2009). Therefore, the drought susceptibility index (DSI), geometric mean (GM), and percentage of seed yield reduction (PR) can be used as additional indices to evaluate the response to drought by genotypes. Drought susceptibility index is an estimation that helps to determine and select drought-tolerant germplasm for further evaluation and inclusion in breeding programs (Ramírez-Vallejo and Kelly, 1998; Porch et al., 2009; McClean et al., 2011). The drought susceptibility index is based on the change in seed yield under NS and DS environments for a genotype:

$$\text{DSI} = \left(1 - \frac{Y_d}{Y_i}\right) \div DII$$

where Y_d is mean seed yield of the same genotype under DS, and Y_i is mean seed yield for the same genotype under NS (Fischer and Maurer, 1978). A small difference (low DSI value) suggests greater drought tolerance.

However, DSI does not differentiate between genotypes that perform well under both environments (e.g., tolerant to drought stress and high yielding under non-stress) and those that perform poorly under both environments (e.g., poor adaptation) (Schneider et al., 1997a; White and Singh, 1991; Clarke et al., 1992; Abebe et al., 1998; Urrea et al., 2009). Furthermore, Rosielle and Hamblin (1981) suggest that selections based on DSI alone will lead to reduced productivity. Therefore, percentage of yield reduction is the estimation of the actual seed yield reductions from limited water supply, as well as loss from other factors affecting crop production, such as nutrient deficiencies or imbalances, poor soil quality, root and/or shoot diseases, insect pests, weed competition, water logging, and lodging, among others. The percentage of seed yield reduction for this study was calculated as:

$$\text{PYR} = \left[\frac{\text{mean seed yield of a genotype under NS} - \text{mean seed yield of the same genotype under DS}}{\text{mean seed yield of the genotype under NS}}\right] \times 100.$$

Higher values for percent of yield reduction suggest that the RIL does not have good stability across different treatments, while low values indicate better stability.

The geometric mean is an index based on the performance under both DS and NS conditions:

$$\text{GM} = \sqrt{Y_s \times Y_i}$$

where Y_s is the mean seed yield of a genotype under DS conditions and Y_i is the mean seed yield of a genotype under NS conditions (Schneider et al., 1997a; Urrea et al., 2009; Porch et al., 2009). All tests were considered significant at $p < 0.05$.

Statistical analysis

Data was analyzed using the MIXED procedure of SAS v9.3 (SAS Institute, Inc., 2011), using a model where replications within block and sub-blocks within treatments were considered as random effects, and growing conditions (water treatment) and genotypes were treated as fixed effects. Due to natural precipitation in both treatments (NS and DS), soil water regime experienced by plants varied among years. Therefore, each year was analyzed separately. Environments were considered homogeneous when the ratio of the effective error variances for each trait was less than tenfold (Barlett, 1947). If the environments were homogeneous, the data was combined across years. For this study, all data was combined across years, allowing for a significant reduction of the variation due to the environmental effects and its interactions with the genotypes. In the combined analyses, environment, block, and replication within environments were random effects and drought stress level and genotype were fixed effects.

Adjusted means were separated using the F-protected Least Significant Difference at $p \leq 0.05$ level of significance for each evaluated trait. Simple phenotypic correlation coefficients (r) between the adjusted means were used in order to verify if a pair of traits were associated. Only significant interactions were discussed ($r > 0.5$ were considered strong, $r \leq 0.49$ were considered weak).

DNA isolation and genotyping

The entire RIL population, as well as the parents 'Buster' and SER 22, was sown in the winter/spring 2013 greenhouse season in North Dakota to obtain leaf tissue samples for DNA extraction. Two seeds per genotype were sown in 11.43-cm-tall Traditional Square pots (Hummert ™ International, MO) filled with a potting media of Sunshine Soil Mix #1 (Sun Gro Horticulture, Canada). The first trifoliolate leaves were collected from four individual plants per

genotype, bulked, and then frozen in liquid nitrogen. A modified cetyltrimethylammonium bromide (CTAB) method was used to perform the DNA extraction as described by Rogers and Bendich (1985). For each genotype, 50 µl of DNA (100 ng/µl) was sent to the USDA-ARS Soybean Genomics and Improvement Lab Facilities at Beltsville, MD (http://www.ars.usda.gov) for Single Nucleotide Polymorphism (SNP) analysis utilizing a customized 6,000-Infinium BeadChip panel (Hynten et al., 2010) and the Infinium genotyping assay protocol developed by Illumina, Inc. (San Diego, CA).

A cutoff threshold 'GenCall' of 0.25 was used for the clustering and normalization of the data through the implementation of the software GenomeStudio™ v1.0 (Illumina, Inc., San Diego, CA). Heterozygous genotypes were rare (0.008%), as expected from the process of production of an $F_{5:9}$ recombinant inbred lines population. This line (RIL 234) was discarded from the analysis.

Linkage analysis and mapping

Segregation ratios of individual markers were assessed statistically at an individual marker locus for deviations from the expected Mendelian ratio (1:1) by an X^2-test. If the marker deviated from the expected 1:1 ratio at a *p* value of 0.001 then the marker was removed from further analysis.

Linkage analysis was performed using the software CarthaGène v1.3.beta (de Givry et al., 2005, www.inra.fr/mia/T/CarthaGene) on genotypic data previously generated with the use of a 6,000-Infinium iSelect BeadChip. To generate the linkage map, the following steps were performed: first, the *mrkmerges* command was used to merge all pairs of co-segregating markers or those that have no recombination/breakage. Second, the *group* command was used with a minimum LOD threshold of 3.0 and recombination frequency of 30 to try to group the markers

into linkage groups. Next, the *mrkselset [groupget x]* command was used to automatically select the markers of the group desired to work with, and at the same time a default markers order was determined. The *Buildfw* command was used to build a good framework map, where weakly ordered markers were not included in stepwise marker insertion method. *Keep and adding threshold* were set to a minimal LOD threshold of 3.0. Once the map was built, determination of whether or not this was a good map was done by a "verification algorithm" with the *FLIPS* option. Then the option *POLISH* was used to check for the reliability of the map by swapping pairs of markers.

QTL analysis

QTL analysis for each trait was performed using the software WinQTLCartographer v2.5_011 (Wang et al., 2012, http://statgen.ncsu.edu/qtlcart/WQTLCart.htm) and the phenotypic adjusted means were used in each location-year-growing condition. The population distribution of all traits were plotted and tested for normality using the Kolmogorov-Smirnov test. Data were considered normally distributed if $p \geq 0.05$. Single Marker Analysis (SMA) was initially done to identify markers associated with drought tolerance. Markers were considered significantly associated at a $p \leq 0.05$. Next, Composite Interval Mapping (CIM) was done to identify QTL regions more accurately. The parameters used included a window size of 10 cM, 5 background markers, 1 cM walk speed, and a Backward and Forward regression model was used for the identification of QTL with the default parameters for cofactor selection suggested in Silva et al. (2012). Significant QTL for each trait were determined by the location of the peak LOD score at the empirical threshold of $p \geq 0.05$ after 1,000 permutations (Churchill and Doerge, 1994). Linkage maps and QTL were displayed using Mapchart v2.2 (Voorrips, 2002), and map

locations for the identified QTL were estimated based on the more recently published consensus map by Schmutz et al. (2013).

QTL putative gene annotation

To do the QTL annotation, the Pv annotation data (Schmutz et al., 2013; unpublished) was used. First, estimation of how many genes per QTL were estimated. The physical positions (Mb) of the flanking markers for each QTL were used as the range. Then genes close to the QTL peak position (cM) were selected. Because of the extent of the information generated in this process the results obtained can be found as a supplemental tables (S1 to S9).

The National Center for Biotechnology Information (NCBI) web page (http://www.ncbi.nlm.nih.gov) was used to search for gene function information such as protein process, and metabolic process, among others. The intent of this exercise was to have an idea of what metabolic processes could be related to those genomic regions.

Results and Discussion

Phenotypic data

The seed yield across environments for the recombinant inbred lines ranged from 108.73 to 1,838.29 kg ha^{-1} under drought stress conditions and from 1,264 to 3,970 kg ha^{-1} under non-stress conditions for the combined analysis. Average seed yield for Buster across environments was 2,010 and 4,371 kg ha^{-1}, and for SER 22 2, 814 and 3,396 kg ha^{-1} under DS and NS, respectively. Average seed yield were higher in Mitchell, NE than in Juana Diaz, PR (data not shown). Transgressive segregation for seed yield was therefore observed (Figure 2.2). Some were better or worse in yield than the drought-tolerant parent SER 22 or the drought-susceptible parent Buster. Similarly, transgressive segregation was evident for seed size and as well as for the phenological traits. SER 22 had higher yield than Buster under drought. However, under

irrigated conditions Buster had a high yield than SER 22 (Table 2.3). Also, typical heat-drought stress symptoms such as curly pods and pod abortion were observed among the RILs and parental lines. Differences were significant ($P < 0.05$) between the parents in terms of seed weight and also significant for seed yield. The effect of drought caused a 1–10 g weight significant decrease per seed weight when comparing irrigated and drought treatments. SER 22 was earlier maturing than Buster by 2–5 days under both treatments across all environments. Meanwhile, under irrigation both parental genotypes matured at the same time in some environments, but overall SER tends to mature earlier. In terms of correlations, the phenological traits do not tend to be correlated among each other when compared to correlations between yield and seed weight traits. Meanwhile, seed weight was positively correlated with days to maturity in the drought treatment and yield was negatively correlated with days to maturity in the irrigated treatment only. Correlations were highest for the comparisons of the same trait across drought and irrigated treatments and were especially significant ($P < 0.001$) for seed weight and days to flowering across treatments in all environments and for days to maturity across treatments.

Table 2.3. Means for traits evaluated cross 12 environments.

Trt†	Parental lines		Recombinant inbred lines			$LSD_{0.05}$	Checks						
	Buster	SER 22	Mean	Min	Max		Beryl	Matterhorn	Morales	UI 114	Bucksin	Raven	Verano
						Days to flowering							
						d							
DS	47	45	47	41	48	3	46	46	50	46	46	50	47
NS	48	41	46	41	48	4	47	48	48	48	41	48	49
						Days to maturity							
						d							
DS	88	79	79	60	95	6	80	85	86	80	80	91	62
NS	90	84	85	70	90	7	83	86	90	85	84	95	65
						Seed yield							
						kg ha $^{-1}$							
DS	2010	2814	1956	60	4564	923	1719	1308	1021	1750	1661	674	624
NS	4371	3396	4107	260	7534	1025	4236	7043	3210	5440	2914	1606	1542
						Seed weight							
						g 100^{-1}							
DS	34.8	25.3	29.4	12.3	46.7	3.2	26.9	31.7	19.4	26.9	26.9	17.1	21.2
NS	43.2	25.4	31.8	16.2	49.6	4.5	30.5	32.4	20.4	40.2	39.3	24.1	23.5
						Leaf Temperature							
						^{0}F							
DS	82.86	82.62	83.45	83.85	84.00	0.78	84.37	84.91	83.10	83.45	82.09	82.87	81.11
NS	81.38	81.93	81.59	83.00	84.00	1.03	84.28	82.30	82.12	81.62	82.72	82.30	82.12

†Trt: treatment, DS: Drought Stress and NS: Non-stress.

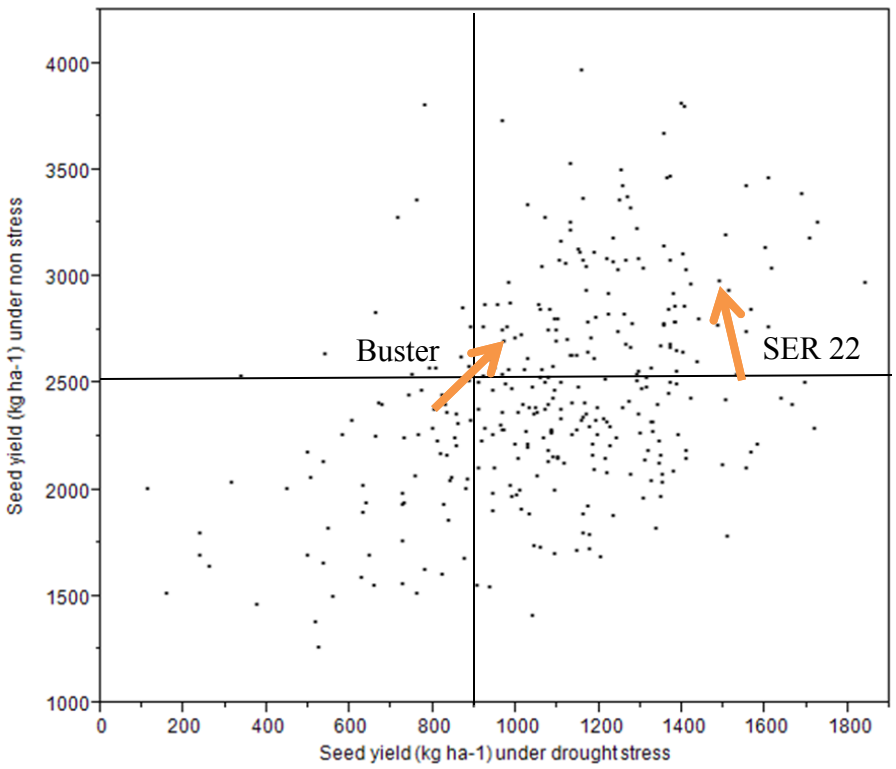

Figure 2.2. Mean seed yield (kg ha^{-1}) of 367 genotypes for combined analysis across 12 environments under drought stress (x-axis) and non-stress (y-axis). Parental lines are identified by an arrow.

Drought parameters

Nebraska 2011 showed the most severe stress across all environments. Higher values (DII=0.8) for Mitchell, NE have been reported by Urrea et al. (2009). However, drought intensity in Juana Diaz was moderate when compared to other evaluations performed in the same location where intermittent (value not published) drought has been reported (Porch et al., 2009) (Table 2.4). The same trend is observed for percent yield reduction and drought susceptibility index. The geometric mean indicates how the RILs performed under both growing conditions. NE2012 was the environment with the highest geometric mean, while PR2013 was lowest. However, PR2013 data have considerable missing values in the irrigated trial, for that reason the values in Table 2.4 are estimates and are shown in bold.

Table 2.4. Drought intensity index (DII), percent yield reduction (PYR in %), drought susceptibility index (DSI), and geometric mean (GM) due to drought stress calculated within each within environment.

Environment	DII	PYR	DSI	GM (kg ha^{-1})
NE 2011	0.67	76	1.45	1365
PR 2012	0.42	45	0.85	844
NE2012	0.53	35	0.68	2505
PR 2013	**0.57**	**51**	**0.62**	**722**
Average	0.54	52	0.9	1359

Among genotypes in the combined analysis, the top ten are reported herein for the main indices calculated. Detail information for all genotypes can be found in the Supplemental Table 10. RIL 28 showed the highest yield under DS, followed by 119, 137, 305, 44, 158, SER22, 57, 230, and 205. For yield reduction genotypes 104, 137, 45, 10, 258, 29, 123, 222, 161 and 57 had the lowest yield reduction. Those with the highest geometric mean were RILs 158, 119, 227, 28, 305, 149, 324, 170, 298, and 195.

Linkage map analysis

Of the 5,398 SNP markers, 3,635 markers (67%) were monomorphic. In other words, relatively few of the markers screened across the parental lines were polymorphic, and based on X^2 *p* value*,* 876 SNP markers were removed. Similar results using three different populations were found and reported by McConnell et al. (2010). A total of 887 SNP loci were included in the linkage analysis. The linkage map comprised a total of 378 SNP markers (at a LOD of 3.0) for a total map distance of 778.4 cM divided among twelve linkage groups (Table 2.5; Figure 2.4 and Figure 2.5) representing 47% of the estimated genome size of the 'Redhawk'/'Stampede' (1665.5 cM) map based on 7,000 SNP markers (Schmutz et al., 2013; unpublished). The number of markers per linkage group varied from 5 to 99 (Table 2.5), and the average distance between marker was 2.06 cM. The largest interval was 23.5cM, located on Pv7. Based on Schmutz et al. (2013), the 11 bean chromosomes are represented herein, with two linkage groups representing

chromosome Pv8 (Pv8.1 and Pv8.2). The relative small size of the map indicates a lack of genome coverage, especially on Pv9, and Pv10 (Table 2.4; Figure 2.3, Figure 2.4, and Figure 2.5). The consensus map used for this study was developed using the 'Redhawk'/'Stampede' population (n=245, F_2 generation) and do not present gaps larger than 30 cM with low recombination stretches (Cregan, 2011; McClean, 2011). Therefore, a lack of coverage in this map can be due to the high level of monomorphic markers, resulting in a map with few numbers of markers on Pv9, and Pv10.

However, Khanal et al. (2013) mapped less (63) SNP markers and got a map with a length of 1,056.14 cM using a F_2 population of 'Othello'/'Redhawk' (Mesoamerican x Andean cross) to perform evaluations for folate content, while Hagerty (2013) mapped 1,689 SNP markers, obtaining a map length of 1,196 cM using the 10K Infinium iSelect Beadchip for the evaluation of root traits and root rot disease resistance on a RIL population of two Mesoamerican parental lines (OSU5446 and RR6950). Unfortunately, the origin of OSU5446, which was derived from a cross between Smilo and ORG91G, is uncertain. According to Hagerty (2013), phylogenetic studies (unpublished) OSU5446 may contain a mixture of Mesoamerican and Andean derived genes. Assuming OSU5446 has indicate introgressions from the Andean genepool, both of the previous studies have shown a better coverage of the consensus map, which is the same one used for the 'Buster'/SER 22 RIL linkage map.

Table 2.5. A summary of SNP markers integrated into the 'Buster'/SER 22 population genetic map and average distance of markers within each linkage group.

Chromosome	Linkage group	Number of markers	Kosambi	Avg distance (cM)
1	1	30	104.9	3.50
2	2	99	88.1	0.89
3	3	35	124.2	3.55
4	4	17	20.3	1.19
5	5	34	89.9	2.64
6	6	24	44.7	1.86
7	7	35	106.9	3.05
8	8.1	9	6.2	0.69
8	8.2	39	76.9	1.97
9	9	5	14.6	2.92
10	10	7	35.9	5.13
11	11	44	65.8	1.50
Combined		378	778.4	2.06

Segregation distortion

Segregation distortion was observed in this population on a group of 61 SNP markers ($X^2_{0.05}$ = 7%) clustered mainly in Pv2, and Pv7 of the consensus map (Table A.11). When considering an $X^2_{0.01}$, only 0.11% (one marker) were distorted among the SNP markers included in the linkage map (Table S.11). The segregation distortion for the 61 SNP markers was toward the maternal allele (AA= 'Buster'). Due to co-segregation, 55 out of 61 SNP markers are not included in the linkage map (Table A.11, Figures 2.4 and 2.5). The six distorted markers, represented in bold in Table A.11, are the only ones that are included in the linkage map (Figure 2.4 and 2.5).

This phenomenon of segregation distortion occurs as the result of gametic selection, zygotic selection, or both (Xu, 2008) and has been reported in dry beans previously (Vallejos et al., 1992; Jung et al.,1996; Jung et al.,1997; Beattie et al., 2003). QTL analysis performed on mapping populations derived from the crosses XR-235-1-1/'Calima', WO3391/ 'OAC Speedvale', BAC6/HT7719, PC-50/XAN159, among others, had reports of 8, 12, 24, and 31% of

distortion segregation, respectively (Vallejos et al., 1992; Beattie et al., 2003; Jung et al.,1996; Jung et al.,1997). Among them, the PC-50/XAN159 RIL population had the highest number of distorted markers (Jung et al., 1997). The level of segregation distortion reported herein ($X^2_{0.05}$ = 7%) is similar to the lower proportions (8% and 9%) of RFLP markers with segregation distortion using an F_2 population reported by Vallejos et al. (1992), and Nodari et al. (1993), respectively. On the other hand, reports of segregation distortion using RAPD markers on F_5 and F_6 RIL populations were relatively high (12, 24, and 31%) when compared to SNP and RFLP markers (Beattie et al., 2003; Jung et al.,1996; Jung et al.,1997; respectively). Previously, the effect of distorted markers in QTL analysis was unknown, and for that reason markers were discarded as a preventive solution. However, recent studies have shown that distorted markers can be safely used for the purpose of QTL mapping with low or no detrimental effect on the QTL detection power (Xu, 2008; Zhang et al., 2010).

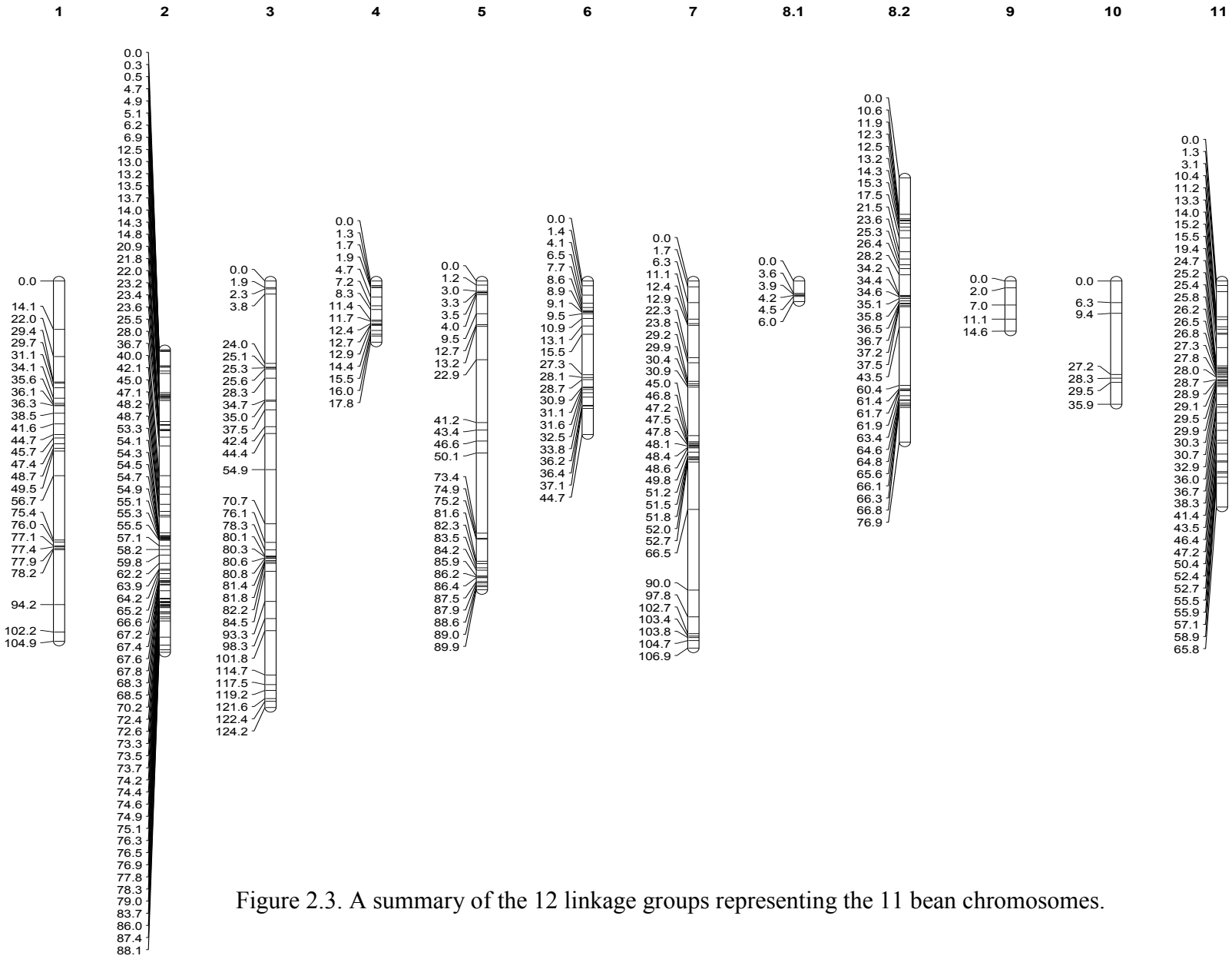

Figure 2.3. A summary of the 12 linkage groups representing the 11 bean chromosomes.

QTL analysis

Composite interval mapping (CIM) identified a total of 12 QTL for agronomic traits, including seed yield, and ten for physiological traits on seven chromosomes under drought or non-stress conditions (Table 2.6). Specifically, three QTL for seed yield, six for 100-seed weight, one for days to flowering, two for days to maturity, and ten for leaf temperature were detected. The R^2 values were used to determinate the total phenotypic variance explained by each QTL. In this study, the total phenotypic variance ranged from 3% (seed yield, 100-seed weight, and days to flower) to 10% (leaf temperature) for a given trait in a specific environment and explained by single loci. Individual environments varied in regards to the total number of QTL and the traits for which those loci were detected (Table 2.6). The total phenotypic variance explained for a trait by all QTL detected in the same environment ranged from 3% (PRNS12) to 25% (NENS11). The R^2 values did not differ in magnitude when taking into consideration growing conditions. Based strictly on R^2 values, the most important QTL was for leaf temperature with up to 10% of phenotypic variation explained by a single locus (Table 2.6).

Several of the QTL represent similar genetic regions associated with the same trait across environments, while others represent a genetic region that was only associated with a trait in one or two environments. For example, overlapping QTLs were found on Pv8.1 for days to flower and seed weight (Table 2.6). Positive allelic contributions for QTL detection came from each parent, indicating that both parents contributed to a trait either under drought or non-stress conditions. For seed yield under drought stress for example, all allelic contributions came from SER 22, but for seed weight it came from both parents.

Seed yield

Three significant QTL for seed yield were found in linkage groups Pv3 and Pv7 (2 and 1, respectively) in three environments (Table 2.6). Two of them, SYNEDS11 and SYNENS11, were detected under drought stress, and non-stress conditions, respectively. SYNEC11 was detected using the data combined from both growing conditions, indicating a QTL for seed yield (Table 2.4). The R^2 values for seed yield QTL ranged from 0.03 to 0.09, and additive effects varied from 23 to 112 kg ha^{-1}. Markers sc00034ln878273_431537 (51.86) and sc00034ln878273_572052 (51.72) flanked the SYNEDS11 QTL on chromosome Pv3 (Figures 2.5 and. 2.6), and it spanned 2.2 cM. SYNEDS11 had the largest effect on seed yield under DS conditions, accounting for up to 9% of seed yield variation, and was detected only in NEDS11 environment with an allele contribution of SER 22 that accounts for up to 23 kg ha^{-1}. QTL for seed yield have been previously reported in dry beans on chromosome Pv3 (Schneider et al., 1997b; Beattie et al., 2003; Wright and Kelly, 2011; Blair et al., 2012). Particularly, Schneider et al. (1997b) reported two QTL for seed yield, one under drought conditions and the other one under non-stress. Blair et al. (2012) reported up to four QTL on Pv3 for seed yield under irrigated conditions, contrasting the results presented herein, where the QTL on Pv3 was detected under drought-stress conditions.

From a breeding standpoint, SYNEDS11QTL may not contribute with a high phenotypic variation to be considered a major QTL, but this genomic region may include an important gene cluster for drought improvement based on the *Pv* annotation data (Schmutz et al., 2013; unpublished). The *Arabidopsis thaliana* pTCAC5 (Plastid Transcriptionally Active5) gene is found in the genomic region of the QTL and close to its peak position (3.31 cM). This gene is

essential for chloroplast development in *Arabidopsis* under heat stress by maintaining PEP function (Zhong et al., 2013).

The second QTL on Pv3, SYNEC11, flanked by markers sc00062ln709754_363848 (47.81) and sc00019ln1150109_1018006 (45.59) spanned 9.5 cM, and does not depend on growing conditions since it was detected in the NEC11 environment (Table 2.6). This QTL accounts for up to 5% of the seed yield phenotypic variation (104.16 kg ha^{-1}) with an allele contribution of 'Buster', and its genomic region based on *Pv* annotation data accounts for up to 93 *Arabidopsis* genes (Schmutz et al., 2013; unpublished). From those 93 genes, 47 are near to the peak position of SYNEC11 (39.51cM). One interesting gene in this region is the HCF107 (high chlorophyll fluorescent 107) which provides a link between the intercistronic RNA processing and the accumulation of translation-competent psbH transcripts in chloroplasts (Felder et al., 2001). To understand the functional significance of RNA processing for the expression of plastome-encoded photosynthesis genes, this group investigated the nuclear mutation HCF107 of *Arabidopsis*. Results show that PsbH and PsbB are essential requirements for Photosystem II (PSII) assembly in photosynthetic eukaryotes. Overall, HCF107 thus represents a new member of the growing helical repeat family of proteins that seem to play a gene-specific role in regulating plastidial gene expression and biogenesis (Sane et al., 2005).

On the other hand, the seed yield QTL on chromosome Pv7, SYNENS11, spanned 5.6 cM, and had the lowest effect on seed yield under NS conditions, accounting for up to 3% of the seed yield variation. Markers flanking this QTL can be found in Table 2.5. NENS11 was the only environment where SYNENS11was detected and is associated with 'Buster', the drought-susceptible parental line (Table 2.6; Figure 2. and Figure 2.4). Blair et al. (2012) also found a unique QTL for seed yield under non-stress conditions on Pv7, but in their study, BAT477, the

drought-tolerant parent line, was the one conferring the alleles with a contribution of up to 132.32 kg ha^{-1}. Schneider et al. (1997b) also detected three QTL for seed yield under drought and non-stress conditions on Pv7. Based on annotation, 12 genes can be found close to the SYNENS11QTL peak position (99.81cM). Among them, the most related to plants is the ATVAMP727 (Vesicle-Associated membrane protein) gene, which encodes a member of the Synaptobrevin-like protein family. The SNARE ‘‘**SNA**P (Soluble NSF Attachment Protein) **Re**ceptor’’ complex is a key regulator of vesicular traffic, executing membrane fusion between transport vesicles or organelles and target membranes. A functional SNARE complex consists of four coiled-coil helical bundles, three of which are supplied by Q-SNAREs and another from an R-SNARE. *Arabidopsis thaliana* VAMP727 is an R-SNARE, with homologs only in seed plants. Scientists have found that VAMP727 forms a complex with SYP22, VTI11, and SYP51, and that this complex plays a crucial role in vacuolar transport, seed maturation, and vacuole biogenesis. Based on that, the VAMP727 complex mediates the membrane fusion between the prevacuolar compartment and the vacuole, and because of this action scientists thinks that this process has evolved as an essential step for seed development (Ebine et al., 2008).

100-seed weight

Six QTL were found for 100-seed weight in six environments on four chromosomes (Pv2; Pv8.1; Pv8.2; and Pv11). However, just one QTL was detected in two out of 12 environments (Table 2. 4). R^2 values ranged from 0.03 to 0.06, and additive effects varied from 0.79 g 100^{-1} to 1.30 g 100^{-1}. Three 100-seed weight QTL were associated to a ‘Buster’ allele on Pv2, Pv8.1, and Pv8.2, and the other three QTL were associated to SER 22 on Pv2, Pv8.2, and Pv11. Previous studies reported similar QTL for seed weight on Pv2, Pv6, Pv5, and Pv9 (Blair et al., 2012), as well Pv5, Pv6, Pv8, and Pv11 (Wright and Kelly, 2011), Pv6, B8, and Pv11 (Blair

et al., 2006), and Pv11 (Tar'an et al., 2002). Markers sc00089ln640327_50900 (0.18) and sc00089ln640327_268606 (0.39) flanked the SYNEC11 QTL on chromosome Pv8.1 (Table 2.4; Figure 2.3 and Figure 2.4), and it spanned 4.5 cM. SYNEC11 had the lowest effect on seed weight, accounting for up to 3% of the seed weight variation. Detection of SYNEC11 QTL was only in one environment (NEC11), with an allele contribution of 'Buster' that accounts for up to 1.30 g 100^{-1}. SYNEC11 QTL overlaps the DTFPRNS12 QTL, and its genomic region accounts for up to seven genes (Schmutz et al., 2013; unpublished). Among them, the RPI2 (ribose-5-phosphate isomerase 2) gene in *Arabidopsis* causes chloroplast dysfunction, late flowering, and premature cell death (Howles et al., 2006). The QSOX1 (quiescin-sulfhydryl oxidase 1) gene is involved in the regulation of cation homeostasis. This gene positively regulates shoot accumulation of K^+ and inhibits the accumulation of toxic cations. It also, acts at the level of root K^+ efflux systems involved in xylem loading (root symplast-xylem interface) (Alejandro et al., 2007). The LPAT1 (Phospholipid/glycerol acyltransferase family protein) converts lysophosphatidic acid (LPA) into phosphatidic acid by incorporating acyl moiety at the 2 position. LPAT1 has preference for C-16-CoA substrates compared to C-18-CoA substrates and is essential for embryo development during the transition from when chloroplasts begin to form, it is widely expressed, can be found in higher proportions in leaves and at lower level in the silique walls when compared to leaves (Kim and Huang, 2004). Recently, studies in Lipoxygenase activity in pinto beans show significant differences in three different growing locations in North Dakota (Hatton, Forest River, and Johnstown). Pinto beans from Hatton had the highest activity (285.08 μM) compared to beans from Johnstown (99.30 μM) and Forest River (174 μM). A group from NDSU Cereal Sciences Department thinks that the difference could be due to the more severe drought conditions experienced at Hatton (Simmons et al.,

2013). Lipoxygenase gene expression has been reported to increase in plants exposed to stress, including water deficit (Bell et al., 1991).

Among all seed weight QTL, the most promising one can be found on Pv2. QTL SWPRDS12 and SWPRC12, flanked by sc00160ln486724_338720 (44.64) and sc00160ln486724_301841 (44.60) markers, account for 5% of the total phenotypic variation under stress conditions with an additive effect from the maternal allele of 1.03g 100^{-1} and 0.79g 100^{-1}, respectively (Table 2.4). SWPRDS12 and SWPRC12 were detected at the same peak position (23.61cM) on PRDS12 and PRC12 environments (Table 2.4), and it spanned 7 and 6.2 cM, respectively (Table 2.5). Based on the *Pv* annotation (Schmutz et al., 2013; unpublished), 21 *Arabidopsis* genes are in this region, but just two (TUB7, and ATFAH1) are close to the QTL peak position (23.61 cM). TUB7 (tubulin beta-7 chain) is involved in different biological process such as the cytoskeleton organization, regulation of the meristem growth, response to cadmium ion, and to salt stress (Yamada et al., 2003). Meanwhile, AtFH1 (formin-like protein 1) might be involved in the organization and polarity of the actin cytoskeleton, and is also involved in the pollen cell growth process by maintaining tip-focused cell membrane expansion for the polar extension of pollen tubes (Cheung et al., 2004).

SWPRC12, flanked by sc00296ln326650_106196 (52.73) and sc00678ln168824_163688 (8.31), was also found in the PRC12 environment, and accounts for 6% of the seed size variation, with SER 22 contributing with 1.18g per sample. SWPRC12 spanned 7.2 cM and this genomic region accounts up to 421 genes; from those, 69 are close to the QTL peak position (59.51cM). For example, two of them, AtEBP and FUM1, are expressed when plants are under stress probably acting as a transcriptional activator and may be involved in the regulation of gene expression by stress factors and by components of stress signal transduction pathways (Buettner

and Singh, 1997). Some of the biological processes where these genes are involved are heat acclimation and cell death. Particularly, FUM1 is expressed in response to salt and oxidative stress.

SWNENS12, flanked by sc00113ln562714_289656 (46.82) and sc00113ln562714_374686 (46.74), spanned 5.5 cM on Pv2 and its peak position is at 12.51 cM. This marker was only detected under non-stress conditions at the NENS12 environment (Table 2.6), and accounts for 4% of the total phenotypic variation for seed weight, with 38 genes in its genomic region (Schmutz et al, 2013). A contribution of 0.93 g 100^{-1} from the SER 22 allele is present in this marker. It is unique that this marker was detected under irrigated conditions, but it is the drought-tolerant parent that is contributing to the seed weight of the RIL. The same thing happens to the SWNEC11, where the SER 22 allele in the NEC12 environment adds up to 0.95 g per sample, and at the same time accounts for the largest (6%) phenotypic variation for seed weight. SWNEC13 spanned 5.2 cM and has 17 genes in its genomic region (Schmutz et al, 2013). Most of the genes in this region are involved in the primary root development, and in the regulation of the anthocyanin metabolic process, the chlorophyll biosynthetic process, and the root meristem and seed growth (Mason et al., 2004).

Overall, more QTL were found for seed weight than for seed yield, and it may be due to heritability that tends to be higher for seed weight under drought stress (Schneider et al., 2007b). For example, QTL for seed yield were only found in the Nebraska 2011growing season (NEDS11, NENS11 and NEC11), while for seed weight the presence of QTL among and within environments was more diverse (Table 2.6). Among all traits, seed weight was also the only trait that had an overlapping QTL for different traits (pleotropic effect), in this case for days to flower (Pv8.1). However, no conclusions can be made about pleotropic effect at this point. To be able to

corroborate this information, more markers will be needed for the saturation of SWNEC11 and DTFPRNS12 region on Pv8.1 (Figure 2.4 and Figure 2.5). Taking into consideration that seed weight is a component of seed yield, it does not seem to be an unrealistic association at the cellular level since seed yield in most crops is controlled by multiple loci.

The fact that more QTL were found for seed weight than for seed yield does not seem to be an uncommon situation. Similar results were obtained by Schneider et al. (1997a, b), and more recently by Blair et al. (2012). If the inheritance of seed weight is taken into consideration, as shown in the 'Buster'/SER 22population, seed weight tends to be a highly heritable trait regardless of the growing conditions when compared to seed yield, which may have a direct impact on the detection of QTL. An interesting fact in this study is the allele contribution of SER 22, the drought-tolerant parent, and from 'Buster', the drought-susceptible parent, to the detection of the QTL, and the substantial transgressive segregation for seed weight indicating that seed filling may be one mechanisms by which SER 22 contributes to yield performance in the RIL population.

Days to flowering

Two QTL were detected for days to flower on chromosome Pv6 (DTFNEC11) and Pv8.1 (DTFPRNS12) (Table 2.6). The R^2 values for days to flower QTL ranged from 0.03 to 0.05, and additive effects varied from 0.32 to 0.6 days. Previous studies reported QTL for days to flower on Pv6 (Blair et al., 2012; Wright and Kelly, 2011), but not for Pv8. Markers sc00058ln727328_87916 (24.48) and sc00355ln290049_84131 (21.92) flanked the DTFNEC11 QTL on Pv6, with a peak position at 21.51cM that accounts for up to 91 genes based on the *Pv* annotation data (Schmutz et al., 2013; unpublished). The YAB2 (Putative axial regulator YABBY 2) gene is close to its peak position and is involved in the abaxial cell fate

determination during embryogenesis and organogenesis (Siegfried et al., 1999). DTFNEC11spaned 11.3 cM and accounts for up to 5% of the total phenotypic variation for days to flower. While markers sc00089ln640327_50900 (0.18) and sc00089ln640327_268606 (0.39) flanked the DTFPRNS12 QTL, it spanned 4.5 cM overlapping the SWNEC11 QTL. For both QTL, the allele contribution was from 'Buster'.

Days to maturity

One QTL, DTMNEPRNS1112, was detected for days to maturity on chromosome Pv2 (Table 2.4), suggesting that may not be environmentally specific since it was detected using the combined adjusted means for non-stress over the years, growing conditions, and locations. However, detection of QTL within locations, growing conditions, and years was null. The R^2 value for days to maturity QTL was 0.04, and additive effects 0.37 days (Table 2.6). Markers sc00411ln257942_127705 (3.28) and sc00240ln364462_178258 (3.57) flanked the DTMNEPRNS1112 QTL on Pv2, with a peak position at 82.01cM, and it spanned 4.2 cM (Table 2.5). Beattie et al. (2003) report three QTL from days to maturity on Pv4, Pv6, and Pv8, while Blair et al. (2013) report QTL on Pv2, Pv5, Pv6, and Pv7, and Wright and Kelly (2011) report four QTL on Pv2, Pv3, Pv5, and Pv7. Co-localized QTL for days to flowering and days to maturity were not detected on the 'Buster'/SER 22 RIL population. Previous studies have reported co-localized QTL on Pv2 when populations of similar genetic background have been used. This confirms the broad genetic diversity within the 'Buster'/SER 22 RIL population.

Based on the *Pv* annotation data (Schmutz et al., 2013; unpublished), the DTMNEPRNS1112 genomic region accounts for up to 32 genes. Close to its peak position (82.01 cM), the Arabidopsis AtCHX3, AtGRP2, RALFL24 genes can be found, among others. These proteins play a role in a cell signaling peptide that may regulate plant stress, growth, and

development, and also regulates flowering transition, flower and seed development. They also promote seed germination under salt stress, may regulate respiratory oxygen uptake, and may confer cold and freezing tolerance (Olsen et al., 2002).

Leaf Temperature

Ten significant QTL for leaf temperature were found on chromosomes Pv1, Pv2, Pv3, and Pv5, (3, 3, 2, and 2, respectively) in four environments (Table 2.6). Two of them, LTNEPRDS1112 and LTPRDS12 were detected under drought stress conditions, on Pv1 and Pv2, respectively. The other five were detected under non-stress conditions on Pv1, Pv2, and Pv5 (2, 2, and 1, respectively), and the three remaining QTL were detected using the data combined from different growing conditions (Table 2.6). The R^2 values for leaf temperature QTL ranged from 0.01 to 0.10, and additive effects varied from 0.26 to 0.71 ^{o}C.

The QTL for leaf temperature with the largest phenotypic variation (10%) was LTNENS11 on Pv1 with a peak position at 84.21cM, and it spanned 12.9 cM with a SER 22 allele contribution that accounts for up to 0.30^{o}C (Table 2.4). On the other hand, a second QTL, LTNENS11, was detected under NENS11 environment on Pv1 (Table 2.4), this one accounts for up to 8% of the phenotypic variation, and it spanned 12.3 cM. Those two QTL are very close to each other and the region accounts for up to 208 genes based on the *Pv* annotation data (Schmutz et al., 2013; unpublished). Another QTL for leaf temperature was detected on Pv1 (LTNEPRNS1112) but under drought conditions. LTNEPRDS1112, flanked by markers sc00135ln517288_390319 (15.67) and sc01618ln66072_45822 (36.58) spanned 23.1 cM and accounts for 2% of the total phenotypic variance for leaf temperature with a 'Buster' allele contribution (Table 2.4; Table 2.5). In its genomic region eight genes can be found close to its peak position (44.71 cm) (Schmutz et al., 2013; unpublished). One of them, the TINY2

(dehydratation-responsive element binding protein) gene may be involved in the regulation of gene expression by stress factors and by components of stress signal transduction pathways (Wei et al., 2005).

Three significant QTL for leaf temperature were detected on Pv2. LTNENS11, LTNEC11, and LTPRDS12, and they spanned 2.2, 7.4, and 0.05 cM, respectively. LTNENS11 was detected under non-stress conditions, while LTNEC11 was detected using the combined data among growing conditions in Nebraska, growing season from 2011, while LTPRDS12 was detected under drought stress in PRDS12 environment. LTNENS11, LTNEC11, and LTPRDS12, account for up to 5, 1, 4% of the total phenotypic variance for leaf temperature, respectively. Markers sc00329ln305942_245750 (48.80) and sc00445ln245016_243467 (48.62) flanked the LTPRDS12 QTL, and its genomic region has four genes (Schumtz et al., 2013). The ATCESA8 (Cellulose synthase A catalytic subunit 8) is a protein required for beta-1,4-glucan microfibril crystallization, a major mechanism of the cell wall formation involved in the secondary cell wall formation required for the xylem cell wall thickening (Turner and Somerville, 1997). QTL for leaf temperature with very small phenotypic variation were detected and its detailed information can be found in Table 2.6 and Table 2.7.

Among leaf temperature QTL, 'Buster' contributed to those QTL detected under drought conditions and SER 22 contributed to those detected under non-stress conditions. No previous detection of QTL for leaf temperature have been found in dry beans, but according to van Schoonhoven and Voysest (1991), canopy temperature is related to drought tolerance. Lower temperature presumably greater root growth, permitting a larger uptake of water, combined with lesser effects of leaf size, movement and reflectivity.

QTL analysis was also performed for percentages of yield reduction, harvest index, pod harvest index, but no QTL were detected for these indices.

Future and on-going work will be the validation of the QTL detected herein in a subset of extreme phenotypes (30-50 lines) from the 'Buster'/SER 22 RIL population. The phenotypic evaluation of this extreme genotypes should be done close to bean production areas where drought stress has been already or could be a major constraint.

Number of replications should be increase since this may reduce the experimental error and a better and more accurate estimation of the genotypic variances could be done. Evaluation of root traits should be considered for the 'BUSTER'/SER 22 RIL population, and it could be done under greenhouse conditions. Since this population is evaluated at reproductive stage, evaluation of roots in the field together with the agronomic and physiological data collection would be very labor intensive. Currently, the first subset of extreme genotypes was evaluated in Juana Diaz, PR and has been evaluated at Mitchell, NE.

Table 2.6. QTL information for seed yield, seed weight, days to maturity, days to flower, and leaf temperature over 12 environments in the 'Buster'/SER 22 RIL population.

Trait	Environment	Pv[†]	Peak Position (cM)	LOD score	Additive effect[‡]	R^2
Seed Yield	NEDS11	3	3.31	1.92	-23.00	0.09
	NENS11	7	99.81	1.66	111.97	0.03
	NEC11	3	39.51	2.92	104.16	0.05
Seed weight	NEC11	8.1	0.01	2.12	1.30	0.03
	PRDS12	2	23.61	3.42	1.03	0.05
	PRC12	2	23.61	3.61	0.79	0.05
	PRC12	8.2	59.51	3.02	-1.18	0.06
	NENS13	2	12.51	2.91	-0.93	0.04
	NEC13	11	60.91	3.54	-0.95	0.06
Days to flower	NEC11	6	21.51	2.95	0.60	0.05
	PRNS12	8.1	3.91	2.14	0.32	0.03
Days to maturity	NEPRNS1112	2	82.01	2.92	0.37	0.04
Leaf temperature	NENS11	1	73.71	5.71	-0.28	0.08
	NENS11	1	84.21	5.52	-0.30	0.10
	NENS11	2	76.91	3.79	-0.28	0.05
	NEC11	2	66.61	1.04	-0.41	0.01
	NEC11	3	76.11	1.35	0.71	0.02
	NEC11	3	101.81	1.07	0.48	0.01
	PRDS12	2	0.01	3.27	0.31	0.04
	NEPRDS112	1	44.71	1.45	0.26	0.02
	NEPRNS1112	5	77.81	1.64	-0.30	0.02
	NEPRNS1112	5	13.21	1.73	-0.42	0.02

[†]Pv = *Phaseolus vulgaris* chromosome based on consensus map.
[‡]Positive values indicate allele from 'Buster' and negative from SER 22.

Table 2.7. Flanking markers from QTL for seed yield, seed weight, days to maturity, days to flower, and leaf temperature over 12 environments in the 'Buster' /SER 22 RIL population.

Trait	Pv[†]	Length of the QTL (cM)	Flanking Markers	
Seed Yield	3	2.20	sc00034ln878273_431537 (51.86)	sc00034ln878273_572052 (51.72)
	7	5.60	sc00002ln2152649_1191485 (50.82)	sc00002ln2152649_1539101 (51.16)
	3	9.50	sc00062ln709754_363848 (47.81)	sc00019ln1150109_1018006 (45.59)
Seed weight	8.1	0.01	sc00089ln640327_50900 (0.18)	sc00089ln640327_268606 (0.39)
	2	7.00	sc00160ln486724_338720 (44.64)	sc00160ln486724_301841 (44.60)
	2	6.20	sc00160ln486724_338720 (44.64)	sc00160ln486724_301841 (44.60)
	8.2	7.20	sc00296ln326650_106196 (52.73)	sc00678ln168824_163688 (8.31)
	2	5.50	sc00113ln562714_289656 (46.82)	sc00113ln562714_374686 (46.74)
	11	5.20	sc00005ln1829281_1378783 (1.47)	sc00005ln1829281_769790 (0.86)
Days to flower	6	7.10	sc00058ln727328_87916 (24.48)	sc00355ln290049_84131 (21.92)
	8.1	04.50	sc00089ln640327_50900 (0.18)	sc00089ln640327_268606 (0.39)
Days to maturity	2	4.20	sc00411ln257942_127705 (3.28)	sc00240ln364462_178258 (3.57)
Leaf temperature	1	12.30	sc00587ln197790_102673 (43.7)	sc00022ln1003704_288203 (49.48)
	1	12.90	sc00022ln1003704_757321 (49.00)	sc00003ln2130026_591835 (51.29)
	2	2.20	sc00255ln354264_9387 (5.03)	sc00413ln257938_32444 (4.64)
	2	7.40	sc00118ln552338_522535 (28.17)	sc00137ln512899_430228 (25.84)
	3	2.10	sc00049ln755052_384902 (11.57)	sc00950ln123884_39400 (28.54)
	3	15.70	sc00216ln396195_319474 (2.28)	sc00059ln720534_580545 (1.07)
	2	0.05	sc00329ln305942_245750 (48.80)	sc00445ln245016_243467 (48.62)
	1	23.10	sc00135ln517288_390319 (15.67)	sc01618ln66072_45822 (36.58)
	5	0.80	sc00413ln257938_118330 (4.55)	sc00413ln257938_185379 (4.48)
	5	7.10	sc00927ln127007_72084 (1.58)	sc00386ln270379_31411 (2.66)

[†]Pv = *Phaseolus vulgaris* chromosomes based on the consensus map.
[‡]Values in parenthesis indicate the physical distance of marker on genome.

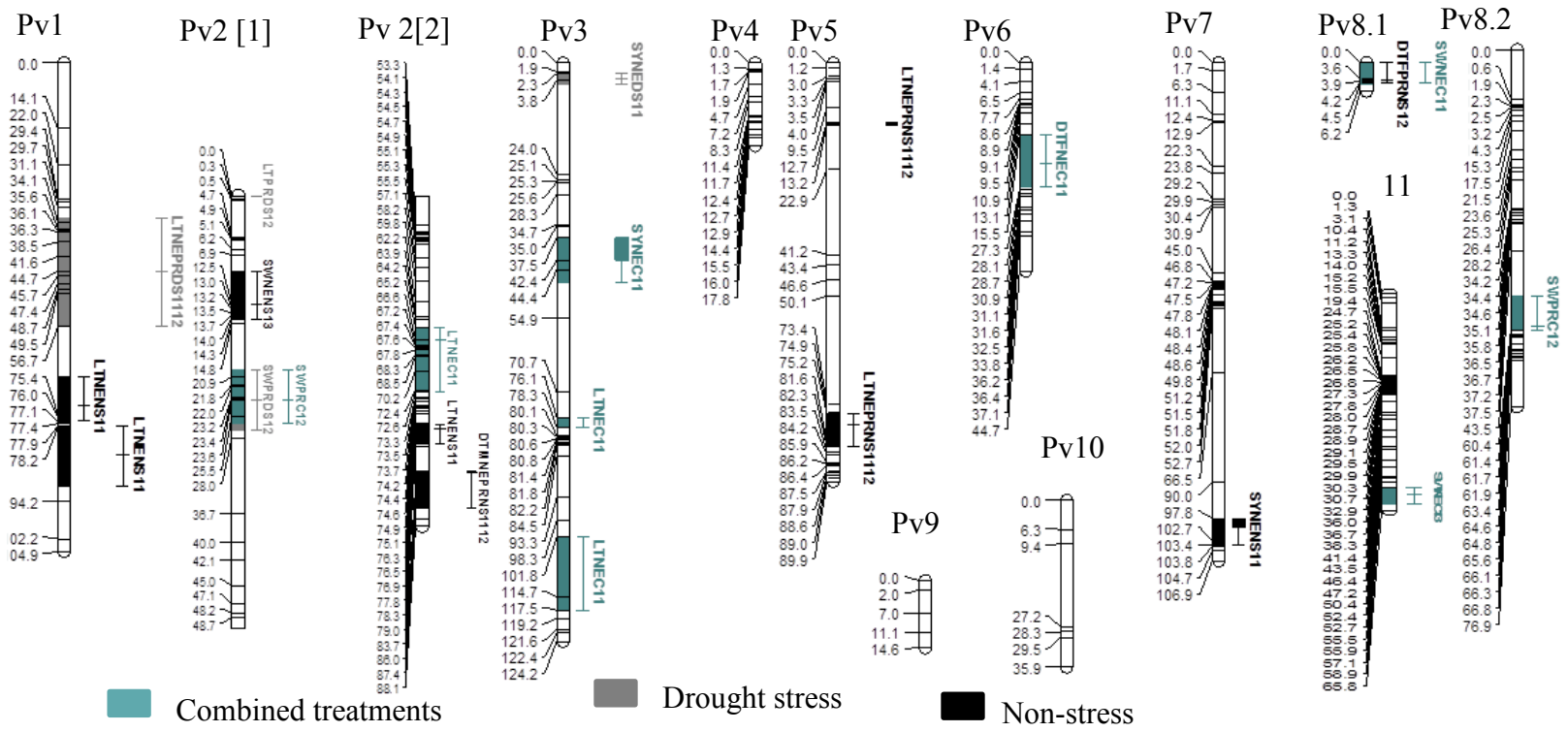

Figure 2.4. A summary of the genetic linkage map of the 'Buster'/SER 22 RIL population, indicating QTL for seed yield (SY), 100-seed weight (SW), days to flower (DTF), days to maturity (DTM), and leaf temperature (LT) traits. Vertical bars to the right of the marker indicates QTL detected with a LOD score > 3.0. The code next to the vertical bars indicates the corresponding trait and environment as shown in Table 2.1. The horizontal lines indicates the LOD peak for the QTL, and QTL found in non-stress are in black, drought stress in gray, and combined in teal. Left: Map distances in centimorgans (Kosambi, 1944).

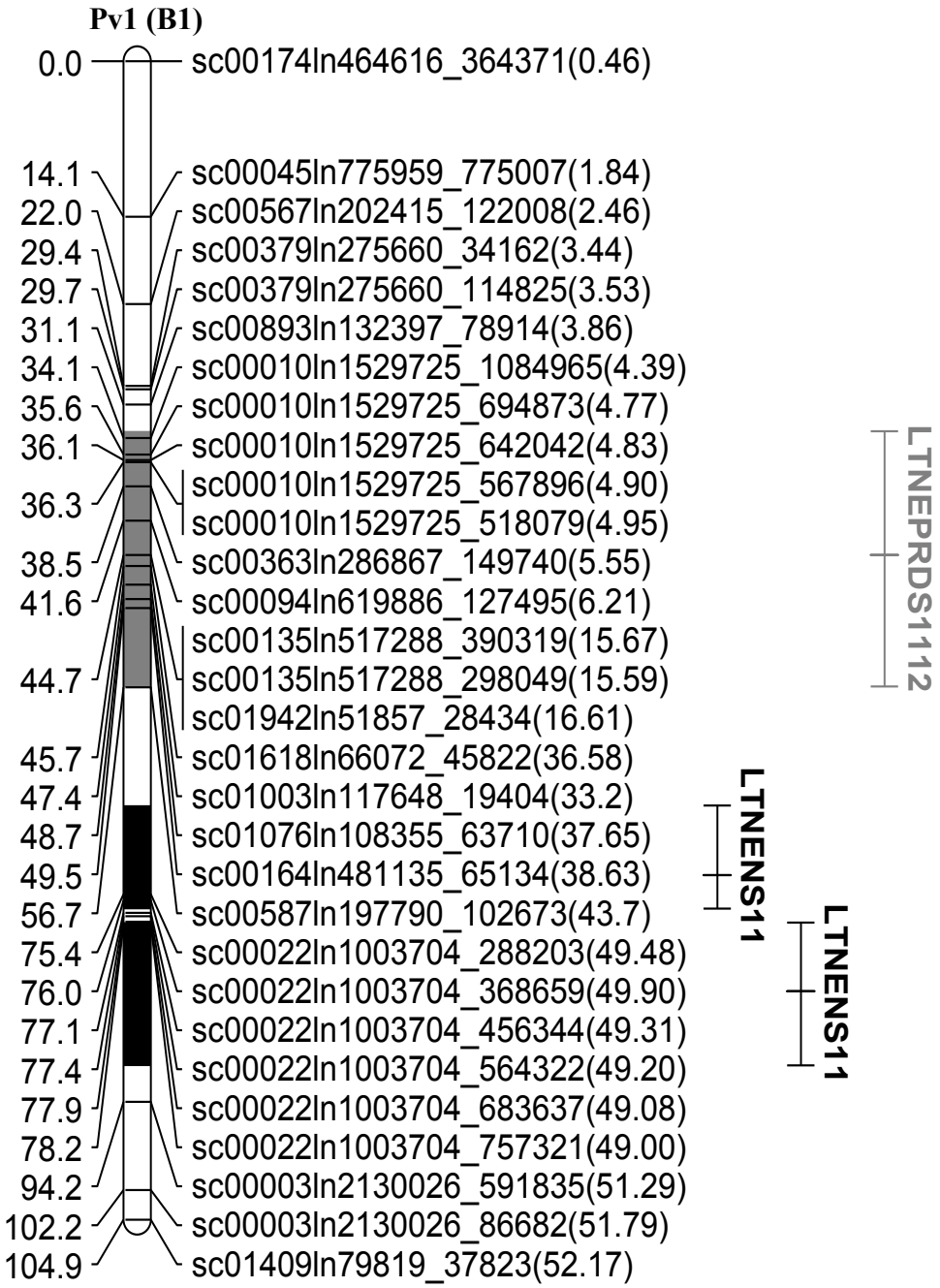

Figure 2.5. A genetic linkage map of the 'Buster'/SER 22 RIL population, indicating QTL for seed yield (SY), 100-seed weight (SW), days to flower (DTF), days to maturity (DTM), and leaf temperature (LT) traits. Marker loci are listed to the right; the number in parenthesis is the 'Redhawk'/'Stampede' physical position in Mb. Vertical bars to the right of the marker indicates QTL detected with a LOD score > 3.0. The code next to the vertical bars indicates the corresponding trait and environment as shown in Table 2.1. The horizontal lines indicate the LOD peak for the QTL, and QTL found in drought are in black, non-stress in gray, and combined in teal. Left: Map distances in centimorgans (Kosambi, 1944).

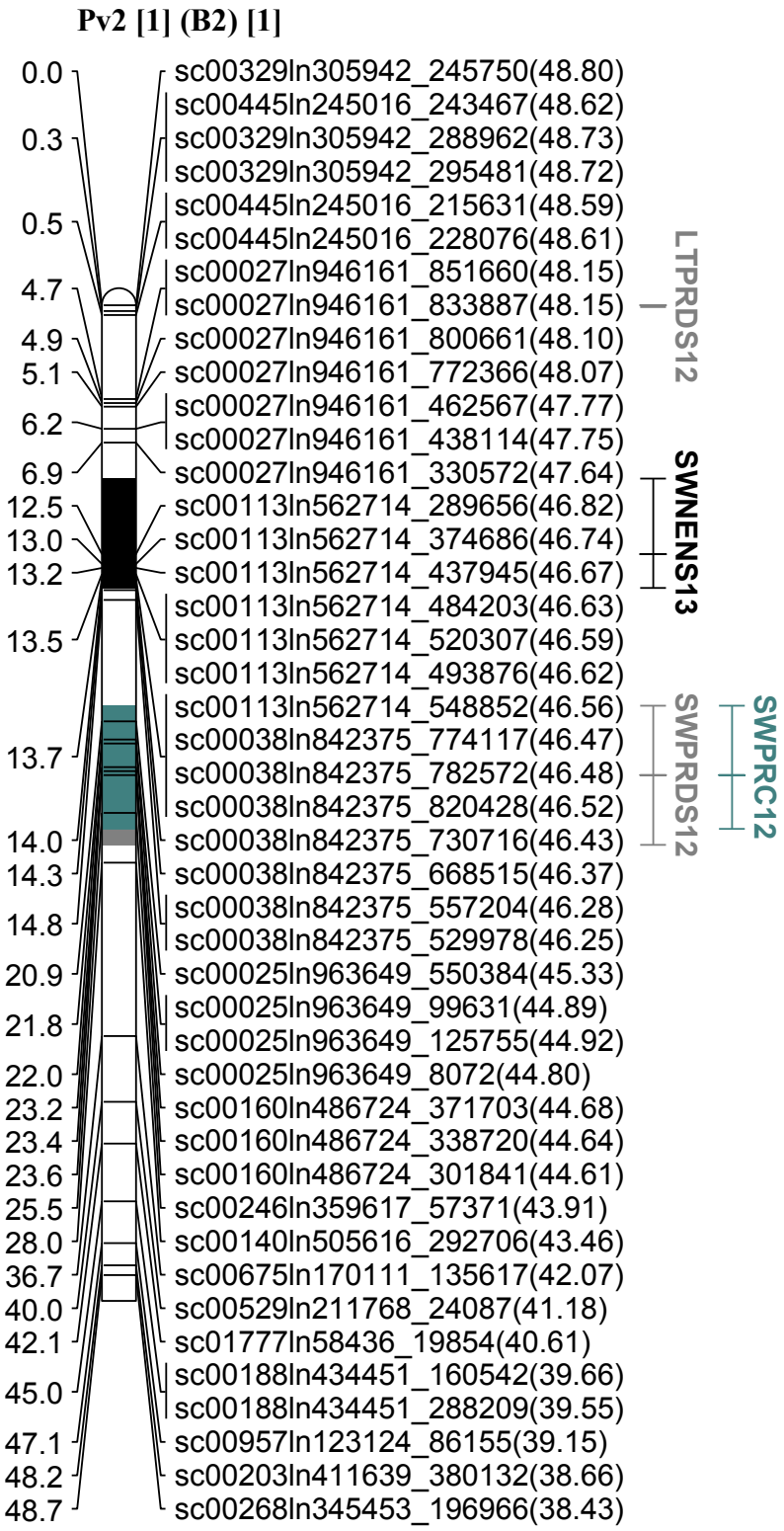

Figure 2.5. A genetic linkage map of the 'Buster'/SER 22 RIL population (cont.).

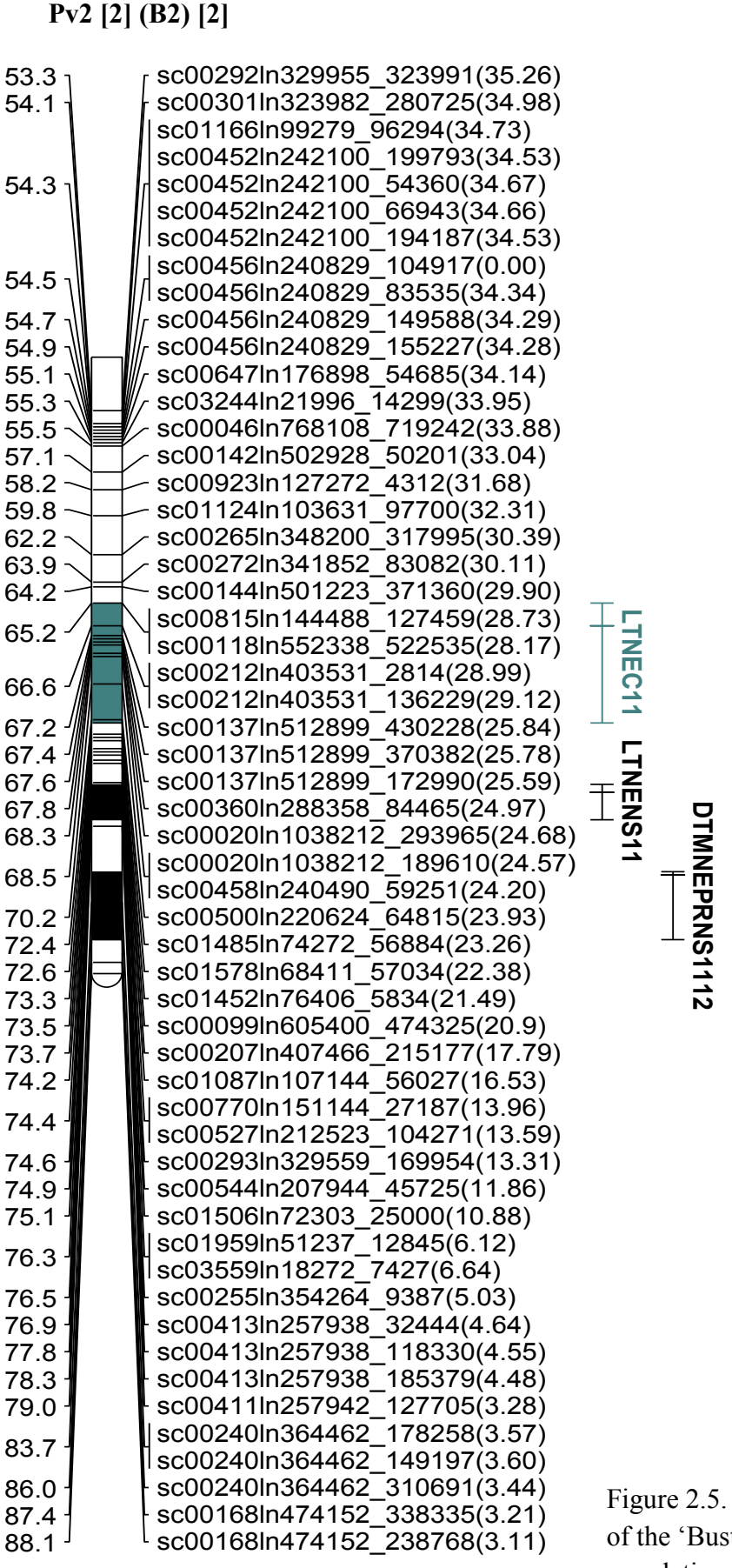

Figure 2.5. A genetic linkage map of the 'Buster'/SER 22 RIL population (cont.).

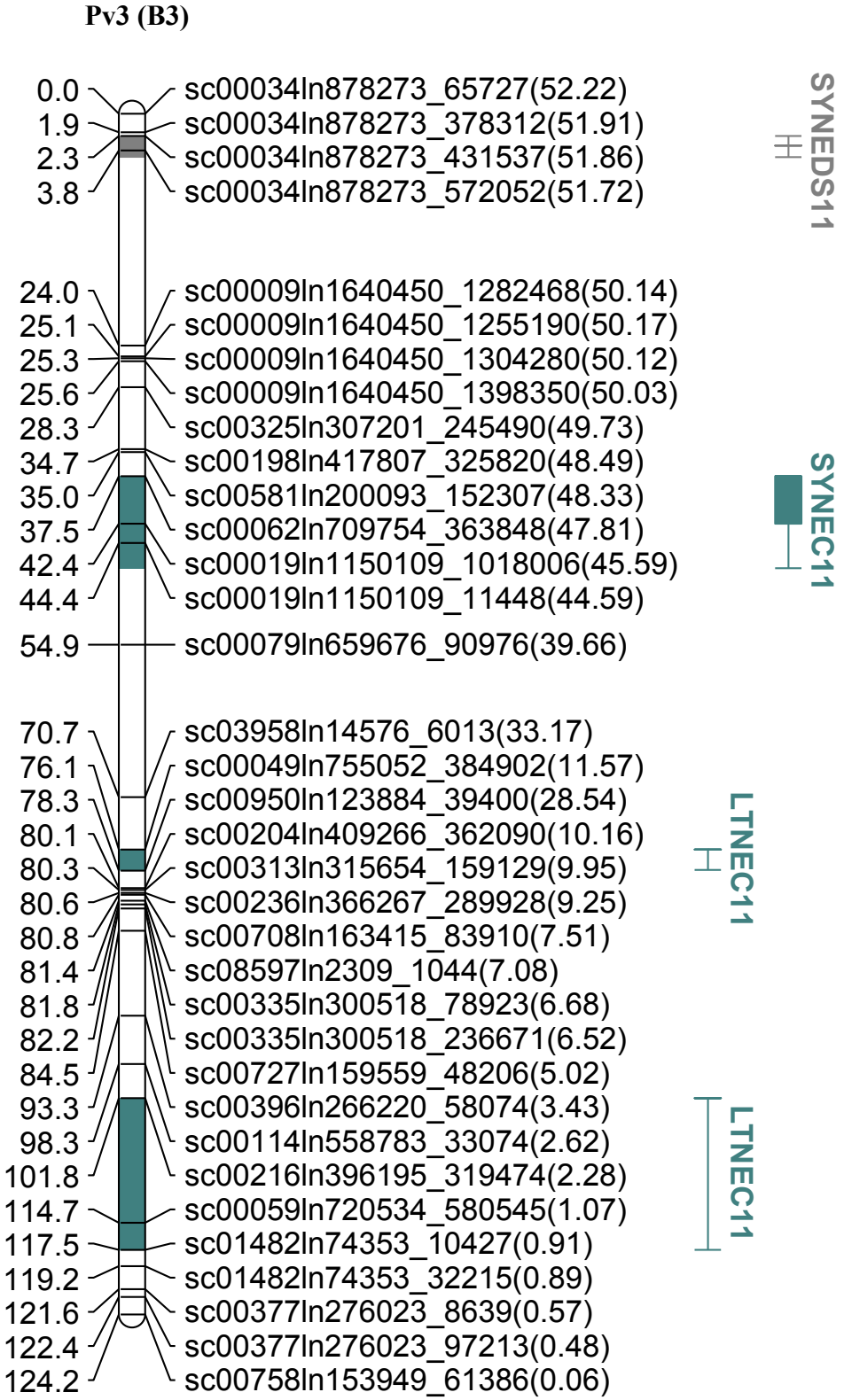

Figure 2.5. A genetic linkage map of the 'Buster'/SER 22 RIL population (cont.).

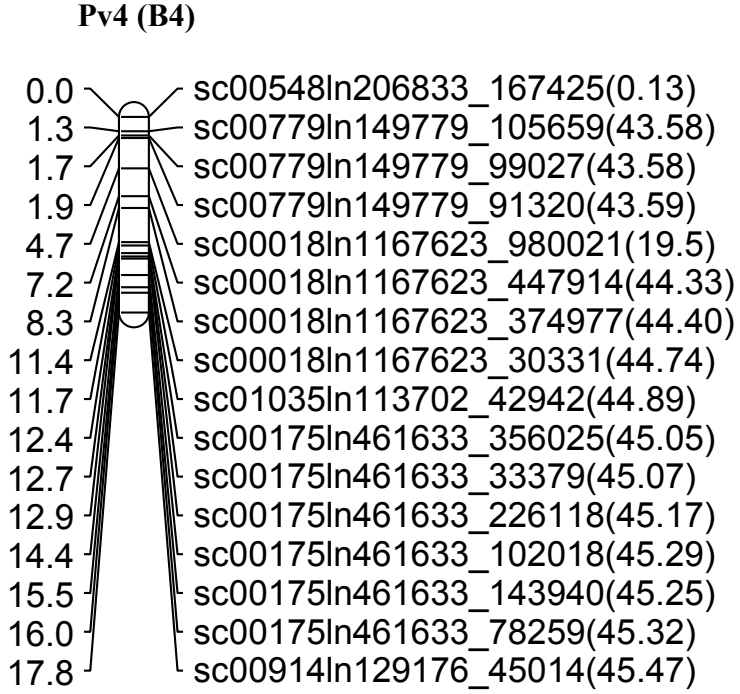

Figure 2.5. A genetic linkage map of the 'Buster'/SER 22 RIL population (cont.).

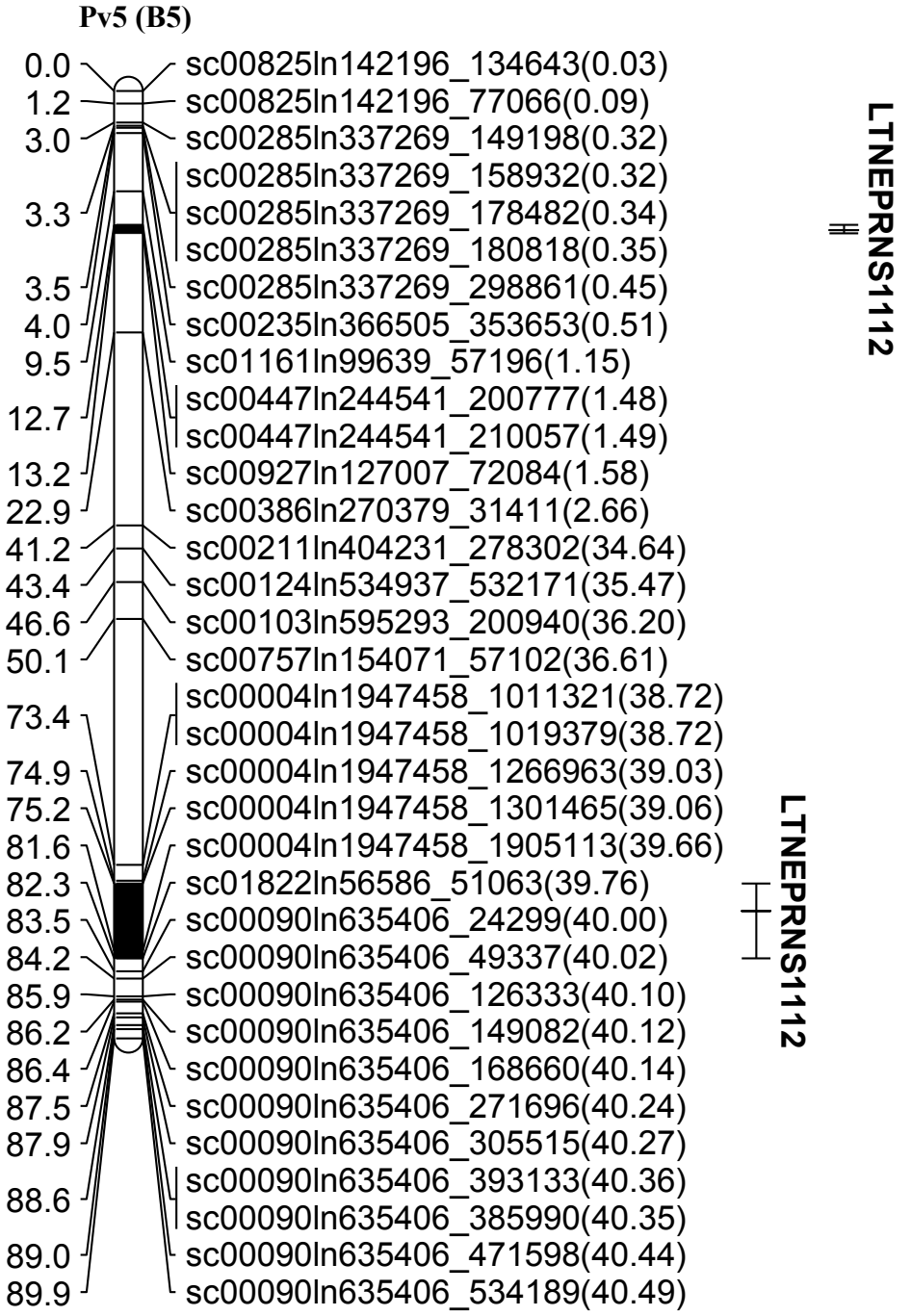

Figure 2.5. A genetic linkage map of the 'Buster'/SER 22 RIL population (cont.).

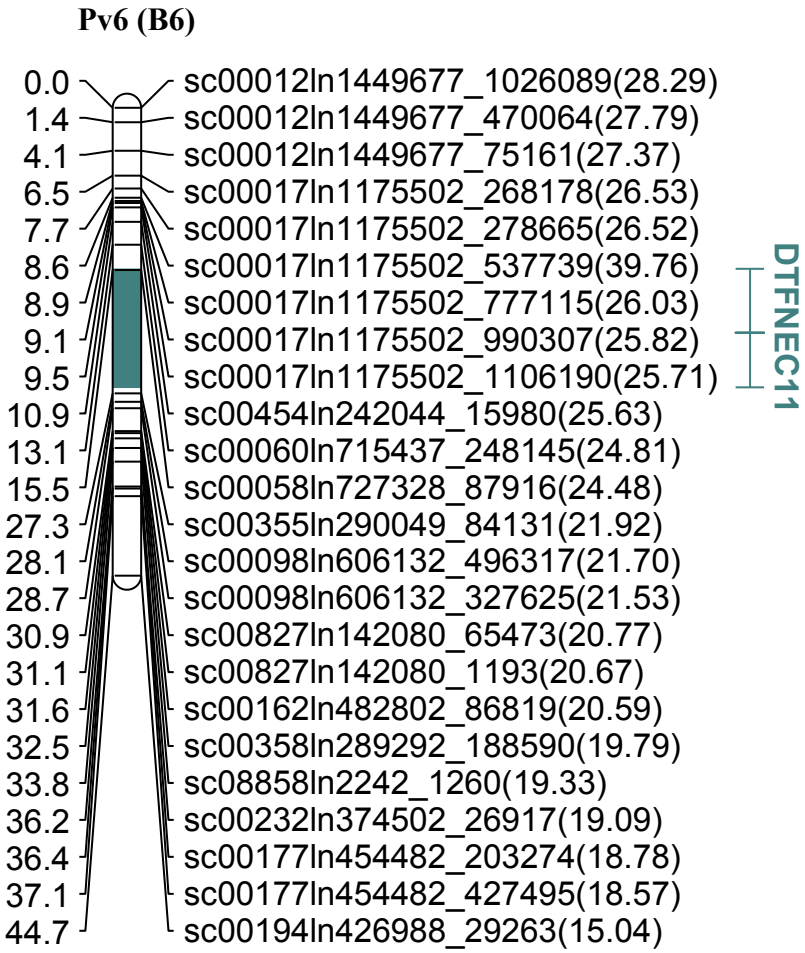

Figure 2.5. A genetic linkage map of the 'Buster'/SER 22 RIL population (cont.).

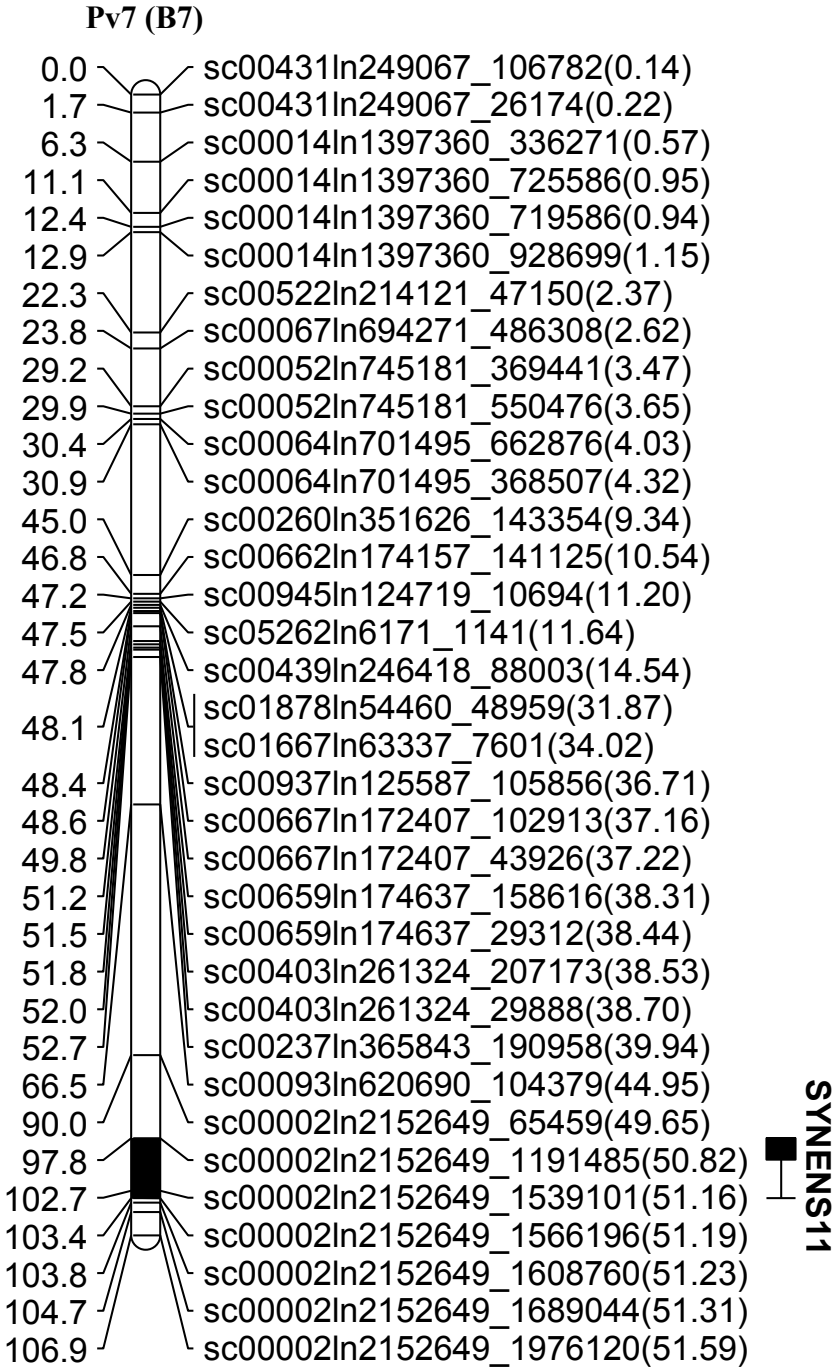

Figure 2.5. A genetic linkage map of the 'Buster'/SER 22 RIL population (cont.).

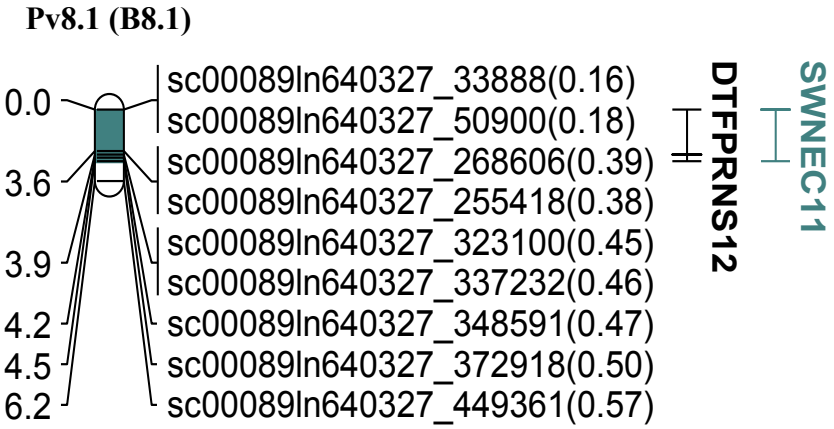

Figure 2.5. A genetic linkage map of the 'Buster'/SER 22 RIL population (cont.).

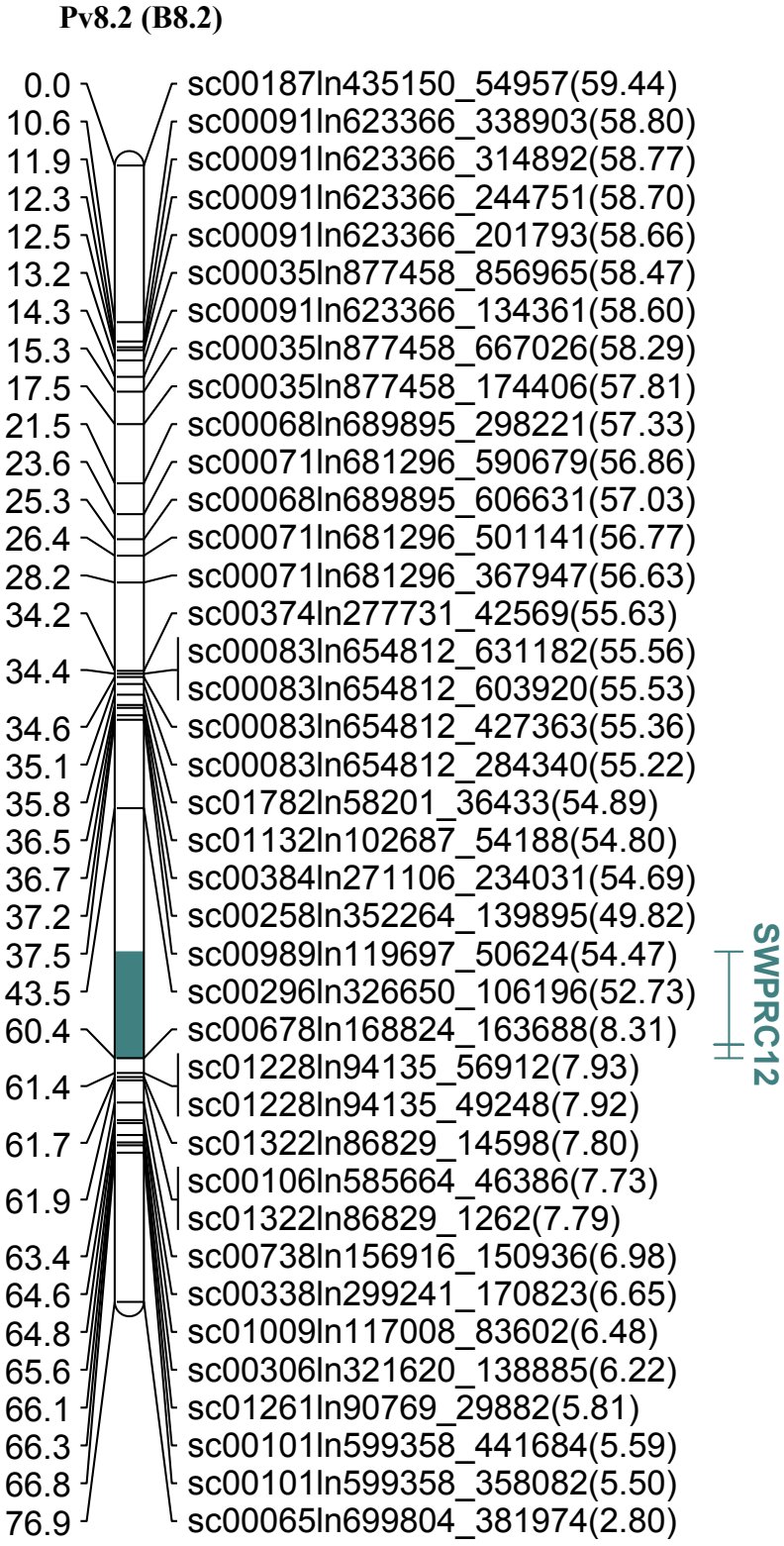

Figure 2.5. A genetic linkage map of the 'Buster'/SER 22 RIL population (cont.).

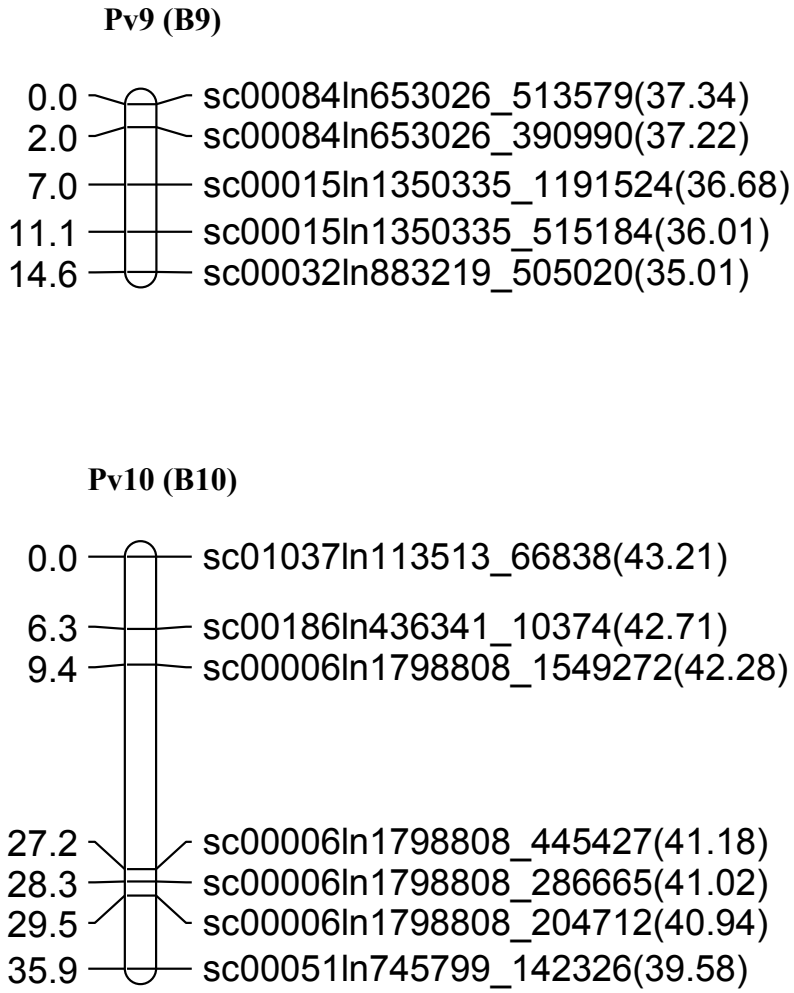

Figure 2.5. A genetic linkage map of the 'Buster'/SER 22 RIL population (cont.).

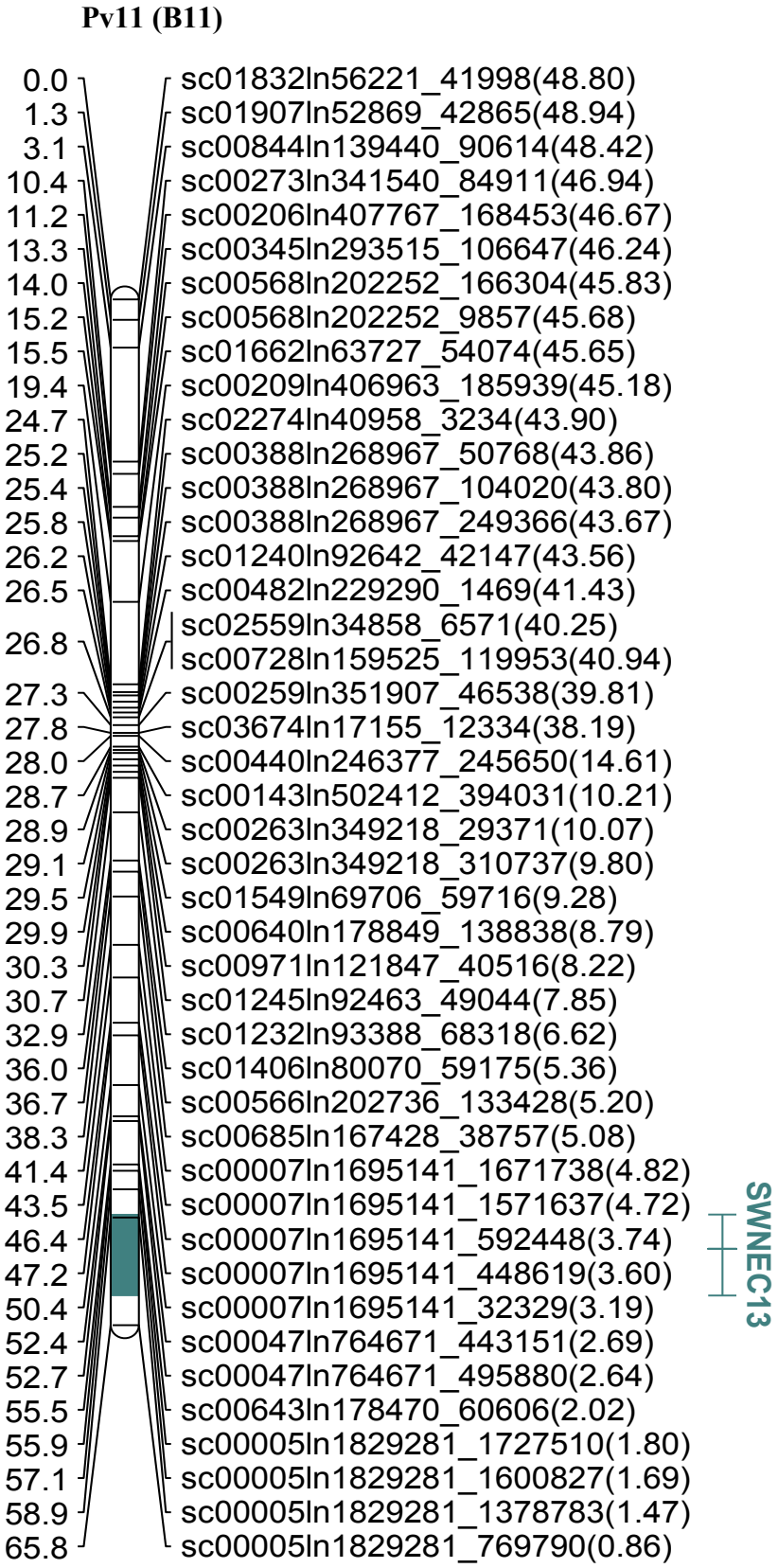

Figure 2.5. A genetic linkage map of the 'Buster'/SER 22 RIL population (cont.).

Summary and Conclusions

Selection for greater tolerance to abiotic stresses such as drought and heat has gained more importance in response to climate change and the increased use of marginal land for bean production. In the U.S. the production has moved to arid environments as soybean and corn production has expanded due to higher prices of these commodities. Tolerance to drought stress could help farmers to produce more stable bean yields in unfavorable environments. Therefore, genome mapping and molecular breeding are tools that can help breeders to identify genomic regions of interest, as well as to understand the genetic mechanisms behind the impact of drought and its interaction with the environment. Marker-assisted selection has been proposed as a means of identifying markers linked to important traits that follow a quantitative inheritance. However, this utility will depend on how reliable marker-trait associations are for predicting the phenotype based on the genotype. Ideally, a marker should explain a considerable portion of the phenotypic variation and the expression of the trait should not be greatly affected by the environment. Due to the nature of drought, validation studies are challenging. Population size should be big enough to detect QTL that are statistically powerful. Intensive field evaluation should be carried out in multiple locations during the same growing season, where the drought intensity will play a major factor in the possible detection of significant QTL, whereas the development of strong predictive methodologies are imperative for the development of molecular applications that take advantage of the genotyping instead of phenotyping, thus benefiting the selection of dry bean genotypes with desirable commercial attributes.

The objective of this study was to evaluate the 'Buster'/SER 22 recombinant inbred line population under drought conditions and to identify loci associated to drought tolerance that could be used in Marker Assisted Selection in the future.

Some important results and conclusions drawn from this research are:

- Composite interval mapping identified a total of 12 QTL for agronomic traits, including seed yield, and ten for physiological traits on seven linkage groups under drought or non-stress conditions.
- High yielding, drought-tolerant RIL with broad adaptation has been identified even though the levels of drought stress in Mitchell and Juana Diaz were not the same across years. These lines could be used in future crosses in breeding programs.
- Seed weight was correlated with seed yield under drought stress.
- According to the list presented by Beebe et al. (2013), the 'Buster'/SER 22 RIL population has been the first population having a considerably good size of individuals (N=345). On the other hand, low recombination regions were detected among parental lines, which is normal for almost any cross due to heterochromatic and euchromatic regions. A considerable set of SNP markers linked to drought-tolerant genes have been identified. Some QTL linked to drought tolerance may also be linked to salinity tolerance, as it may be the case of SWPRDS12 and SWPRC12 where in its genetic region and close to the QTL peak position, the gene TUB7 (tubulin beta-7 chain) is found. TUB7 is involved in different biological processes, such as the cytoskeleton organization, regulation of meristem growth, response to cadmium ion, and response to salt stress.
- Overall, more QTL were detected under severe drought intensity (Mitchell, NE) that under moderate drought intensity (Juana Diaz, PR). However, Juana Diaz, PR

the interaction of edaphic stressors may interfere in the detection of more QTL related to drought stress.

Although a desirable loci from SER 22, the drought parental line, was found for seed yield under drought stress, its total phenotypic variation accounts for just 9% (23 kg ha^{-1}). The *Arabidopsis thaliana* pTCAC5 (Plastid Transcriptionally Active5) gene is found in the genomic region of SYNEDS11. This gene is essential for chloroplast development in *Arabidopsis* under heat stress by maintaining PEP function.

- Linkage group Pv3 seems to play an important role in the improvement of seed yield under drought stress. A seed yield QTL detected in this linkage group could have the potential to be a major QTL.
- Seed weight was influenced by loci on at least five linkage groups with relatively equal effects, ranging from 0.03 to 0.06. Based on the Pv annotation data a single QTL detected on Pv8.2 may have a conservative candidate gene coming from the Mesoamerican race.
- A small number of QTL for days to flowering and days to maturity (2 and 1, respectively) were detected in the 'Buster'/SER 22 RIL population.
- A single QTL on linkage group Pv1 accounted for up to 10% of the variation for leaf temperature under irrigated conditions, a desirable loci from SER 22 helped to acclimate the plants to heat stress. Smaller effects for leaf temperature QTLs were identified independently in some environments in Pv1, Pv2, Pv3, and Pv5.

References

de Givry, S., M. Bouchez, P. Chabrier, D. Milan, and T. Schiex. 2005. CARTHAGENE: multipopulation integrated genetic and radiated hybrid mapping. Bioinformatics 21: 1703-1704.

van Schoonhoven, A. and O. Voysest. 1991. Common Beans: Research for crop improvement p. 517.

Abebe, A., M.A. Brick, and R.A. Kirkby. 1998. Comparison of selection indices to productive dry bean lines under diverse environmental conditions. Field Crops Res. 58:15 23.

Acosta-Díaz, E., C. Trejo-López , L.M. Ruiz-Posadas, J.A Acosta-Gallegos, S. Padilla-Ramírez. 2004. Adaptación del frijol a sequía en la etapa reproductiva. Terra Latinoamericana 22: 49-58.

Acosta-Gallegos, J.A., and M.W. Adams. 1991. Plant traits and yield stability of dry bean (*Phaseolus vulgaris* L.) cultivars under drought stress. J. Agric. Sci. 117:213-219.

Alejandro, S., P.L. Rodriguez, J., M. Belles, L. Yenush., M.J. Garcia-Sanchez, J.A. Fernandez, and R. Serrano. 2007. An Arabidopsis quiescin-sulfhydryl oxidase regulates cation homeostasis at the root symplast-xylem interface. Embo J. 26:3203-3215.

Bartlett, M.S. 1947. The use of transformations. Biometrics 3:39-52.

Beattie, A.D., J. Larsen, T.E. Michaels, and K.P. Pauls. 2003. Mapping quantitative trait loci for a common bean (*Phaseolus vulgaris* L.) ideotype. Genome 46:411-422.

Beaver, J.S., and P.N. Miklas. 1999. Registration of 'Morales' small white bean. Crop Sci. 39:1257.

Beebe, S.E., M. Rojas-Pierce, X. Yan, M.W. Blair, F. Pedraza, F. Muñoz, J. Tohme, J.P. Lynch 2006. Quantitative Trait Loci for root architecture traits correlated with phosphorus acquisition in common bean. Crop Sci 46:413-423.

Beebe, S.E., M.R. Idupulapati, C. Cajiao, and M. Grajales. 2008. Selection for drought resistance in common bean also improves yield in phosphorus limited and favorable environments. Crop Sci. 48: 582-592.

Beebe, S.E. 2012. Common bean breeding in the tropics. Plant Breed. Rev. 36:357-426.

Bell, E.M., and J.E. Mullet. 1991. Lipoxygenase gene expression is modulated in plants by water deficit, wounding, and methyl jasmonate. Mol. Gen. Genet. 230:456-462.

Blair, M. W., C.H. Galeano, E. Tovar, M.C. Muñoz Torres, A. Velasco Castrillón, S. Beebe, and I.M. Rao. 2012. Development of a Mesoamerican intra-genepool genetic map for QTL detection in a drought-tolerant x susceptible common bean (*Phaseolus vulgaris* L.) cross.

Mol. Breed. 29:71-88.

Blair, M.W., M.C. Giraldo, H.F. Buendia, E. Tovar, M.C. Duque. 2006. QTL analysis of seed yield traits in an advanced backcross population derived froma cultivated Andean x wild common bean (*Phaseolus vulgaris* L.). Theor Appl Genet 113:100-109.

Boyer, J.S. 1982. Plant Productivity and Environment. Science. 218 (4571): 443-448.

Brick, M.A., J.B. Ogg, S.P. Singh, H.F. Schwartz, J.J. Johnson JJ, and M.A. Pastor- Corrales. 2008. Registration of drought-tolerant, rust-resistant, high-yielding pinto bean germplasm line CO46348. J Plant Registrations 2:120-124.

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

Buettner, M., and K.B. Singh. 1997Arabidopsis thaliana ethylene-responsive element binding protein (AtEBP), an ethylene-inducible, GCC box DNA-binding protein interacts with an ocs element binding protein. Proc. Natl. Acad. Sci. USA. 94:5961-5966.

Chaves, M.M., J.P. Maroco, and J.S. Pereira. 2003. Understanding plant responses to drought from genes to whole plant. Functional Plant Biol. 30:239-264.

Cheung, A. Y., and K.B. Singh. 2004. Overexpression of an Arabidopsis formin stimulates supernumerary actin cable formation from pollen tube cell membrane. Plant Cell 16:257-269.

Churchill, G.A., and R.W. Doerge. 1994. Empirical threshold values for quantitative trait mapping. Genetics. 138:963-971.

Clarke, J.M., R.M. DePauw, and T.F Townley-Smith. 1992. Evaluation of methods for quantification of drought tolerance in wheat. Crop Sci. 32:723-728.

Cregan, P. 2011. BeanCAP-2011-Cregan-Beltsville ARS -SNP markers. http://www.beancap.org/Meetings.cfm (accessed 28 July 2013).

Ebine, K., Y. Okatani, T. Uemura, T. Goh, K. Shoda, M. Niihama, M. Terao Morita, C. Spitzer, M.S. Otegui, A. Nakano, and T. Ueda. 2008. A SNARE complex unique to seed plants is required for protein storage vacuole biogenesis and seed development of *Arabidopsis thaliana.* Plant Cell. 20:3006-3021.

Felder, S., K. Meierhoff, A.P. Sane, J. Meurer, C. Driemel, H. Plücken, P. Klaff, B. Stein, N. Bechtold, P. Westhoff. 2001. The nucleus-encoded HCF107 gene of Arabidopsis provides a link between intercistronic RNA processing and the accumulation of translation-competent psbH transcript in chloroplasts. Plant Cell. 13:2127-2142.

Fischer, R.A., and R. Maurer. 1978. Drought resistance in spring wheat cultivars: I. Grain yield responses. Aust. J. Agric. Res. 29:897-912.

Hagerty, C.H. 2013.Mapping QTL for root rot resistance, root traits, and morphological trait in a

common bean recombinant inbred population. M.S. Thesis. Oregon State University.

Ho, M.D., J.C. Rosas, K.M. Brown, and J.P. Lynch. 2005. Root architectural tradeoffs for water and phosphorus acquisition. Functional Plant Biol. 32:737-748.

Holland, J.B. 2006. Estimating genotypic correlations and their standard errors using multivariate restricted maximum likelihood estimation with SAS Proc MIXED. Crop Sci. 46:642-654.

Holland, J.B., W.E. Nyquist, and C.T. Cervantes-Martínez. 2003. Estimating and interpreting heritability for plant breeding: An update. Plant Breeding Rev. 22:9-112.

Howles, P.A., J.B. Birch, D. A.Collings, L.K. Gebbie, U. A. Hurley, C.H. Hocart, T. Arioli, R.E. Williamson. 2006. A mutation in an Arabidopsis ribose 5-phosphate isomerase reduces cellulose synthesis and is rescued by exogenous uridine. Plant J. 48:606-618.

Hynten, D.L., Q. Song, E.W. Fickus, C.V. Quigley, J.S. Lim, I.Y. Choi, E.Y. Hwang, M. Pastor-Corrales, and P.B. Cregan. 2010. High-throughput SNP discovery and assay development in common bean. BMC Genomics 11:475-482.

Jung, G., D.P. Coyne, P.W. Skroch, J. Nienhuis, E. Arnaud-Santana, J. Bokosi, H.M. Ariyarathne, J.R. Steadman, J.S. Beaver, and S.M. Kaeppler. 1996. Molecular markers associated with plant architecture and resistance to common blight, web blight, and rust in common beans. J. Am. Soc. Hort. Sci. 121: 794-803.

Jung, G., P.W. Skroch, D.P. Coyne, J. Nienhuis, E. Arnaud-Santana, H.M. Ariyarathne, S.M. Kaeppler, and M.J Bassett. 1997. Molecular-marker-based genetic analysis of tepary bean-derived common bacterial blight resistance in different developmental stages of common bean. J. Am. Soc. Hort. Sci. 122: 329-337.

Khanal, S., J. Xue, R. Khanal, W. Xie, J. Shi, K.P. Pauls, and A. Navabi. 2013. Quantitative Trait Loci Analysis of Folate Content in Dry Beans, *Phaseolus vulgaris* L. Inter. J. of Agro. 2013:1-9.

Kim, H.U., and A.H.C. Huang. 2004. Plastid lysophosphatidyl acyltransferase is essential for embryo development in Arabidopsis. Plant Physiol. 134:1206-1216.

Kosambi 1944. The estimation of map distances from recombination values. AnnEugen. 12:172-175.

Lynch, J.P. 2007. Roots of the second green revolution. Australian J. of Bot. 55:493-512.

Lynch, M., and B. Walsh. 1998. Genetics and analysis of quantitative traits. Underland, MA*:* Sinauer Associates, Inc. p. 980.

Mason, M.G., D.E. Mathews, J.J Kieber, and G.E Schaller. 2004. Type-B response regulators display overlapping expression patterns in *Arabidopsis*. Plant Physiol. 135:927- 937.

McClean, P.E. 2011. BeanCAP-2011-McClean-NDSU-PAG-Talk. http://www.beancap.org/Meetings.cfm (accessed 28 July 2013).

McClean, P.E., J. Burridge, S. Beebe, I.M. Rao and T.G. Porch. 2011. Crop improvement in the era of climate change: an integrated, multi-disciplinary approach for common bean (*Phaseolus vulgaris*) Funct. Pl. Biol. 38:927-933.

McConnell, M., S. Mamidi, R. Lee, S. Chikara, M. Rossi, R. Papa, and P. McClean. 2010. Syntenic relationships among legumes revealed using a gene-based genetic linkage map of common bean (*Phaseolus vulgaris* L.). Theor. Appl. Genet. 121: 1103-1116.

Muñoz-Perea, C.G., H. Terán, R.G. Allen, J.L. Wright, D.T. Westermann, and S.P. Singh. 2006. Selection for drought resistance in dry bean landraces and cultivars. Crop Sci. 46:2111-2120.

Nielson, D.V., and N.O. Nelson. 1998. Black bean sensitivity to water stress at various growth stages. Crop Sci. 38:422-427.

Nodari , R.O., S.M. Tsai, P. Guzman, R.L Gilbertson, and P. Gepts. 1993. Toward an integrated linkage map of common bean. III. Mapping genetic factors controlling host-bacteria interactions. Genetics 134: 341-350.

NOAA. 2012. State of the Climate National Oceanic and Atmospheric Administration National Climatic Data Center. July 2012: hottest month on record for contiguous United States. http://www.ncdc.noaa.gov/sotc/index.php. (accessed 9 Aug 2012).

Olsen, A.N., J. Mundy, and K. Skriver. 2002. Peptomics, identification of novel cationic Arabidopsis peptides with conserved sequence motifs. In Silico Biol. 2:441-451.

Osorno, J. M., K.F. Grafton, G.A. Rojas-Cifuentes, J.R. Gelin and A.J. VanderWal. 2010. Registration of 'Lariat' and 'Stampede' pinto beans. J. Plant Reg. 4:5-11.

Porch, T.G., C.A. Urrea, J.S. Beaver , S. Valentin, P.A. Peña, and J.R. Smith. 2012. Registration of TARS-MST1 and SB-DT1 Multiple-Stress-tolerant Black Bean Germplasm. J. Plant Reg. 6:75-80.

Porch, T.G., R. Bernsten, J.C. Rosas, and M. Jahn. 2007. Climate change and the potential economic benefits of heat tolerant bean varieties for farmers in Atlántida, Honduras J. of Agric. Univ. of Puerto Rico 91:133-148.

Porch, T.G., V.H. Ramirez, D. Santana, and E.W. Harmsen. 2009. Evaluation of common bean for drought tolerance in Juana Diaz, Puerto Rico. J. Agro. & Crop Sci. 195:328-334.

Ramirez, V.H. 2007. Plant-water relationships for several common bean (*Phaseolus vulgaris* L.) genotypes with and without drought stress conditions. M.S. Thesis University of Puerto Rico, Mayaguez.

Ramirez-Vallejo, P., and J.D. Kelly. 1998. Traits related to drought resistance in common bean.

Euphytica 99:127-136.

Rao, I.M., J.A. Polania, M. Rivera, and J. Ricaurte. 2008. Phenotyping common beans for adaptation to drought: Protocol for field evaluation. Centro Internacional de Agricultura Tropical (CIAT). Cali, Colombia http://www.flickr.com/photos/ciatevents/sets/72157629659518859/detail/ (accessed 10 April 2012).

Rogers, S.O., and A.J. Bendich. 1985. Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissues. Plant Mol. Biol. 5:69-76.

Rosas, J.C., A. Castro, J.S. Beaver, C.A. Perez, A. Morales-Gomez, R. Lepiz. 2000. Genetic improvement of the tolerance to high temperature and resistance to bean golden mosaic virus on common beans. Agron Mesoam. 11:1-10.

Rosas, J.C., J.C. Hernandez, R. Araya. 2003. Registration of 'Bribri' small red bean (race mesoamerica). Crop Sci. 43:430–43.

Rosielle, A.A., and J. Hamblin. 1981. Theoretical aspects of selection for yield in stress and non-stress environments. Crop Sci. 21:943-946.

SAS Institute Inc., 2011. SAS/STAT Users' Guide. SAS Institute Inc., Cary, NC, USA.

Sane, A.P., B. Stein, and P. Westhoff. 2005. The nuclear gene HCF107 encodes a membrane-associated R-TPR (RNA tetratricopeptide repeat)-containing protein involved in expression of the plastidial psbH gene in Arabidopsis. Plant J. 42:720-730.

Schmutz, J., P. McClean, S. Mamidi, G.A. Wu, S.B. Cannon, J. Grimwood, J. Jenkins, S. Shu, Q. Song, C. Chavarro, V. Geffroy, S.M. Moghaddam, D. Gao, B. Abernathy, K. Barry, M. Blair, M.A. Brick, M. Chovatia, P. Gepts, D.M. Goodstein, M. Gonzales, U. Hellsten, D.L. Hyten, G. Jia, J.D. Kelly, D. Kudrna, R. Lee, M.M.S. Richard, P.N. Miklas, J.M. Osorno, J. Rodrigues, V. Thareau, C.A. Urrea, M. Wang, Y. Yu, M. Zhang, R.A. Wing, P.B. Cregan, D.S. Rokhsar, and S.A. Jackson. 2013. A reference genome and domestication of common bean. Nature. In review.

Schneider, K.A., M.E. Brothers, and J.D. Kelly. 1997a. Marker-assisted selection to improve drought resistance in common bean. Crop Sci. 37:51-60.

Schneider, K.A., R. Rosales-Serna, F. Ibarra-Perez, B. Cazares-Enriquez, J. Acosta-Gallegos, J. Ramirez-Vallejo, N. Wassimi, and J.D. Kelly. 1997b. Improving common bean performance under drought stress. Crop Sci. 37: 43-50.

Siegfried, K.R., Y. Eshed, S.F. Baum, D. Otsuga, G.N. Drews, and J.L. Bowman. 1999. Members of the YABBY gene family specify abaxial cell fate in Arabidopsis. Development 126:4117-4128.

Silva, LDC, E., S. Wang, and Z.B. Zeng. 2012. Composite interval mapping and multiple interval mapping: Procedures and guidelines for using Windows QTL Cartographer. In: S.A.

Rifkin editors, Quantitative Trait Loci (QTL): Methods and protocols, Methods in Molecular Biology. Springer Science, New York. p. 75 -119.

Simmons, C.W., C. Hall III, and J.M. Osorno. Lipoxygenase activity and hexanal and hexanol concentration of Lariat pinto beans grown at various locations. AACC Meeting. Unpublished.

Sinclair T.R., C.B. Tanner, and J.M. Bennet. 1984. Water-use efficiency in crop production. BioScience 34:36-40.

Singh, S.P. 1991. Breeding for seed yield. In: van A. Schoonhoven, O. Voysest, editors, Common beans. Research for crop improvement. CAB Int., CIAT, Colombia.

Singh, S.P. 1995. Selection for water-stress tolerance in interracial populations of common bean. Crop Sci. 35:118-124.

Singh, R.K. and B.D. Chaudhary. 1985. Biometrical methods in quantitative genetic analysis Kalyani Publishers, Rajinder Nagar, Ludhiana, India. p. 318.

Spech, J.E., J.H. William, C.J. and Weidenbenner. 1986. Differential responses of soybean genotypes subjected to a seasonal soil water gradient. Crop Sci. 26: 922-934.

Sponchiado, B.N., J.W. White, J.A. Castillo, P.G. Jones. 1989. Root growth of four common bean cultivars in relation to drought tolerance in environments with contrasting soil types. Exp. Agric. 25:249-257.

Tar'an B., T.P. Michaels, K.P. Pauls. 2002. Genetic mapping of agronomic traits in common bean. Crop Sci. 42:544-556.

Terán, H., and S.P. Singh. 2002. Selection for drought resistance in early generations of common bean population. Can J. Plant Sci. 82:493-497.

Turner, S.R., and C.R. Somerville. 1997. Collapsed xylem phenotype of Arabidopsis identifies mutants deficient in cellulose deposition in the secondary cell wall. Plant Cell 9:689-701.

USDA-NRCS. 2013. Web Soil Surveys. Natural Resources Conservation Service. https://soilseries.sc.egov.usda.gov/OSD_Docs/J/JUANA_DIAZ.htm (accessed 2 Oct 2013).

USDA-NASS. 2013. North Dakota Agricultural statistics 2012. North Dakota Agricultural Statistics Service, North Dakota field office, U.S. Department of Agriculture, National Agricultural Statistics Service. http://www.nass.usda.gov/Statistics by State/ North Dakota/Publications/Annual Statistical Bulletin/index.asp. (accessed 29 Sept 2013).

Urrea, C.A., and T.G. Porch. 2009. Phenotypic evaluation of a subset of the *Phaseolus vulgaris* core collections and the *P. acutifolius* germplasm collection, and advanced common bean lines for drought tolerance in Nebraska. Annu. Rep. Bean Improv. Coop. 52:104-105.

Urrea, C.A., C.D. Yonts, D.J. Lyon, and A.E. Koehler. 2009. Selection for drought tolerance in dry bean derived from the Mesoamerican gene pool in western Nebraska. Crop Sci. 49:2005-

2010.

Vallejos, C.E., N.S Sakiyama, and C.D. Chase. 1992. A molecular marker-based linkage map of *Phaseolus vulgaris* L. Genetics 131: 733-740.

Voorrips, R.E., 2002. MapChart: Software for the graphical presentation of linkage maps and QTLs. The Journal of Heredity 93: 77-78.

Wallace, D.H. 1980. The genetics of photosynthesis and crop productivity -with emphasis on beans. In: Proc 14th Int. Congr. Genet. V.1, book 2. MIR Publ, Moscow. p 306-317.

Wang S., C.J. Basten, and Z.-B. Zeng. 2012. Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC. (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm).

Wei, G., Y. Pan, J. Lei, and Y.X. Zhu. 2005. Molecular cloning, phylogenetic analysis, expressional profiling and in vitro studies of TINY2 from Arabidopsis thaliana. J. Biochem. Mol. Biol. 38:440-446.

White, J.W., M. Ochoa, P. Ibarra, and S.P. Singh. 1994. Inheritance of seed yield, maturity and seed weight of common bean (*Phaseolus vulgaris*) under semi-arid rainfed conditions. J. Agric. Sci. 122:265–273.

White, J.W., and S.P. Singh. 1991. Breeding for adaptation to drought. p. 501-506. In A. van Schoonhoven, and O. Voyset (ed.) Common beans: Research for crop improvement. CAB International. Wallingford, UK & CIAT, Cali, Colombia.

Wright, E.M., and J.D. Kelly. 2011. Mapping QTL for seedand canning quality following processing of black bean (*Phaseolus vulga*ris L.). Euphytica 179:471-484.

Wortmann, C.S., R.A, Kirkby, C.A. Eledu, D.J. Allen.1998. Atlas of common bean (*Phaseolus vulgaris* L.) production in Africa. CIAT publication no. 297. Centro Internacional de Agricultura Tropical (CIAT). Cali, Colombia. 131 p.

Xu, S. 2008. Quantitative trait locus mapping can benefit from segregation distortion. Genetics 180:2201–2208.

Yamada, K., J. Lim, J.M. Dale, H. Chen, P. Shinn, C. J. Palm, A.M. Southwick, H.C. Wu, C.J. Kim, S.X. Liu, B. Lam, H. Sakano, T. Wu. G. Yu and J.R. Ecker. 2003. Empirical analysis of transcriptional activity in the Arabidopsis genome. Science 302:842-846.

Zhang, L., S. Wang, H. Li, Q. Deng, A. Zheng, S. Li, P. Li, Z. Li, and, J. Wang. 2010. Effects of missing marker and segregation distortion on QTL mapping in F_2 populations. Theor. Appl. Genet.121:1071-1082.

Zhong, L., W. Zhou, H. Wang, S. Ding, Q. Lu, X.Wen, L.Peng, L.Zhang, and C. Lu. Chloroplast. 2003. Small Heat Shock Protein HSP21 Interacts with Plastid Nucleoid Protein pTAC5 and Its Essential for Chloroplast Development in Arabidopsis under Heat Stress. Plant . Cell 25:2925-2943.