Selection of commercial and heirloom common bean (*Phaseolus vulgaris* L.) for organic production in Minnesota

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"Well, you just can't be an island out here," he said. One of my first days at the University of Minnesota, a scientist, unbeknown to me at the time, made it clear that my success at the U of M would be dependent on two things: personal initiative and support from the academic community. As my time at the university comes to an end, I reflect on how grateful I am to have had the support of so many energetic minds.

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DEDICATION

To anyone who has ever saved a seed…

ABSTRACT

The common dry bean (*Phaseolus vulgaris* L.) is an annual pulse crop produced and consumed around the world. Recent trends in the sales and consumption of organic food within the United States has led to an increase in organic cropland dedicated to the production of organic dry beans. Minnesota, in particular, has seen nearly a four-fold increase in organic dry bean production since 2008. Research dedicated to the evaluation and selection of a) current commercial market class cultivars and b) niche-market heirloom seed types is critical to enhance accessibility of productive seed that complies with organic regulation standards. Between 2012 and 2014, three trials were conducted in southern Minnesota and eastern North Dakota to evaluate commercial and heirloom common bean (*Phaseolus vulgaris* L.) performance in organic production.

Twenty-eight commercial market class cultivars were evaluated at five locations between 2012-2014 using a randomized complete block design. Yield data were subject to stability analysis using linear mixed model methodology, and gross revenue ac^{-1} was calculated from average yield within a market class. Yield across all seed classes and cultivars ranged from 1181 kg ha^{-1} to 2839 kg ha^{-1}. Analyses based on small, medium, and large seed size classes indicated increased yield and yield stability in the small and medium seed types. The large environmental effects and lower gross revenues exhibited by larger seed types suggest that growers interested in production of these types should have well-established soil and crop management practices.

Yield evaluation of seventeen heirloom cultivars was performed using a randomized complete block design in 2013 and 2014 at four locations around the Twin

Cities Metro region. Yields of heirloom cultivars were drastically lower than the commercial market class check included in the trial. Within the heirloom cultivars, yields ranged from 825 kg ha^{-1} to 2127 kg ha^{-1} to, with a mean of 1362 kg ha^{-1}. In contrast, commercial check cultivars yielded approximately 44% greater than heirloom cultivars. Stability analyses and economic incentives, however, suggest that production of heirloom cultivars, especially 'Jacob's Cattle Gold', 'Lina Sisco's Bird Egg', 'Peregion', and 'Tiger's Eye', may be a feasible enterprise for local growers.

Four heirloom dry bean cultivars, 'Jacob's Cattle Gold', 'Lina Sisco's Bird Egg', 'Peregion', and 'Tiger's Eye', were selected for pure line evaluation in 2013-2014 on the basis of market potential, yield, stability across locations in the heirloom dry bean yield trials. Sixty random plants were selected within each cultivar in 2012 and bulked seed from each plant (i.e. "pure line") was grown in 2013-2014 as a single plant rows. Sampling plants within each plant row for eight morphological traits provided estimates of genetic variation, including the standard deviation (*s*) of pure line means, coefficient of variation (CV) among pure lines, and broad-sense heritability (H^2) on an entry-mean basis. Selection for improved pure lines within 'Jacob's Cattle Gold', 'Lina Sisco's Bird Egg', 'Peregion', and 'Tiger's Eye' was performed after the 2013 season. A gain from selection trial was established during the 2014 season to compare the performance of selected improved pure lines within each cultivar to original heirloom populations. The pure line trial of 2013-2014 and the gain from selection trial in 2014 revealed that genetic variation within heirloom dry bean cultivars was sufficient to allow for selection of traits associated with maturity, yield, and plant architecture within an heirloom population.

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INTRODUCTION AND LITERATURE REVIEW

Taxonomy and Reproductive Biology

The common bean (*Phaseolus vulgaris* L.) is a member of the genus *Phaseolus* belonging to the subtribe Phaseolinae in the Leguminosae-Papilionaoideae family-subfamily designation (Delgado-Salinas et. al., 2006). Members of the Fabaceae (Leguminosae) family are typically characterized by leguminous fruits, compound leaves, and the ability to fix atmospheric nitrogen. The genus *Phaseolus* is represented by just over 70 diploid species ($2n=2x=22$), five of which are commonly cultivated: *P. vulgaris* (common bean), *P. lunatus* (lima bean), *P. coccineus* (scarlet-runner bean), *P. acutifolius* (tepary bean), and *P. polyanthus* (year-bean) (Andersson and de Vincente, 2010; Debouck, 1991; Evans, 1976). In their wild form, *P. vulgaris, P. lunatus, and P. acutifolius* are primarily self-pollinated with a limited amount of outcrossing; self-pollination of *P. coccineus* and *P. polyanthus* is typically inhibited in the wild (Evans, 1976; Smartt, 1988).

Debouck (1991) reviewed the genetic relationships among members of *Phaseolus*, framing species in relation to the primary gene pools of *P. vulgaris* (Harlan, 1975b; Smartt, 1981). Recent literature has indicated that two distinct primary gene pools exist within *P. vulgaris*, corresponding to the two centers of domestication in Mesoamerica and the Andean region of South America (Kwak and Gepts, 2009). *P. coccineus* and *P. polyanthus* have been characterized secondary gene pools for *P. vulgaris*, and numerous studies have reported successful hybridization between *P. vulgaris* and *P. coccineus* (Al-

Yasiri and Coyne, 1966; Smartt, 1970). Fertile crosses between *P. vulgaris* and its tertiary gene pool, *P. acutifolius*, have been obtained, though methods of embryo rescue and congruity backcrossing are frequently used (Anderson et. al., 1996; Singh, 2001; Waines et. al., 1988). Successful interspecific crosses between tepary and common bean germplasm are of particular interest, given the potential for enhanced abiotic stress resistance in common bean (Porch et. al., 2013). Though Honma and Heeckt (1959) reported a successful hybrid of *P. vulgaris* and *P. lunatus*, a successful hybridization has not since been reported. *P. lunatus* is generally understood to be a quaternary gene pool to *P. vulgaris* (Debouck, 1991; Singh, 2001).

Morphology and Classification of Common Bean

Developmental phases of the common bean (*P. vulgaris* L.) are categorized into vegetative (V0 - $V_{(n)}$) and reproductive (R5 - R9) growth stages (Kandel et. al., 2013; Pastor Corrales and van Schoonhoven, 1987). During the V0 and V1 stages, the common bean is noted for its epigeal germination and simultaneous taproot formation, followed shortly thereafter by adventitious growth of basal roots from the crown (Graham and Ranalli, 1997; Lynch, 1995). The vegetative nomenclature and morphological numbering system of the common bean has previously been described in detail by Debouck (1991). Stem elongation, formation of primary leaves, extension of lateral axes, and development of trifoliolate leaves are encompassed within the V2 -$V_{(n)}$ stages of vegetative growth. The R5 reproductive stage begins with the formation of the first flower bud. Determinate growth habits exhibit floral inflorescences at the terminal end of the main stem and lateral branches; indeterminate growth habits produce inflorescences at the nodes along the main stem (Debouck, 1991). Implications of these growth habits are discussed below. Stages R6-R9 are distinguished by flowering, pod formation, pod fill, and physiological maturation (Pastor Corrales and van Schoonhoven, 1987).

As previously mentioned, the annual form of the cultivated common bean exists in two primary forms: determinate and indeterminate. Differences in length of internodes, total number of nodes, leaf size, and growth of the axillary buds differentiate the two primary habits of common bean (Debouck, 1991). The four-tiered classification system proposed by Singh (1982) remains the most effective way to illustrate the variability in common bean growth habits. This system categorizes determinate cultivars as Type I on the basis of few nodes (3-7 vs. 7-15 total nodes), bush architecture, and a rapid decline in vegetative growth after flowering (Debouck , 1991; Singh, 1982). Types II, III, and IV are classified as indeterminate growth habits differentiated according to plant architecture: indeterminate-bush, climbing, or prostrate (Voysest and Dessert, 1991). Classification of growth habits is crucial to understand the potential of a cultivar in a cropping systems or environment, given the impact of photoperiod and temperature on the vegetative and reproductive development of the plant (Masaya and White, 1991).

Similarities in seed type and extensive use of common names for species within the *Phaseolus* genus have made the classification and documentation of common bean cultivars difficult. Voysest and Dessert (1991), however, have outlined four approaches of classification: growth habit, mode of consumption, seed characteristics, and duration of growth period. Identification of growth habit (see above) is routinely performed as an initial means of classifying common bean germplasm introduced to a new region or environment. Modes of consumption are perhaps the most practical means of classifying germplasm for end-use purposes; the common bean may be consumed at green (prior to physiological maturity), shell (mature seed), or dry (after physiological maturity) stages (Purseglove, 1968; Singh 1989). Though the green, also known as “snap,” stage is popular where processing facilities are readily available, the dry bean is grown most extensively around the world due to its high nutritive value and storability (Broughton et. al., 2003).

Within the dry bean mode of consumption, germplasm can further be divided into seed type classes on the basis of seed size, shape, and seedcoat characteristics (e.g. color and pattern) (Hidalgo, 1988; Voysest and Dessert, 1991). An immense amount of diversity among seed characteristics of the common bean exists as a result of dual domestications and intense human selection for horticultural traits in isolated regions. Commercial classification of seed types, however, has become standardized in recent years to encompass major seed types. The United States Department of Agriculture (USDA) currently publishes statistics on ten seed types: navy, black, pinto, cranberry, dark red kidney, light red kidney, pink, small red, great northern, small white (USDA, 2011a). These seed types (i.e. market classes) are recognized internationally, but there are numerous other classes recognized on regional scales, including yellow eye, white kidney, white marrow, heirloom, and others. Current commercial market classes are most commonly associated with elite cultivars of common bean bred extensively for favorable agronomic traits and processing quality.

Domestication of the Common Bean (*Phaseolus vulgaris* L.)

The first formal records of the common bean (*Phaseolus vulgaris* L.) coincided with the discovery of the Americas in the late fifteenth century (Gepts, 1988; Hendrick, 1931). The history of the common bean, however, extends back to its domestication nearly 6,000-8,000 years ago in the Tehuacan Valley of Mexico and regions within the Peruvian Andes (Evans, 1976; Gepts, 1998). Sampling of wild plant populations, archeological collections, analysis of phaseolin seed storage protein, and, more recently, molecular marker technology have provided evidence for dual domestication events within the species (Gepts, 1988; Gepts and Debouck, 1991; Kaplan and Kaplan, 1988; Kwak and Gepts, 2009). Gene pools within the Mesoamerican and Andean centers of origin can further be divided into races consisting of individuals with similar characteristics, including growth habit, physiological traits, phaseolin patterns, and distribution (Beebe et. al., 2001; Díaz and Blair, 2006; Singh et. al., 1991).

In its wild form, *P. vulgaris* exists primarily as an annual, multi-branched climbing plant, though a perennial habit has been noted on rare occasions (Gentry, 1969). The Mexican wild form (*P. vulgaris* var. *mexicanus*) is typically distinguished from the Andean (*P. vulgaris* var. *arborigineus*) wild form by its smaller seeds, narrower pods, larger bracteoles, and increased number of flowers per inflorescence (Brücher, 1988; Delgado Salinas et. al., 1988; Gepts and Debouck, 1991). Though independent domestications of *P. vulgaris* var. *mexicanus* and *P. vulgaris* var. *arborigineus* resulted in distinct gene pools, domestication of both followed similar trends.

Smartt (1988) outlined numerous molecular, biochemical, and physiological modifications that occurred within *P. vulgaris* during domestication. Trends of domestication from the wild-to-cultivated form included gigantism of morphological characteristics, loss of seed dormancy mechanisms, reduced seed shattering, and changes in growth habit and photoperiod sensitivity (Genty, 1969; Gepts, 1988; Singh, 2001; Smartt, 1988). Current dry bean cultivars are often warm-season annuals that exhibit photoperiod insensitive flowering response (Masaya and White, 1991).

Diversity between the two gene pools of common bean, however, has led to distinct differences in seed size, growth habits, biochemical markers, and maturity (Singh, 1989). Though there is variability within both the gene pools, Mesoamerian cultivars are typically noted by small (<25g 100 seeds^{-1}) and medium (25-40g 100 seeds^{-1}) seed types, while Andean cultivars are characterized by large (>40g 100 seeds^{-1}) seed types (Hidalgo, 1988). Variation in morphological and molecular traits between the two gene pools is significant enough that F_1 lethality or hybrid breakdown due to incongruity has occasionally been noted in hybrids between the two gene pools (Gepts, 1998; Gepts and Bliss, 1985). As researchers continue to dissect the evolutionary history of the common bean, biochemical and genetic markers will become increasingly important in understanding the underlying genetic diversity within the species today (Gepts et. al., 2008; Schmutz et. al., 2014, Singh et. al. 1991b).

Diversity and Conservation of Common Bean Germplasm

Additional seed type diversity between wild and elite germplasm exists as intermediary forms cultivated in regional settings for thousands of years (Sonnante et. al., 1994). Evolutionary principles (e.g. migration, drift, and genetic bottlenecks) and *in situ* selection pressures (e.g. abiotic stress and culinary attributes) influenced the domestication and selection for intermediary seed types (Gepts and Debouck, 1991; Hidalgo, 1991). A reduction in genetic variation coincided with the domestication and selection of wild types for improved human use (Sonnante et. al., 1994). Intermediary seed types that exhibit a wide range of genetic variation are commonly referred to as 'landrace' populations.

A working definition proposed by Camacho Villa et. al. (2006) is perhaps the most succinct and descriptive definition of a landrace to date: "A landrace is a dynamic population(s) of a cultivated plant that has historical origin, distinct identity and lacks formal crop improvement, as well as often being genetically diverse, locally adapted and associated with traditional farming systems." Terms such as 'heirloom,' 'heritage,' 'folk,' or 'farmer-bred' are commonly used synonyms for 'landrace.' Several authors have proposed alternative definitions for landrace and its respective synonyms (Harlan, 1975a; Preston et. al., 2011; Whealy, 1990). The definition proposed by Camacho Villa et. al. (2006), however, can be extended to all synonyms, depending on the relative emphasis placed on each component.

Though seed saving communities have arguably existed for hundreds of years, specialty N. American seed companies initiated an informal movement in the 1970s to preserve dry bean landrace populations distinguished by their colorful seedcoats and cooking quality (Harlan, 1975a; Seed Savers Exchange, 2014; Navazio, 2012). Today, dry bean landraces that were once region-specific are sold and distributed nationwide (in the U.S.) as heirloom cultivars through specialty seed companies, though the scale of production is minor.

The foundation of the U.S. dry bean seed industry dates back to the late 1700s. It was not until the early 1900s that a concerted effort was made on behalf of public and private programs to improve common bean genetics (Silbernagel and Hannan, 1988). Public breeding programs today focus primarily on the development of dry bean cultivars, while private breeding programs emphasize snap bean improvement (Myers and Baggett, 1999). The majority of common bean diversity accessible to breeding programs is housed in germplasm repositories, both domestically and internationally. The USDA's National Plant Germplasm System's (NPGS) collection of dry beans, curated in Pullman, Washington, maintains approximately 13,000 accessions of wild, landrace, and elite *Phaseolus* species (USDA, 2010). Global germplasm repositories, however, such as the International Center for Tropical Agriculture (CIAT), maintain the majority of *Phaseolus* accessions. It is estimated that 36,000 *Phaseolus* accessions, approximately 85% of which are *P. vulgaris*, are housed in the CIAT headquarters in Columbia (CIAT, 2013).

Preserved *ex situ* landraces are often used by breeders interested in the transfer of single-gene disease resistance, culinary or horticultural traits, and occasionally abiotic stress resistance into elite breeding lines (Cooper et. al., 2001; Silbernagel and Hannan, 1988). Introgression of traits, however, is difficult when the trait is multigenic (i.e. quantitative) and the genetic background of the accession is unfavorable from agronomic and quality perspectives (Sullivan, 1988; Tanksley and Nelson, 1996). As a result, most modern breeding programs do not readily utilize wild and landrace germplasm.

Consumption Patterns and Nutritional Quality of Common Bean

The common dry bean (*Phaseolus vulgaris* L.) is an important pulse crop worldwide, especially in Latin American, East African, and South African countries, where it is grown primarily for its nutritive qualities, cultural connectivity, and storability (Broughton et. al., 2003; FAO, 2011: Gepts et. al., 2008; Gowda et. al., 2009). Dry bean production occurs on an estimated 25-28 million hectares of cropland worldwide, though accurate estimates of world dry bean production are often confounded by alternative pulse crop production figures (Akibode and Maredia, 2011). In 2009, the top-five dry bean producing countries of the world were Brazil, Myanmar, India, China, and the United States, respectively (USDA, 2011e).

The majority of dry bean production occurs on small acreage and subsistence farms in many Latin American and African countries, where dry beans are an important caloric source and can contribute up to 30% of daily protein intake (Gepts et. al., 2008). Seed of common bean is composed of 15-25% protein on a dry-weight basis, though it is typically deficient in sulfur-containing amino acids, methionine, and cysteine (Ma and Bliss, 1978; Sathe, 2002). Dry beans are also known for their complex carbohydrate profile, high fiber content, low fat, and antioxidant properties (Anderson et. al., 1999; Reddy et. al., 1984). The relative nutrient composition of fresh pods/leaves and the dried seed of the common bean are similar when compared on a dry-matter basis (Shellie-Dessert and Bliss, 1991).

Factors related to storability and regional preferences, however, make the dry bean the most consumed pulse crops in the world. Consumption rates around the world vary dramatically for dry bean; the highest consumption rates are in Latin America (~11 kg capita^{-1} year^{-1}) and Sub-Saharan Africa (~5 kg capita^{-1} year^{-1}) (Lucier et. al., 2000). With an average consumption rate of 2.8 kg capita^{-1} year^{-1} (6.2 lb.), the U.S. has the lowest consumption of dry bean in the world (Zahniser, S. and Wells. 2014). Work by Lucier et. al. (2000) indicated that the U.S. Hispanic population, which makes up 11% of the total population, accounts for 33% of the total consumption. In addition, dry beans are typically consumed in lower income households in the U.S. (Lucier et. al., 2000).

Despite the relatively low consumption rates within the U.S., the nutritive qualities and health benefits of dry beans are so well recognized that school lunch programs around the U.S. are now required to serve at least 0.4 oz. (½ cup) of dry beans (or peas) per week (USDA, 2012a). Encouraging studies on the cancer-prevention properties of dry bean suggest cooked beans retain some anticancer properties, though the specific mechanisms have yet to be elucidated (Bennink, 2002; Thompson et. al., 2009). Numerous health professionals tout dry beans as a key part of a healthy diet and a means of preventing chronic illnesses such as heart disease, diabetes, and obesity. Zahnsier and Wells (2014), however, assert that doubling farm land dedicated to dry bean production would be needed in order to comply with current recommended intake of cooked dry beans per week (12 oz. or 1½ cups).

Common Bean Production in the U.S. Midwest

European immigration and the selection for regionally adapted plant material helped establish the foundation for commercial dry bean seed production in Northeastern regions of the U.S. (Silbernagel and Hannan, 1988). The majority of current dry bean cultivars were introduced by native communities to the Southwest U.S., especially those of Mesoamerican origin (Gepts, 1988; Hidalgo, 1988; Silbernagel and Hannan, 1988). Large-seeded, Andean types were introduced to the northeastern U.S. along Native Amercian trading routes and, to some extent, European settlement (Hendrick, 1931). Large-scale production began to slowly expand westward at the start of the twentieth century, as bacterial and fungal diseases were favored under the high-moisture, rainy conditions of the northeast. The dry summers of western U.S. were ideal for commercial production of dry bean seed; areas of eastern Washington, California, and Idaho are still known for their production of certified bean seed (Navazio et. al., 2007).

An estimated 584,851 ha (1,445,200 ac), with a production value of approximately $763 million, of cropland were dedicated to U.S. dry bean production in 2008 (USDA, 2011e; Zahniser and Wells, 2014). The U.S. Midwest, however, is currently the nation's largest producer of dry beans. According to 2010 harvest data, the states with the highest dry bean production included North Dakota (42%), Michigan (13%), Minnesota (10%), Nebraska (8%), and Idaho (7%) (USDA, 2011a).

Reports from USDA-NASS estimated 62,726 ha (155,000 ac) of dry beans were planted in Minnesota in 2014 (USDA, 2015). The majority of dry bean production was located in the northwest region of the state. Production was up nearly 23% over 2013, with total statewide production estimated to be 2.89 million hundredweight (cwt). Top producing counties were Stevens, Kandiyohi, and Swift. Average reported yields were 2,186 kg ha^{-1} (1,950 lbs. ac^{-1}) (USDA, 2015). Minnesota's market class production in 2011 was distributed among navy (40%), red kidney (30%), black bean (14%), and other (16%) (USDA, 2011d).

Dry beans require warm-season temperatures and well-drained soils for Midwest production. To avoid common root rot diseases and fungal growth in cool, wet soils, planting typically occurs in late May or early June after soil temperatures warm to approximately 18°C (65°F) (Navazio, 2012). The majority of dry bean production in the Midwest occurs on dryland, rainfed sites, though irrigated production is noted. Seeding rates (56-135 kg ha^{-1} or 50-120 lb. ac^{-1}) and target plant populations (185,000-247,000 plants ha^{-1} or 75,000-100,000 plants ac^{-1}) vary depending on the relative seed sizes and plant growth habits. Dry beans are commonly planted on 0.38-0.76 meter (15-30 inch) row centers, though production trends indicate narrower rows production is increasingly commonplace (Kandel et. al., 2013). Inoculation with common bean nitrogen-fixing rhizobia, *Rhizobium phaseoli*, is common, though supplemental (typically less than 44.8 kg ha^{-1} or 40 lb. $acre^{-1}$) N is required to attain maximum yields (Myers, 1999; Kandel et. al., 2013). On many midwestern soils, soil phosphorus, zinc, and pH levels may be constraints to achieving maximum yields.

Relative maturities of dry bean are dependent on growing location and yearly conditions, but typically dry beans mature 85-120 days after planting (DAP). Dry beans are harvested after physiological maturity, when the pods are yellow-to-tan in color and seeds retain approximately 15-18% moisture (Kandel et. al., 2013). Balancing seed moisture with shattering and seed splitting is crucial to maintain adequate seed quality standards (Schumacher and Boland, 2011). Under commercial production, Type III, climbing dry beans are swathed and windrowed at harvest, while Type I and Type II beans with upright, bush architecture are well suited to direct harvest equipment (Myers, 1999; Urrea and Ostdiek, 2014). Barriers to small-scale production include production issues such as labor, land required relative to their market value, and lack of processing equipment. Researchers at the Organic Seed Alliance, University of Vermont, and Washington State University, however, have begun to experiment with threshing and cleaning equipment suited to small-scale production (Colley et. al., 2010; Harwood, 2011; Miles, 2015).

Bacterial and fungal diseases remain persistent issues in Midwestern dry bean production, though genetic studies and breeding efforts over the past thirty years have resulted in numerous resistant cultivars (Miklas et. al., 2006; Singh, 1991, 2001). Fungal root rots (*Fusarium, Rhizoctonia, and Pythium*) and white mold (*Sclerotinia sclerotiorum*) are problematic soil-borne diseases in the Midwest, particularly in the production of dry beans (Kandel et. al., 2013). Anthracnose (*Colletotrichum lindemuthianum*), a fungus identified by sunken, circular lesions on the pods and seeds, has also been noted in North Dakota and Minnesota production (Hagedorn and Inglis, 1986). Diseases impacting the ability of farmers to save and replant seed, such as anthracnose, common bacterial blight (*Xanthomonas campestris* pv. *phaseoli*), and bean common mosaic virus (BCMV), are still of particular concern in non-resistant commercial and heirloom cultivars (Navazio et. al., 2007). Best management practices for the control of many diseases include a four-year crop rotation between dry bean crops, use certified disease-free seed, cultivation when plants are dry, and cultivars with resistance to multiple pathogenic and race resistances (Kandel et. al., 2013).

Certified Organic Production in Minnesota

As part of the 1990 Farm Bill, Congress passed the Organic Food Production Act (OFPA, Title XXI) in response to shifts in production ideology and demand for a regulatory framework that certified organic food production (Greene, 2009; USDA, 2014a). The OFPA allowed the USDA to establish the National Organic Program (NOP), under the advisement of a National Organic Standards Board (NOSB). Organic regulation standards aim to identify resilient systems that integrate biodiversity and soil-building tactics (i.e. cover crops, composting, fallow periods) into production (USDA, 2014a). Increased tillage to control weeds, inability to immediately correct nutrient issues, need for longer rotation periods, and use of only organically-approved substances are differentiating characteristics between organic and conventional production settings (Lammerts van Bueren and Meyers, 2012; Moncada and Sheaffer, 2010).

The current USDA "organic" label indicates that the production of crops, livestock, processed products, and wild crops adheres to the established organic regulation standards. Application for a certified organic production is producer-initiated, whereby a producer must submit an application outlining the operation and products under consideration, a three-year history of the production land, and an Organic System Plan (USDA, 2014a). The completed application and Organic System Plan are submitted to an accredited USDA certifying agency that reviews and approves applications in compliance with the established organic regulation standards. According to the NOP, eighty certifying agencies oversee the application and certification process of organic productions; 48 certifying agencies are based in the U.S and 32 are foreign (USDA, 2014a). In total, 12,880 farming operations in the U.S., including livestock, pastureland, poultry, and cropland, were certified organic in 2011 (USDA, 2011b).

Minnesota currently ranks 7th in number of organic farming operations, 10th in total organic acreage, and 12th in organic sales within the U.S. (Hartwig and Lofthus, 2012). As of 2011 estimates, seventeen certifying agencies operating in Minnesota certified 555 farming operations on approximately 61,923 hectares (153,014 acres) of certified organic land (USDA, 2014a; USDA, 2011b). The top five counties with the most organic operations in 2011, in ranked order, were Stearns, Winona, Fillmore, Polk, and Goodhue (Hartwig and Lofthus, 2012).

The majority of organic dry bean production is found in the Red River Valley region of Minnesota, where there is also a high concentration of conventional dry bean production (Northarvest Bean, 2015; USDA, 2015). Organic dry beans were harvested from an estimated 1,011 hectares (2,498 acres) in Minnesota in 2011, which accounted for approximately 8.7% of total U.S. organic dry bean cropland (USDA, 2011c). Unfortunately, few data exist that describe farm size and scale of production, particularly with regard to small-scale growers. It has been noted, however, that acreage dedicated to organic dry bean production nearly quadrupled in the state of Minnesota from 2008-2011 (USDA, 2011c).

Market Outlets for Dry Bean Production in Minnesota

Dry bean producers are typically under contract through a local distributor and/or processor, and the majority of U.S. dry bean production caters to large-scale distribution and export markets (Schumacher and Boland, 2011). Including *Vigna* species, the U.S. exported approximately 359,109 million dollars (20% of total production) worth of dry beans in 2014; approximately 143,445 million dollars (14% of domestic consumption) worth of dry beans were imported (USDA, 2014b) (Schumacher and Boland, 2011).

The University of Minnesota recently identified dry beans as a target food for farm-to-school lunch programs and recent surveys conducted by the Regional Sustainable Development Partnerships (RSDP) (2014, unpublished data) suggest demand for organic beans in direct-to-consumer markets. Survey results indicate that there is new demand among consumers and restaurants for locally produced, organic dry beans in the Twin Cities and Greater Minnesota region (RSDP, 2014, unpublished data). Participating restaurant managers cited an average willingness-to-pay (WTP) of $2.85 per pound for non-heirloom, organic dry beans and $4.78 per pound for heirloom, organic dry beans. According to growers who primarily sell their heirloom dry beans through farmer's markets, consumers exhibit a WTP between $6.00 and $8.00 per pound (John Breslin, 2015, pers. comm., 30 January); Paula Foreman, 2013, pers. comm., 10 October). Coupled with additional marketing research, educational tools, and platforms that connect producers with consumers, local dry bean producers may enter direct-to-consumer markets without difficulty (Grimsbo Jewett et. al., 2007).

Project Rationale

The nutritional profile, storability, local production, and marketability of dry beans, suggest they may be a key player in the farm-to-table movement and food security in Minnesota (Galzki et. al., 2014). In addition, dry beans can serve as a viable economic alternative within an organic rotation and help to diversify production. Though some breeding work specific to dry beans produced in organic systems is ongoing in the U.S., research specific to dry beans in Minnesota organic systems has been minimal. The plant ideotype identified by plant breeders interested in organic production is typically differentiated from that of conventional settings (Lammerts van Bueren and Meyers, 2012); adequate performance in low plant densities, spreading plant canopies, increased biological nitrogen fixation in legumes, vigorous rooting systems, and cultivar stability under heterogeneous environments are all plant traits necessitated by the management strategies and environmental pressures in organic systems (Lammerts van Bueren and Meyers, 2012).

The first objective of this research is to evaluate current commercial cultivars of dry bean for organic production in Minnesota and a) establish the optimal market classes for organic production b) provide organic growers with cultivar recommendations and c) establish the foundation to pinpoint traits specific to Minnesota organic production to target in future breeding efforts. The second objective is to serve and inform producers marketing primarily direct-to-consumer, including restaurants, CSAs, and food coops. Improvement of niche-market heirloom cultivars, through pure line selection methods, will help ensure that producers a) have access to improved heirloom seed in farm-scale quantities and b) maintain the capacity to produce a stable, uniform, and marketable crop.

CHAPTER ONE

Yield Evaluation of Commercial Dry Bean Market Classes (*Phaseolus vulgaris* L.): Cultivar Development for Minnesota Organic Production

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Summary

There has been a four-fold increase in land dedicated to certified organic dry bean production in Minnesota since 2008, and production is expected to increase as demand for organic dry bean rises. Available commercial cultivars have not yet been extensively evaluated in Minnesota organic production systems, necessitating research dedicated to Midwest organic dry bean production. Our objective was to identify suitable market classes for Minnesota organic production on the basis of yield performance and stability across diverse environments. Experiments to determine the yield and stability of 28 commercial dry bean cultivars were conducted from 2012 to 2014 by evaluating at five sites in southern Minnesota and central North Dakota.

Stability analyses comparing yield performance in favorable and unfavorable environments identified specific cultivars with exhibited dynamic (agronomic) stability. The relatively high-yielding small and medium-seeded market classes (i.e. black, pinto, and navy) were well suited to organic production and may provide growers with an adequate economic return. While there is market demand, large seeded market classes (i.e. kidney and cranberry) may be best suited for growers with previous dry bean

production experience, given their comparatively low yields, inferior economic return, and potential for production issues.

Introduction

As the result of dual domestications nearly 8,000 years ago, the common dry bean (*Phaseolus vulgaris* L.) retains an immense amount of genetic diversity that can be exploited to enhance agronomic capabilities and cultural attributes (Gepts, 1998). Though it is difficult to obtain accurate estimates of world production, dry bean production ranges between 25-28 million hectares (62-69 million acres) of cropland (Akibode and Maredia, 2011; USDA, 2011e). The common dry bean is now a staple pulse crop around the world, grown most extensively in tropical climates for its nutritive qualities, cultural connectivity, and storability (FAO, 2011: Gepts et. al., 2008; Gowda et. al., 2009).

Within the United States, dry bean production has remained relatively static over the past ten years and consumption rates are among the lowest in the world (Lucier et. al., 2000; USDA, 2011e; Zahniser and Wells, 2014). The organic sector's production, however, has nearly tripled since 2005. And while it comprises only 2.38% of the total dry bean production area, the positive trend in production mimics the rise in organic sales of fruit and vegetables during the same period (Greene, 2013; USDA, 2011f).

The U.S. Midwest is a major dry bean producing region, comprising nearly 50% of national production. Minnesota accounts for 10% of total dry bean production within the U.S., approximately 9% of the national organic dry bean farmland, and has seen an almost four-fold increase in certified organic dry bean production area since 2008 (USDA, 2011c). This area is expected to increase as demand for organic dry bean rises, justifying an increase in agronomic and plant breeding research dedicated to Midwest organic dry bean production systems.

From a breeding perspective, the ability to support the rise in organic dry bean production depends upon appropriate utilization of available germplasm and genetic diversity that confers an economic advantage in organic production systems. The aforementioned inherent diversity within the dry bean is exemplified by the available market seed classes, such as navy, black, pinto, cranberry, kidney, small red, heirloom, etc. Each market class represents a specific seed type that is associated with a gene pool and race (Kwak and Gepts, 2009; Singh et. al., 1991a), resulting in diversity both within and among each market class. While nearly all market classes are represented in today's U.S. agricultural scene, the navy, black, and pinto bean classes are predominant, especially within the Midwest. Minnesota's market class production is distributed among navy (40%), red kidney (30%), black bean (14%), and other (16%) (USDA, 2011d).

Current commercial dry bean cultivars have been predominantly selected under field conditions consistent with conventional production systems. It has been suggested that the use of conventionally developed cultivars may be contributing to lower yields in organic systems (Burger et. al., 2008; Murphy et. al., 2007; Lammerts van Bueren et. al., 2002). Traits favored in organic systems that would be less important in conventional systems include those that provide the plant with a competitive edge over weeds. These traits include seed emergence vigor, canopy closure, and enhanced rooting systems capable of adequate N-fixation (Lammerts van Bueren and Meyers, 2012). Selection under conventional settings, however, does not always mimic the environmental pressures and conditions typical of organic systems. When breeding cultivars for organic systems, it may therefore be beneficial to follow the well-established practice of making selections only within the target environment (Bernardo, 2010; Fehr, 1987).

Except for a study by Singh et. al. (2011) conducted in the western U.S., the effect of direct selection within U.S. organic dry bean production systems has not been extensively examined. In an earlier study, Singh et. al. (2009) reported significant interactions between dry bean genotypes and production systems in southern Idaho and suggested that accurate estimations of a cultivar's adaptation would require evaluation in multiple diverse production systems. In a comparative study, Heilig and Kelly (2012), estimated that Michigan organic dry bean yields were approximately 20% lower than conventionally produced dry bean yields. Given the deficiencies in current organic dry bean production knowledge, the rising market demand, and apparent yield disparity between dry beans produced in differing systems, trials specific to organic production systems are warranted.

There is a need to evaluate performance of current commercial market class cultivars in organic settings, identify market classes best suited to organic systems, and guide future organic breeding efforts (Lammerts van Bueren and Meyers, 2012). We hypothesize that certain dry bean market classes, and particular cultivars within those market classes, exhibit higher yield and yield stability across environments in organic production systems of Minnesota. This research will provide some of the first cultivar recommendations for growers either currently producing or considering transition to organic dry bean production in Minnesota.

Materials and Methods

Plant Material and Seed Pretreatments

Untreated seed of twenty-eight common dry bean (*P. vulgaris* L.) cultivars were collected from commercial sources and public bean breeding programs in the U.S. and Canada (Table 1.1). These cultivars were selected to represent cultivars and market classes commonly produced in the Upper Midwest (Knodel et. al., 2014). Eleven market classes and twenty-eight commercially registered cultivars, whose registrations spanned forty years, were represented in the trials. Cultivars were assumed to be pure lines with low levels of outcrossing. Germination was tested using the 'Between Paper' method specific to common bean (Rao et. al., 2006). Just prior to planting, seed was inoculated with a commercial source (Novozymes, Franklinton, NC) of N-fixing Rhizobium bacteria (*Rhizobium leguminosarum* biovar *phaseoli*) in a peat-based suspension.

Experimental Design

Yield evaluations were conducted during the 2012-2014 Minnesota growing seasons. Experimental plots were established at five locations: Southwest Research and Outreach Center in Lamberton, MN; A-Frame Farms in Madison, MN; Rosemount Research and Outreach Center in Rosemount, MN (2012-2013); NDSU Agricultural Station in Carrington, ND (2014 only); and the Sand Plain Research Farm in Becker, MN (Table 1.2) (NOAA, 2015). The Lamberton, Madison, and Carrington locations were

USDA certified organic, while the Rosemount and Becker locations were managed according to the UDSA guidelines for two years prior to the onset of the experiment (USDA, 2014a). No fertilizer or insecticides were applied. Soil pH, P, and K levels at all locations were adequate for dryland dry bean production (Franzen, 2013). All locations were maintained as rain fed sites except Becker, MN, which was irrigated per recommended practice in the Anoka Sand Plain.

The experimental design for each experiment was a randomized complete block design with three replications, except for Carrington, ND, which consisted of four replications. A block consisted of sixteen treatments in both the 2012 and 2013 seasons and nineteen treatments in 2014 season, with each treatment corresponding to an edible dry bean cultivar (Table 1.1). Cultivars were not consistent between years, but were consistent among locations within each year. An experimental plot consisted of a single treatment planted in a two-row plot that was 6.1 meters (20 feet) long with 0.76 meters (30 inches) row spacing. Seed count was adjusted for germination and 5% seedling mortality to obtain a target seeding rate of 172,900 plants ha^{-1} (70,000 plants ac^{-1}) for large-seeded cultivars and 222,300 plants ha^{-1} (90,000 plants ac^{-1}) for small and medium seeded cultivars (Hidalgo, 1988). Experiments were seeded between days 145-166 (May 25th - June 15th) on the Julian calendar. The soil had been prepared by chisel plowing and then finished by field cultivating before seeding. Previous crop was corn (*Zea mays* L.), wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), or durum wheat (*Triticum durum*). Weeds were controlled using both mechanical cultivation and occasional hand weeding. At most locations, two cultivation passes were necessary before canopy closure.

Data Collection

Plots were hand harvested when all pods were beyond physiological maturity during late September and early October (Kandel et. al., 2013). The inner 2.44 meters (8 feet) of each two-row plot was harvested by pulling the entire plant. Plant populations within the harvested area were determined. Harvested plants were placed in a low-temperature (35°C) dryer overnight and threshed (Almaco, Nevada, IA); harvested seed was cleaned of debris and diseased seed prior to weighing. Reported per-plot yields were adjusted to 18% moisture and are expressed in kg ha^{-1} (Figure 1.1; Table 1.3).

Statistical Analysis

Environments were defined as specific location-year combinations. To aid in comparisons across market classes, cultivars were grouped according to small, medium, and large seed sizes as classified by Hidalgo (1988). Separate analyses were performed for each seed size class. All data analyses were performed using R-software (version 3.1.2); models were executed and evaluated using the 'lme4' package (Bates et. al., 2014; R Core Team, 2014).

To account for the unbalanced nature of the data set, the following linear mixed effects model was fit using restricted maximum likelihood (REML):

$$y_{ijk} = \mu + \alpha_i + \beta_{(k)j} + (\alpha\beta)_{ij} + \varepsilon_{ijk} \qquad \text{(Eq. 1.1)}$$

where y_{ijk} is the measured yield observation of the i^{th} cultivar in the j^{th} environment ($i = 1, 2, \ldots, g; j = 1, 2, \ldots, e; k = 1, 2, \ldots, r_j$), μ is the overall mean, α_i is the effect of the i^{th} cultivar, $\beta_{(k)j}$ is the effect of the k^{th} replication nested within j^{th} environment, $(\alpha\beta)_{ij}$ is the effect of the i^{th} cultivar with the j^{th} environment, and ε_{ijk} is the random error term. Fixed effects included μ and α_i, whereas $\beta_{(k)j}$, $(\alpha\beta)_{ij}$, and ε_{ijk} were fit as random effects. As such, $\beta_{(k)j}$, $(\alpha\beta))_{ij}$, ε_{ijk} were declared independent and normally distributed random effects and scaled toward zero with a variance of $\sigma_\beta{}^2$, $\sigma_{\alpha\beta}{}^2$, $\sigma_\varepsilon{}^2$, respectively (Yang, 2007). The significance of each random effect variance component was determined from the log-likelihood ratio test statistic, comparing models fit via maximum likelihood with and without the variance component in question, to a Chi square distribution with one degree of freedom (Table 1.4). In the medium and large seed classes, the effect of replication nested within environment was non-significant and excluded from Eq. 1 during downstream analyses, resulting in the model:

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ij} \qquad \text{(Eq. 1.2)}$$

Using the REML procedure allowed for the unbiased estimation of cultivar effects in a highly unbalanced data set. Variance associated with the fixed effects was partitioned into the random effect terms (i.e. $\sigma_\beta{}^2$, $\sigma_{\beta\alpha}{}^2$, $\sigma_\varepsilon{}^2$), thus minimizing the variance among all fixed effect estimates (Bernardo, 2010; Piepho et. al, 2003; Piepho et. al., 2008). Though it has been demonstrated that holding genotype (i.e. cultivar) as a random effect is more advantageous than declaring it a fixed effect, cultivars included in these trials were purposefully selected, had predictable influence on the data, did not conform to the necessary assumption of random sampling (Piepho et. al., 2003; Smith et. al., 2005; Winter, 2013). Therefore, the decision was made to fit cultivar as a fixed effect. Profile confidence intervals (95%) were calculated for each cultivar (Figure 1.1).

Environments were deemed favorable or unfavorable for each seed class according to the predicted effect of environment in the final model; positive β_j

predictions were classified as a favorable environment whereas negative β_j predictions were classified as an unfavorable environment. Final models (i.e. Eq. 2 for the medium and large seed classes) were reevaluated for each set of favorable and unfavorable environments within a seed class and effects of cultivar were again estimated in these environments. To visually assess Type II (dynamic) stability (Becker and Léon, 1988; Lin et. al., 1986), the best linear unbiased estimate (BLUE) of yield for each cultivar within favorable and unfavorable environments was subject to biplot analysis, where BLUEs of entries in favorable and unfavorable environments were plotted on the x- and y-axes, respectively (Figure 1.2). Biplots were divided into four quadrants on the basis of mean yield in each respective environmental grouping.

Due to lack of available information on organic seed pricing and production inputs, a full economic cost analysis was not possible. Average yields of each market class, however, were used to estimate gross revenue per acre. Price per hundred weight ($\$ \text{ cwt}^{-1}$), or the price for one hundred pounds of grain, was estimated according to quotes from two organic dry bean distributors in North Dakota and Minnesota (Table 1.5). Quoted prices represent grower prices out-of-the-field and are subject to market fluctuations. Estimated gross revenues are, in some cases, calculated based on the yield of only one cultivar. Grower prices were unavailable for heirloom, great northern, and cranberry market classes.

Results

Random effects of environment were statistically significant ($p < 0.001$) across all seed size classes (Table 1.4). Large seeded cultivars were most affected by the influence of environment; over 70% of the model's variance was attributed to the effect of environment. Experimental error was a primary contributing factor to the model's variance, particularly in the small and medium seed classes, whereas the cultivar x environment interaction effect, $(\alpha\beta)_{ij}$ contributed the least to the model's variance. In the medium seeded class, the $(\alpha\beta)_{ij}$ was a non-significant variance component, but, despite its insignificance, the $(\alpha\beta)_{ij}$ effect was retained in the medium seeded model so comparisons could be made between seed classes.

Yield across all seed classes and cultivars ranged from 1181 kg ha^{-1} (OAC Lyrik) to 2839 kg ha^{-1} (Maverick). Medium seeded ($\bar{x}$ = 2408 kg ha^{-1}) cultivars outperformed small ($\bar{x}$ = 2202 kg ha^{-1}) and large ($\bar{x}$ = 1546 kg ha^{-1}) seeded cultivars (Table 1.3). Black and pinto were consistently high-yielding market classes within the small and medium seed classes, and pink and small red were the highest yielding market classes within the medium seed class. No observable trend in yield was observed among cultivars in the large seed class.

Examination of 95% confidence intervals associated with the yield BLUEs suggested true differences among cultivars within each seed class (Figure 1.1). Highest yielding cultivars within the small, medium, and large seed classes were 'Zenith' ($\bar{x}$ = 2621 kg ha^{-1}), 'Maverick' ($\bar{x}$ = 2840 kg ha^{-1}), and 'OAC Inferno' ($\bar{x}$ = 2385 kg ha^{-1}), respectively. Lowest yielding cultivars within the small, medium, and large seed classes were 'Lightning' ($\bar{x}$ = 1749 kg ha^{-1}), 'Matterhorn '($\bar{x}$ = 1966 kg ha^{-1}), and 'OAC Lyrik' ($\bar{x}$ = 1181 kg ha^{-1}), respectively. Issues with plant populations were present in all three years of testing. In no instance did a cultivar achieve the target plant population. In all three classes, the lowest yielding cultivar was also associated with the lowest plant population (Table 1.3).

Favorable and unfavorable environments differed between the seed size classes. Seven favorable environments and five unfavorable environments were identified in the small seeded class; five favorable and seven unfavorable environments were identified in the medium and large seeded classes. Across all classes and years, the Rosemount and Carrington locations were considered unfavorable and the Madison location was considered favorable. The Becker location was considered unfavorable in all classes, except for the small seeded class in 2014. The Lamberton location was considered favorable in all classes, except in 2013 for the medium and large seeded classes.

Yield of each cultivar within a seed class was subject to stability biplot analysis by plotting yield in favorable environments on the x-axis and yield in unfavorable environments on the y-axis (Figure 1.2). Cultivars that performed well in both favorable and unfavorable environments were plotted in the upper-right quadrant of the graph. Cultivars that appeared in the upper-left quadrant are of notable exception, as these cultivars performed adequately in unfavorable environments. There were no cases in which a cultivar performed better in an unfavorable than in a favorable environment.

Gross revenue per acre was highly associated with yield (Table 1.5). Maximum gross revenue was associated with the pink market class (\$2378 ac^{-1}), while minimum gross revenue was obtained from the dark red kidney market class (\$1402 ac^{-1}). It should be noted that the pink and small red market classes consisted of only one cultivar;

reservation must be taken in evaluation of these revenue estimates. According to price estimates, kidney seed types received \$0.10-\$0.25 premium cwt^{-1} over black, navy, pinto, small red, and pink market classes. Despite this price differential, yield disparities still made small seeded market classes more profitable than all kidney types. The one deviation from this trend was the light red kidney, which was slightly more profitable than the navy market class (Table 1.5).

Discussion

The observed higher yields and stability trends of small/medium seed classes, versus large seeded types, concur with previously reported comparisons (Kelly et. al., 1987; Singh et. al., 2007). Medium seeded cultivars, often displaying Type II or III upright short vine growth habit, typically exhibit the highest yield and stability across environments (Kelly et. al., 1987). This is in contrast to large seeded, Type I, bush growth habits that are typically more prone to greater environmental effects and lower yields, as was the case in this data set. The small seeded class, however, exhibited the greatest cultivar x environment interaction effect within this experiment. Direct comparisons between stability of edible dry beans with different growth habits must be executed with caution. The relatively small number of cultivars present within each market class, however, prohibited intra-market class stability analyses in this study. Regardless, stability analyses were informative when comparing cultivars grouped according to seed size.

Under the Type II (dynamic) concept of stability, a cultivar is considered stable if its yield response mimics that of the environment in which it was grown (Becker and Léon, 1988; Lin et. al., 1986). The dynamic concept places greater emphasis on the effect of the environment (versus a purely genotypic effect) in determining the stability of a cultivar. This can also be interpreted as an “agronomic” form of stability, whereby a cultivar responds favorably to increased inputs and good management practices (Becker and Léon, 1988). Cultivars found in the upper-right quadrant of the stability plots, such as ‘Lariat’, ‘Maverick’, ‘OAC Inferno’, ‘Rosetta’, and ‘Super Jet’, exhibited Type II stability (Figure 1.2). Dynamic yield stability of these cultivars suggests adaptation and potential in a cultivar of organic environments.

While the Type II approach is highly correlated with yield, it also allows for the identification of cultivars that might perform well in marginal or unfavorable environments. Cultivars ‘OAC Rex’, ‘Stampede’, ‘Merlot’, ‘Krimson’, and ‘Snowdon’ were all located in the upper-left quadrant of the stability biplots, suggesting some adaptability or preference to the unfavorable environments (e.g. sandy soil at Becker, increased weed pressure at Rosemount, or cooler average temperatures in Carrington). Whether their performance in unfavorable environments was sufficient to compensate for their lack of performance in favorable environments, however, is likely dependent on grower-specific circumstances.

It is also important to note that stability and adaptability are not defined by yield alone. To properly declare adaption to a specific region or production system, additional traits must be examined (Kelly et. al., 1998). Such traits may include maturity, harvest index, growth habit, and seed size; none of which were evaluated in this trial. Yield must also be balanced with additional traits that are advantageous in organic systems, such as vigorous emergence, large canopy, disease resistance, and enhanced biological N fixation (Lammerts van Bueren and Meyers, 2012). It is crucial that these yield components be evaluated as a next step, even before quality issues such as canning quality, color, shape, and seedcoat quality are evaluated. Further, the lack of available organic seed and ill-defined market prices make it difficult to compile a comprehensive enterprise budget; details regarding production inputs would contribute to a complete economic analysis.

Growers new to Minnesota dry bean production and/or organic management should first consider reliable, stable market classes such as pinto, pink, and black. In doing so, growers are prepared to obtain stable yield and a reliable economic return. Cultivars representative of the small/medium seed classes, such as ‘Maverick’, ‘Rosetta’, and ‘Zenith’, exhibited adequate dynamic stability and were less influenced by effects of environment. Because large seeded market classes were subject to larger environmental effects and provided less economic return, the production of large seeded cultivars, such as ‘OAC Inferno’ or ‘Majesty’, may be best suited for organic producers with well-established management strategies or previous dry bean production experience. It is of the utmost importance, however, that growers evaluate their system, soil, and experience in conjunction with cultivar recommendations.

Table 1.1 Cultivars descriptions (cultivar names, market class, registration, origin, seed size, plant habit) of the twenty-eight dry bean (*P. vulgaris*) cultivars included in 2012-2014 yield trials.

Entry	Cultivar Name	Market Class[1]	Registration[2]	Origin	Seed Size[3]	Plant Habit[4]
1	Alpena	Navy	2014	MSU	S	USV
2	Avalanche	Navy	2011	NDSU	S	USV
3	Cabernet	DRK	1999	Seminis	L	B
4	Coyne	GN	2009	U. Nebraska	M	V
5	Eclipse	Black	2009	NDSU	S	USV
6	Etna	Cranberry	-	Seminis	L	B
7	Jaguar	Black	2000	MSU	S	USV
8	Krimson	Cranberry	2012	USDA-ARS	L	B
9	Lariat	Pinto	2010	NDSU	M	USV
10	Lightning	Navy	2009	U. of Guelph	S	UV
11	Majesty	DRK	2005	Ag. Can.	L	B
12	Matterhorn	GN	1998	MSU	M	USV
13	Maverick	Pinto	1997	NDSU	M	V
14	Merlot	Small Red	2005	MSU	M	USV
15	Montcalm	DRK	1974	MSU	L	B
16	OAC Inferno	LRK	2012	U. of Guelph	L	B
17	OAC Lyrik	LRK	2009	U. of Guelph	L	B
18	OAC Rex	Navy	2006	U. of Guelph	S	USV
19	OAC Thunder	Navy	1999	U. of Guelph	S	USV
20	Peregion	Heirloom	NA	VT Bean Co.	S	V
21	Red Hawk	DRK	1997	MSU	L	B
22	Rosetta	Pink	2012	MSU/ARS	M	USV
23	Santa Fe	Pinto	2010	MSU	M	USV
24	Snowdon	WK	2012	MSU	L	B
25	Stampede	Pinto	2010	NDSU	M	USV
26	CDC Super Jet	Black	2011	U. Sask.	S	USV
27	Zorro	Black	2009	MSU	S	USV
28	Zenith	Black	2014	MSU	S	USV

[1] DRK = Dark Red Kidney; LRK = Light Red Kidney; WK = White Kidney; GN = Great Northern

[2] Cultivar release date often precedes the date of published registration.

[3] Seed size divisions based on Hidalgo, 1988. S = Small (<25g 100 seeds); M = Medium (25-40g per 100 seeds); L = Large (>40g per 100 seeds)

[4] V = Vine; UV = Upright Vine; USV = Upright Short Vine; B = Bush

Table 1.2 Description (site locations, soil types, geographic coordinates, elevation, temperature, and rainfall) of trial sites of the 2012-2014 dry bean (*P. vulgaris*) yield trials. National Weather Service (NOAA Online Weather Data, 2015) estimates represent accumulated rainfall precipitation (mm) and mean temperature (°C) during the Julian period 152-174 (June 1st - Oct. 1st).

Trial Site	Soil	Coord.	Elevation	2012		2013		2014	
			- m -	°C	- mm -	°C	- mm -	°C	- mm -
Becker, MN[a]	Hubbard-Mosford Complex	45°39'N, 93°88'W	291	70.1	230	69.1	328	67.9	424
Carrington, ND[a]	Heimdal-Emrick Loams	47°51'N, 99°12'W	490	-	-	-	-	64.6	301
Madison, MN	Colvin Silty Clay Loam	45°01'N, 96°12'W	322	69.5	132	70	324	67.3	410
Rosemount, MN	Waukegan Silt Loam	44°71'N, 93°10'W	290	69.4	382	67.9	304	-	-
Lamberton, MN	Webster/Revere Clay Loams	44°23'N, 95°34'W	345	69.8	157	68.8	238	67.1	466

[a] Becker and Carrington estimates acquired from Elk River, MN and Jamestown, ND stations, respectively.

Table 1.4 Random effect variance components and their respective contribution to the model's variance. Variances were estimated from the original full model. Models were fit via maximum likelihood with and without the variance component in question and compared using a Chi-square (χ^2) distribution (df=1).

	SMALL SEED CLASS		
Effect[a]	**Variance**	**Contribution**	**$\chi^{2\,b}$**
$\sigma_{\beta(k)}^2$	37991	7.30%	11.415***
σ_{β}^2	226724	43.50%	65.571***
$\sigma_{\beta\alpha}^2$	83547	16.00%	15.283***
σ_{ε}^2	173054	33.20%	-
	MEDIUM SEED CLASS		
Effect[a]	**Variance**	**Contribution**	**$\chi^{2\,b}$**
$\sigma_{\beta(k)}^2$	27324	4.60%	2.7302^{ns}
σ_{β}^2	301991	51.30%	57.13***
$\sigma_{\beta\alpha}^2$	34412	5.80%	1.0031^{ns}
σ_{ε}^2	225511	38.30%	-
	LARGE SEED CLASS		
Effect[a]	**Variance**	**Contribution**	**$\chi^{2\,b}$**
$\sigma_{\beta(k)}^2$	15857	2.70%	3.0697^{ns}
σ_{β}^2	406992	70.00%	60.496***
$\sigma_{\beta\alpha}^2$	47279	8.10%	6.1657*
σ_{ε}^2	111486	19.20%	-

[a] Where $\sigma_{\beta(k)}^2$, σ_{β}^2, $\sigma_{\beta\alpha}^2$, and σ_{ε}^2 represent the variance associated with replication nested within environment, environment, cultivar x environment interaction, and random error, respectively. Note that cultivar effects are fixed.

[b] Statistical significance indicated at probability levels 0.05, 0.01, and 0.001 by '*', **', '***', respectively. Non-significant effects denoted by '^{ns}'.

Table 1.3 Ranked yield results (cultivar, market class, tested number of environments, plant population, estimated yield, and yield in favorable and unfavorable environments) from the 2012-2014 dry bean (*P. vulgaris*) yield trials. Rankings are sub-divided according to seed size classes.

Small Seeded (<25g per 100 seed) Cultivars

Code	Cultivar	Market Class[a]	Tested Envir.	Plant Popln.[b] (plants ha^{-1})	Yield (kg ha^{-1})	Yield Stability Analysis	
						Favorable (kg ha^{-1})	Unfavorable (kg ha^{-1})
27	Zenith	Black	4	141964	2621	2977	1961
7	Jaguar	Black	4	182867	2578	2915	1968
26	Super Jet	Black	4	148303	2527	2648	2499
1	Alpena	Navy	4	148542	2532	2928	1764
28	Zorro	Black	12	118025	2226	2618	1680
5	Eclipse	Black	12	125117	2138	2499	1632
18	OAC Rex	Navy	12	103152	1991	2167	1752
19	OAC Thunder	Navy	8	110377	1985	2408	1468
20	Peregion	Heirloom	8	127963	1939	2300	1480
2	Avalanche	Navy	8	106310	1934	2491	1283
10	Lightning	Navy	8	97807	1751	2089	1319
	Mean			**128221**	**2202**	**2549**	**1710**

Medium Seeded (25-40g per 100 seed) Cultivars

Code	Cultivar	Market Class[a]	Tested Envir.	Plant Popln.[b] (plants ha^{-1})	Yield (kg ha^{-1})	Yield Stability Analysis	
						Favorable (kg ha^{-1})	Unfavorable (kg ha^{-1})
13	Maverick	Pinto	12	134042	2840	3459	2397
22	Rosetta	Pink	4	161339	2803	3590	2170
9	Lariat	Pinto	12	149157	2697	3154	2367
14	Merlot	Small Red	4	151950	2507	2928	2190
25	Stampede	Pinto	12	108241	2306	2782	1965
4	Coyne	GN	4	113380	2138	2887	1529
23	Santa Fe	Pinto	8	105632	2013	2724	1540
12	Matterhorn	GN	8	98697	1966	2783	1428
	Mean			**127812**	**2408**	**3039**	**1948**

Large Seeded (>40g per 100 seed) Cultivars

Code	Cultivar	Market Class[a]	Tested Envir.	Plant Popln.[b] (plants ha^{-1})	Yield (kg ha^{-1})	Yield Stability Analysis	
						Favorable (kg ha^{-1})	Unfavorable (kg ha^{-1})
16	OAC Inferno	LRK	4	125399	2385	3294	1681
11	Majesty	DRK	4	84138	1743	2515	1165
21	Red Hawk	DRK	7	126762	1574	2258	1098
8	Krimson	Cranberry	4	110330	1573	2126	1203
24	Snowdon	WK	4	100284	1513	1853	1334
15	Montcalm	DRK	12	106850	1382	2124	855
6	Etna	Cranberry	8	106030	1282	2050	748
3	Cabernet	DRK	4	97533	1281	1729	1012
17	OAC Lyrik	LRK	8	88296	1181	1934	654
	Mean			**105069**	**1546**	**2209**	**1083**

[a] Codes: GN=Great Northern; DRK=Dark Red Kidney; LRK=Light Red Kidney; WK=White Kidney

[b] Target Plant Populations = 172,900 plants ha^{-1} (70,000 plants ac^{-1}) for large-seeded and 222,300 plants ha^{-1} (90,000 plants ac^{-1}) for small and medium seeded.

Table 1.5 Estimated organic gross revenue per acre for commercial dry bean (*P. vulgaris*) market classes. Yields presented were averaged across cultivars included in the 2012-2014 dry bean yield trials; $ cwt^{-1} was obtained from commercial dry bean distributors in MN and ND.

Market Class	Yield (kg ha^{-1})	Yield (lb ac^{-1})	Distributor Price ($ cwt^{-1})	Gross Revenue ($ ac^{-1})
Pink[1]	2803	2503	$95.00	$2,378
Small Red[1]	2507	2239	$95.00	$2,127
Pinto	2464	2200	$80.00	$1,760
Black	2420	2160	$84.50	$1,826
Great Northern[2]	2052	1832	-	-
Navy	2038	1821	$83.50	$1,520
Heirloom[1, 2]	1937	1729	-	-
Light Red Kidney	1783	1592	$105.00	$1,672
White Kidney[1]	1513	1351	$110.00	$1,486
Dark Red Kidney	1495	1335	$105.00	$1,402
Cranberry[2]	1428	1275	-	-

[1] Yield estimated from one representative cultivar.

[2] Price estimates were not adequately available.

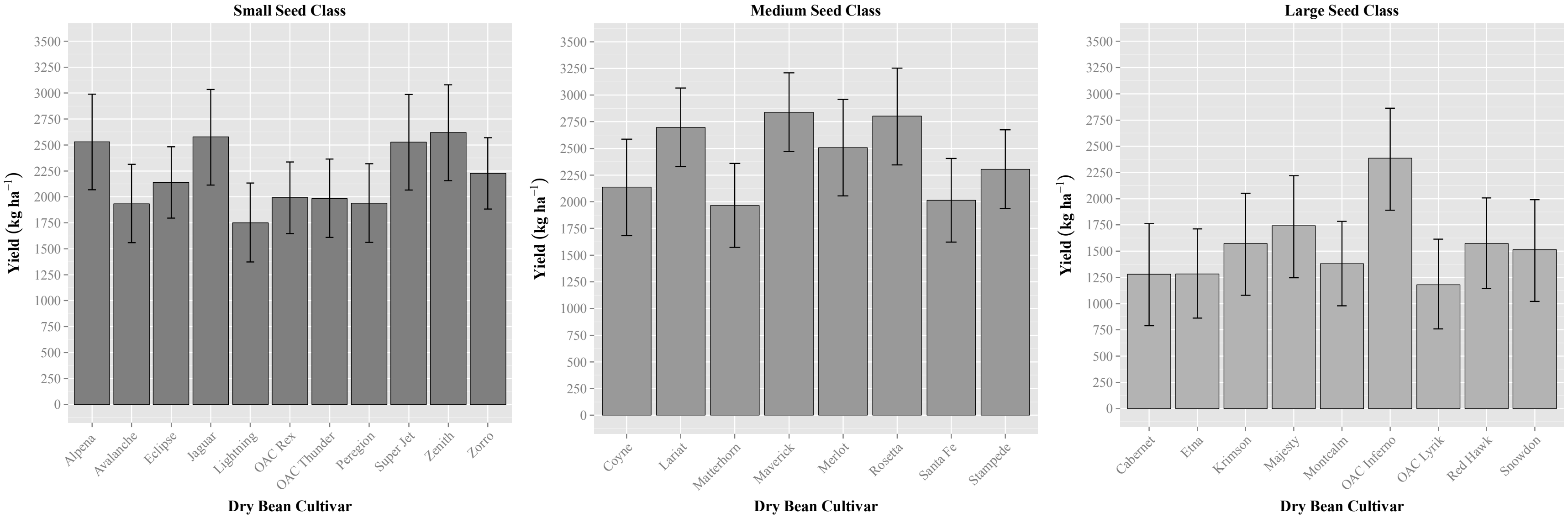

Figure 1.1 Yield estimates (kg ha^{-1}) of dry bean (*P. vulgaris*) cultivars within respective seed classes. Confidence intervals ($\alpha = 0.05$) are indicated by vertical black bars around each estimated mean.

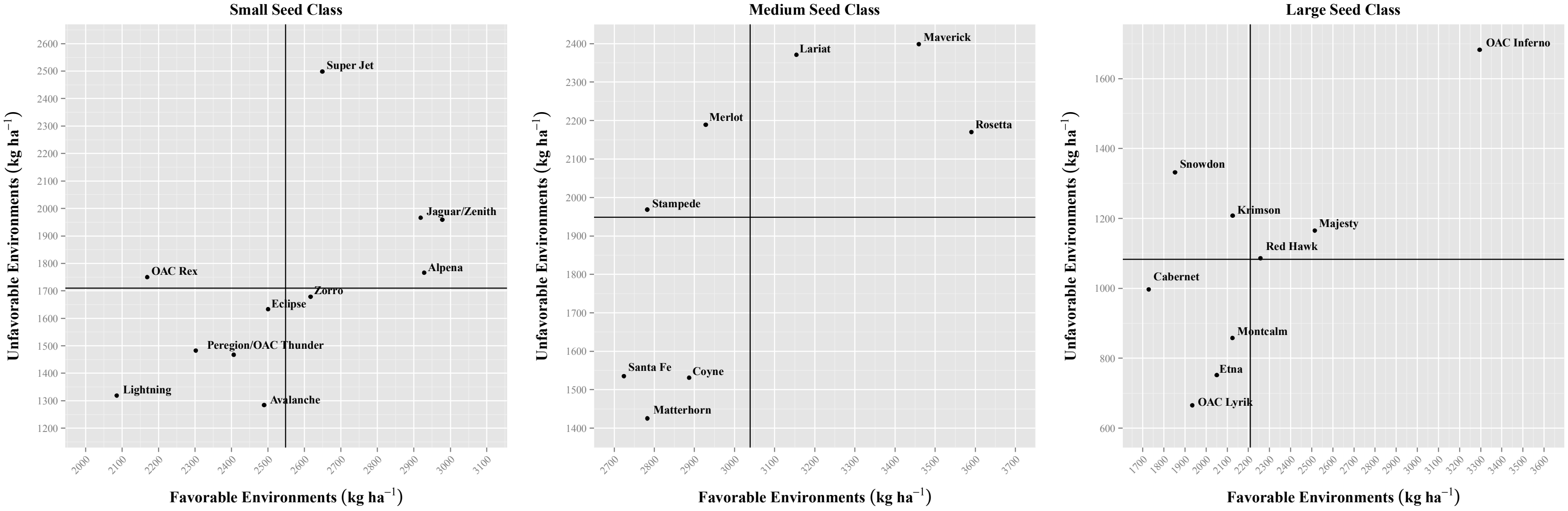

Figure 1.2 Stability biplot of estimated yields ($kg\ ha^{-1}$) in favorable (x-axis) and unfavorable (y-axis) environments within respective dry bean (*P. vulgaris*) seed size classes. Solid black lines denote mean yield within each environmental grouping. Axes vary among seed classes. Type II (dynamic) stability is indicated by cultivars in the upper right-hand quadrant.

CHAPTER TWO

Yield Performance and Stability of Heirloom Dry Bean (*Phaseolus vulgaris* L.):

(Formatted for Publication in *HortScience)*

Summary

Heirloom dry bean (*Phaseolus vulgaris* L.) cultivars are distinctive in their horticultural traits, though little information regarding their performance at the field-scale is currently available. High demand for organic heirloom dry beans from restaurants and food co-ops in Minnesota necessitates evaluation for local production. Yield evaluation of seventeen heirloom cultivars under organic management was performed using a randomized complete block design in 2013 and 2014 at four locations surrounding the Twin Cities Metro region.

Yield data were subject to static and dynamic stability biplot analyses. Yields of heirloom cultivars were approximately 44% lower than commercial market class checks included in the trial; heirloom yields ranged from 825 kg ha^{-1} to 2127 kg ha^{-1}, with a mean of 1362 kg ha^{-1}. Stability analyses and economic incentives, however, suggest that the production of heirloom cultivars, especially ‘Jacob’s Cattle Gold, ‘Lina Sisco’s Bird Egg’, ‘Peregion’, and ‘Tiger’s Eye’, could provide local growers with the opportunity to diversify their production, differentiate themselves in local markets, and maintain economic return.

Introduction

In response to consumer demand, land dedicated to certified organic dry bean production has nearly quadrupled in the state of Minnesota between 2008-2012 (Greene, 2009, 2013; USDA, 2011c). Organic dry bean price premiums, which can more than double gross revenues (Swegarden, 2015, Table 1.5), and ecological system services serve as primary incentives to adopt organic production practices (Mäder et. al., 2002; Marriot and Wander, 2006; USDA, 2015). Western and southern regions of Minnesota have seen recent growth of diversified organic vegetable producers catering to urban outlets and direct-to-consumer markets, including restaurants, community supported agriculture (CSA), or farmer's markets (Adam, 2006; Schnell, 2007; USDA, 2012c).

For the past two years, the Regional Sustainable Development Partnerships (RSDP) (2014, unpublished data) at the University of Minnesota has conducted surveys regarding supply chains and local production of dry beans in Minnesota. There is new demand among consumers and restaurants for locally produced, organic dry beans (RSDP, 2014, unpublished data). In particular, there was an expressed demand for heirloom cultivars known for their horticultural traits, such as cooking quality, flavor, and identifiable seedcoats. Participating restaurant managers cited an average willingness-to-pay (WTP) of $4.78 per pound for heirloom, organic dry beans (RSDP, 2014, unpublished data). According to growers who primarily sell their heirloom dry beans through farmer's markets, consumers exhibit a WTP between $6.00 and $8.00 per pound (John Breslin, 2015, pers. comm., 30 January); Paula Foreman, 2013, pers. comm., 10 October).

Current heirloom cultivars are landrace plant populations that were informally exchanged and cultivated throughout the U.S. (Gepts, 1988; Hendrick, 1931; Silbernagel and Hannan, 1988). Seed savers, farmers, and specialty seed companies, however, more often refer to landrace populations as ‘heirloom,’ ‘heritage,’ ‘folk,’ or ‘farmer-bred,’ all of which emphasize a horticultural or anthropological component (Camacho et. al., 2006). Heirloom populations are known for their deep cultural connections, traditional uses, and ecological adaptation to the region in which they were first cultivated (Burgess, 1994; Nazarea, 2005). As a result of exchange, migration, and human selection, heirloom cultivars today are characterized as populations of highly variable plants lacking in performance and desired agronomic traits (Lioi et. al., 2005; Rodino et. al., 2009).

Previous studies conducted in northern Colorado and northwest Washington indicated the yields of heirloom dry beans were substantially lower than commercial market class cultivars (Walters et. al., 2011; Wagner et. al., 2006). To date, however, yield performance of heirloom cultivars has not been evaluated at the field-scale in Minnesota. In addition, the diversity of organic production environments and the potential for variability within heirloom cultivars necessitate an analysis of stability across growing environments. We hypothesize that heirloom dry bean cultivars, in comparison to commercial check cultivars, exhibit equivalent yield and yield stability across small-scale organic vegetable production environments in southeast Minnesota. Small-scale vegetable growers supplying novel dry beans to local markets will benefit from the recommendations presented in this research regarding favorable heirloom dry bean cultivars.

Materials and Methods

Plant Material and Seed Pretreatments

Untreated seed of seventeen heirloom dry bean (*P. vulgaris* L.) cultivars were sourced from commercial sources (Osborne Family Farms, 2014; Purcell Mountain Farms, 2014; Seed Savers Exchange, 2014; Vermont Bean Company, 2014) (Table 2.1; Figure 2.1). Heirloom cultivars were assumed to be heterogeneous populations of homozygous plants with low levels of outcrossing (Rodiño et. al., 2009). These cultivars were selected due to their novel seed appearance, bush-type architecture, and potential as a cultivar for local niche markets. Seed size descriptors, including one hundred seed weight (g) average seed length (mm), width (mm), and thickness (mm) were collected from original seed stock in 2012 and yield trial stock from 2013 trials (Table 2.1). In addition, seedcoat characteristics such as coat color, corona color, and seedcoat pattern were recorded (Leaky, 1988). Three commercial market class cultivars, ‘Eclipse,’ ‘Lariat,’ and ‘OAC Rex’ were included as commercial checks in the trial; commercial check cultivars were assumed to be pure lines with low levels of outcrossing (Michaels et. al., 2006; Osorno et. al., 2009, 2010). Germination of all seed stock was tested using the ‘Between Paper’ method specific to common bean (Rao et.al., 2006). Just prior to planting, seed was inoculated with a commercial source (Novozymes, Franklinton, NC) of nitrogen-fixing Rhizobium bacteria (*Rhizobium leguminosarum* biovar *phaseoli*) in a peat-based suspension.

Experimental Design

Experiments to determine the yield and stability of heirloom dry bean cultivars were conducted during 2013 and 2014. Plots were established at four locations: Fresh Earth Farm in Afton, MN; Foxtail Farm in Osceola, WI; Gardens of Eagan in Northfield, MN; and The Cornercopia Student Organic Farm in St. Paul, MN (Table 2.2). The Northfield, MN and St. Paul, MN locations were USDA certified organic, while the Afton, MN and Osceola, WI locations had been managed according to the UDSA guidelines for more than ten years prior to the onset of the experiment (Paul Burkhouse, 2013, pers. comm.; Chris James, 2013, pers. comm.; USDA, 2014a). All locations were maintained as non-irrigated, rain fed sites. Previous crops varied and nutrient composition differed dramatically among locations (Table 2.3). No fertilizer or insecticides were applied. The University of Minnesota's Research Analytical Laboratory performed soil fertility tests (pH, OM, P, K, Ca, and Mg) in the fall of 2013 and 2014 (Table 2.3).

The experimental design for each experiment was a randomized complete block with three replications. A block consisted of twenty dry bean cultivars as treatments. Heirloom cultivar 'Lina Sisco's Bird Egg' was omitted from the 2014 trials as a result of improper planting. An experimental plot consisted of a single treatment planted in a two-row plot that was 4.6 meters (15 feet) long with 0.61 meters (24 inches) row spacing. Seed count was adjusted for germination percentage plus 5% seedling mortality in order to obtain a target seeding rate of 87,120 plants acre^{-1} (215,186 plants ha^{-1}) or four plants per 0.304 meters (1 foot). The soil had been prepared by chisel plowing and then finished by field cultivating or rototilling prior to seeding. Experiments were seeded between Julian days 152 and 161 (June 1st - June 10th) in both seasons. Weed-free plots were maintained using a wheel hoe (Valley Oak, Chico, CA) in the early season and bi-weekly hand cultivation mid-to-late season.

Data Collection

Plots were hand harvested when all pods were beyond physiological maturity in late September or early October (Kandel et. al, 2013). The inner 2.44 meters (8 feet) of each two-row plot was hand harvested. Plant populations within the harvested area were determined during the 2014 growing season. Harvested pods were placed in a low-temperature (35°C) dryer overnight and then threshed with a small belt thresher (Agriculex, Guelph, Ontario). To maintain a marketable yield for direct-to-consumer markets, harvested seed was cleaned of debris, splits, and diseased seed prior to weighing. Reported per-plot yields represent weights at ambient relative humidity expressed in kg ha^{-1}.

Progression of within-row canopy coverage was measured at three time points (four, six, and eight weeks after planting) using a modified form of Canfield's 'line intercept' method (Canfield, 1941). In this modification of Canfield’s method, a 0.3 meter wide (half the row width) transect was pressed again the base of the bean stem along 2.44 meters (8 feet) of row. Parallel lines were drawn across the width of the transect to indicate coverage at 10% intervals. A canopy that intersected the edge of the transect at 0.3 meters was noted to have 100% canopy coverage. At each time point, the within row cover of a cultivar was an average of two coverage measurements: the inner left row and the inner right row. Measurements were repeated across all replications and locations. While the six and eight week measurements were taken in both 2013 and 2014 seasons, the four week measurements were measured only during the 2014 growing season (Figure 2.2). Mean canopy coverage at each time point was correlated with yield estimates using a Pearson product-moment correlation coefficient.

Statistical Analysis

Environments were defined as a specific location-year combination. To account for heterogeneity in variances between heirloom cultivars and commercial checks, yield data were subject to a square root transformation, on the basis of a Box-Cox test (Box and Cox, 1964). All data analyses were performed using R-software (version 3.1.2); models were executed and evaluated using the ‘lme4’ package (Bates et. al., 2014; R Core Team, 2014). To account for the unbalanced nature of the data set, the following linear mixed effects model was fit using restricted maximum likelihood (REML):

$$y_{ijk} = \mu + \alpha_i + \beta_{(k)j} + (\alpha\beta)_{ij} + \varepsilon_{ijk} \qquad \text{(Eq. 2.1)}$$

where y_{ijk} is the measured yield observation of the i^{th} cultivar in the j^{th} environment ($i = 1, 2, \ldots, g; j = 1, 2, \ldots, e; k = 1, 2, \ldots, r_j$), μ is the overall mean, α_i is the effect of the i^{th} cultivar, $\beta_{(k)j}$ is the effect of the k^{th} replication nested within j^{th} environment, $(\alpha\beta)_{ij}$ is the effect of the i^{th} cultivar with the j^{th} environment, and ε_{ijk} is the random error term. Fixed effects included μ and α_i whereas $\beta_{(k)j}$, $(\alpha\beta)_{ij}$, and ε_{ijk} were fit as random effects. As random effects, $\beta_{(k)j}$, $(\alpha\beta))_{ij}$, ε_{ijk} were declared independent and normally distributed and scaled toward zero with a variance of σ_{β}^2, $\sigma_{\alpha\beta}^2$, σ_{ε}^2, respectively (Yang, 2007). The significance of each random effect variance component was determined from the log-likelihood ratio test statistic, comparing models fit via maximum likelihood with and without the variance component in question, with a Chi-square distribution and one degree of freedom (Table 2.4).

Using the REML procedure allowed for the best linear unbiased estimation (BLUE) of cultivar effects in an unbalanced data set (Figure 2.3). Variance associated with the fixed effects was partitioned into the random effect terms (i.e. σ_{β}^{2}, $\sigma_{\beta_\alpha}^{2}$, σ_{ε}^{2}), thus minimizing the variance among all fixed effect estimates (Bernardo, 2010; Piepho et. al., 2003, Piepho et. al., 2008). Though it has been demonstrated that holding cultivar as a random effect is more advantageous than declaring it a fixed effect, heirloom cultivars included in these trials were purposefully selected and do not conform to the necessary assumption of random sampling (Piepho et. al., 2003; Smith et. al., 2005).

Stability Analyses

Heirloom cultivar stability was evaluated according to the Type I (static) and Type II (dynamic) concepts of stability classified by Lin and Binns (1986) and described further by Becker and Leon (1988). Stability under the Type I concept can be evaluated by calculating a cultivar's coefficient of variation (CV): $\frac{\sqrt{S_i^2}}{\bar{X}}$, where S_i^2 is equivalent to the cultivar's variance among environments and $\bar{X}$ is the grand mean (Francis and Kannenberg, 1978). To visually assess Type I stability, a cultivar's CV was plotted on the x-axis against its estimated mean yield (kg ha^{-1}) on the y-axis (Figure 2.4a). Plots were divided into four quadrants based on the mean CV (23.5%) and mean observed yield (1525 kg ha^{-1}) across cultivars. Stable cultivars, according to the Type I definition of stability, exhibited high yield, a low coefficient of variation, and appeared in the upper-left quadrant of the biplot.

Visual assessment of Type II (dynamic) stability (Becker and Léon, 1988; Lin and Binns, 1986), was achieved by plotting a environmental regression coefficient (b_i) (x-axis) against the estimated yield (y-axis) of each heirloom cultivar (Figure 2.4b). The environmental regression coefficient was calculated from the regression of the predicted random effects of cultivar x environment $(\alpha\beta))_{ij}$ against the predicted random effect of environment $\beta_{(k)j}$. A negative regression coefficient indicated superior performance in poor (negative $\beta_{(k)j}$) environments, a positive coefficient indicated superior performance in favorable (positive $\beta_{(k)j}$) environments, and a coefficient of zero indicated stability across all environments. A regression method of stability using an environmental index, presented here as $\beta_{(k)j}$, was first proposed by Eberhart and Russell (1966). This environmental index, however, was not directly compared to the yield. Rather, the cultivar x environment $(\alpha\beta))_{ij}$ effect was first regressed on the environmental index $\beta_{(k)j}$ as a means of evaluating stability without directly using yield. Dynamic stability in this biplot was best illustrated by cultivars with high yield (>1525 kg ha^{-1}) and regression coefficient (b_i) near zero.

Results

The final model reported random effect variances of replication nested within environment, environment, and cultivar x environment interaction that significantly ($p<0.001$) contributed to the model's variance (Table 2.4). Effect of environment and cultivar x environment both accounted for approximately 22% of the exhibited variance, where just over 47% of the model's variance was attributed to random error.

Yield of commercial check cultivars was 44% greater than yield of heirloom cultivars (Table 2.5). Commercial check cultivar yields ranged from 2355 kg ha^{-1} ('OAC Rex') to 2617 kg ha^{-1} ('Lariat'), with an average yield of 2447 kg ha^{-1}. Within the heirloom cultivars, yields ranged from 825 kg ha^{-1} ('Dapple Grey') to 2127 kg ha^{-1} ('Peregion'), with an average of 1362 kg ha^{-1} over all entries. The yield performance of heirloom cultivar 'Peregion' was 36% greater than the mean yield of all other heirloom cultivars and 530 kg ha^{-1} greater than the next highest yielding heirloom cultivar, 'Lina Sisco's Bird Egg'. Examination of 95% profile confidence intervals suggested differences among the yield performance of cultivars (Figure 2.3). Both commercial checks and heirloom cultivars did not attain target plant populations; commercial checks and heirloom cultivars were 7% and 17%, respectively, below target plant population levels.

Trends in the progression of canopy closure suggest a majority of cultivars developed half of their final canopy by the four-week time point (Figure 2.2). This development, however, was not correlated with the yield BLUEs (r = -0.029). A small increase in canopy coverage occurred between the four-week and six-week time points, and, in most cultivars, approximately 25% of the canopy developed between the six- and eight-week time points. A moderate correlation (r = 0.466) was observed between the yield and canopy coverage at the eight-week time point. In no case did a cultivar's canopy coverage reach 90% by eight-weeks; 'Peregion' and 'Lariat' exhibited the highest canopy coverage with 86% and 84%, respectively. Heirloom cultivar 'Lina Sisco's Bird Egg', despite having the second highest heirloom yield (1597 kg ha^{-1}), had the lowest total canopy coverage (54%). A consistent trend was not observed in this study's canopy progression data, there is merit in exploring vigorous emergence and early (i.e. before the four-week time point) canopy formation in future studies.

The average coefficient of variation (CV) for yield was similar for both commercial (23.5%) and heirloom cultivars (23.5%) (Table 2.5). 'Jacob's Cattle Gold' exhibited the lowest CV (15.4%) and third highest yield of the heirloom cultivars (1543 kg ha^{-1}). In contrast, 'Dapple Grey' exhibited the highest CV (36.6%) and lowest yield (924 kg ha^{-1}) of all heirloom cultivars. Five cultivars, 'Lariat', "OAC Rex', 'Peregion', 'Jacob's Cattle Gold, and 'Lina Sisco's Bird Egg' displayed Type I (static) stability, though only 'Jacob's Cattle Gold' and 'Peregion' exhibited a CV less than 20% within that group (Figure 2.4a). An additional seven heirloom cultivars exhibited CVs less than the average CV (23.5%) across cultivars, though all seven were below the mean yield (1362 kg ha^{-1}) of all cultivars in the trial.

Calculated environmental regression coefficients ranged from b_i = -0.568 ('Lariat') to b_i = 0.512 ('Yin Yang'). Visual assessment of Type II (dynamic) stability biplot identified three heirloom cultivars with an environmental regression coefficient near zero: 'Jacob's Cattle Gold', 'Tiger's Eye', and 'Jacob's Cattle' (Figure 2.4b). Of those three, only 'Jacob's Cattle Gold' was above the mean yield of the heirloom cultivars in the trial (Figure 2.4b). Three cultivars ('Eclipse', 'Peregion', and 'Lina Sisco's Bird Egg') were placed in the upper right-hand quadrant of the plot, indicating superior performance in favorable environments (Figure 2.4b). Two commercial cultivars, 'Lariat' and 'OAC Rex' appeared in the upper left-hand quadrant, suggesting a competitive advantage in unfavorable environments; ‘Eclipse’ exhibited the smallest environmental regression coefficient of all three commercial cultivars. Though heirloom cultivars ‘Steuben Yellow Eye’ and ‘Koronis Purple’ had yields below the trial mean, their environmental regression coefficients also suggest an advantage in unfavorable environments. In no instance, however, was a cultivar’s mean yield performance in unfavorable environments superior to its mean yield performance in favorable environments (Figure 2.4b).

Discussion

The assessment of heirloom cultivar yield performance was accented by yield stability evaluation. Examination of the 95% confidence intervals indicated a difference between ‘Peregion’ and ‘Dapple Grey’, the highest and lowest yielding cultivars, but there were 11 cultivars with intermediary (1200-1500 kg ha^{-1}) yield performance that were not readily distinct in their performance (Figure 2.3). Cultivars ‘Koronis Purple’, ‘Steuben Yellow Eye’, and ‘Ying Yang’, would have been selected as superior based on yield alone. The cultivars, however, did not appear readily stable according to either the Type I or Type II stability assessments.

Under the Type I (static) stability concept, a cultivar is considered stable if its among-environment variance is relatively small and its performance is unaffected by changing environmental conditions (Becker and Leon, 1988; Lin and Binns, 1986). In that sense, this stability parameter is the best estimate of a cultivar’s biological stability, independent of other cultivar treatments in the trial. Lin and Binns (1986) indicated that a satisfactory Type I stability parameter (i.e. CV) is often associated with poor yield performance, which makes the results of the heirloom yield trials somewhat counterintuitive. Half of the heirloom cultivars in this trial exhibited low variation and (relatively) high yield (Figure 2.4a). This trend may be unique to heirloom dry bean cultivars, whereby greater plant-to-plant variation may exist within the cultivar and, as a result, lower variation across environments. Further breeding work is required to estimate variation within these populations, but, given that the mean CV of all heirloom cultivars was the same as the commercial check cultivars, it can be assumed that heirloom cultivars exhibit adequate static stability.

The Type II stability biplot (Figure 2.4b) is a unique way to use the predicted environmental effects in a linear mixed effects model as a means of simultaneously selecting a cultivar for high yield and stability across environments. This biplot depicts dynamic stability that incorporates the predicted effect of cultivar x environment regressed on an environmental index, which, in this case, was the predicted effect of environment (Eberhart and Russell, 1966; Lin and Binns, 1986). The Type II biplot is convenient for identifying cultivars that respond favorably to increased agronomic inputs and/or management practices (Furtado Ferreira et. al., 2006). In addition, this biplot allows for visualization of cultivars that may retain some selective advantage to abiotic stressors or are better adapted to poor environmental conditions (i.e. those that appear in the upper-left quadrant).

Both Type I and Type II stability analyses suggested that heirloom cultivars ‘Jacob’s Cattle Gold’ and ‘Tiger’s Eye’ are relatively stable from both a biological and agronomic perspective (Becker and Leon, 1988; Lin and Binns, 1986). The relatively high yield of ‘Lina Sisco’s Bird Egg’ and ‘Peregion’ make them interesting cultivars to explore further. The large percentage of variation attributed to error was most likely due to seed processing after harvest, in which all diseased and split seed was removed in order to represent a yield for direct-to-consumer markets. Additional work regarding small-scale threshing and cleaning equipment still needs to be addressed in future research. All four of these heirloom cultivars, however, are suitable for small-scale, local organic production according to the yield and stability analyses performed herein.

Differences between the yield performance of commercial check cultivars and heirloom cultivars may seem drastic, but, when the economic incentives are considered, heirloom cultivars become a viable marketing option. The Regional Sustainable Development Partnerships’ (RSDP, 2014, unpublished data) restaurant survey conducted in 2013 indicated that restaurants cited a WTP of $2.85 per pound for non-heirloom, organic dry beans and $4.78 per pound of heirloom, organic dry beans. Given that the mean yield of commercial checks was 44% greater than the mean yield of heirloom cultivars, this price differential accounts for all but 6% of the yield discrepancy. Further, small-scale producers that sell their beans to farmer’s markets stand to make an even greater profit. These results lead to the conclusion that heirloom dry beans offer small-scale organic producers economic incentives and support their direct-to-consumer markets within the Twin Cities and Greater Minnesota regions.

Table 2.1 Descriptions (cultivar name, seed origin, plant habit) of commercial and heirloom dry bean (*P. vulgaris*) cultivars included in the 2013-2014 yield trials. Seed descriptors (seed size, shape, weight, coat color, coat patter, corona color, length, width, and thickness) were evaluated from 2012 original seed stock (n=15) and 2013 yield trial stock (n=15).

Cultivar Code	**Cultivar Name**	**Seed Stock Origin**	**Plant Habit**[a]	**Seed Size**[b]	**Seed Shape**	**100-Seed Weight**[c] (g)	**Seedcoat Color**	**Seedcoat Pattern**[d]	**Corona Color**	**Length** (mm)	**Width** (mm)	**Thickness** (mm)
BB	**Bumble Bee**	Seed Savers	B	L	cuboid	55.7	white; dark red	maximus virgarcus	dark red	14.4	8.8	6.6
CAL	**Calypso**	Seed Savers	B	M	round	38.3	black; white	stellatus majus	black	11.3	8.3	6.8
DG	**Dapple Grey**	Purcell Mt.	B	M	oval	36.3	white; mixed grey	major punctatus	dark grey	12.5	7.5	6.0
ECL	**Eclipse**	NDSU	USV	S	oval	18.4	black	uniform	black	9.1	6.1	4.7
HS	**Hutterite Soup**	Seed Savers		M	round	28.1	yellow	uniform	light grey	10.7	6.8	5.7
ICA	**Ireland Creek Annie**	Seed Savers	B	M	kidney; cuboid	35.4	yellow	uniform	grey	13.9	7.0	5.6
JC	**Jacob's Cattle**	Osborne Family	B	L	kidney	52	red-purple; white	major punctatus	red-purple	15.9	7.5	6.2
JCG	**Jacob's Cattle Gold**	VT Bean Co.	B	L	kidney	41	gold; white	major punctatus	dark grey	14.7	7.3	6.1
KP	**Koronis Purple**	Seed Savers	B	L	kidney	48	purple; white	uniform; punctatus	ochre	16.4	7.1	5.7

[a] Plant Habit: V = Vine; UV = Upright Vine; USV = Upright Short Vine; B = Bush Seedcoat Color

[b] S = Small (<25g 100 seeds); M = Medium (25-40g per 100 seeds); L = Large (>40g per 100 seeds) as defined by Hidalgo (1988)

[c] Weight (g) of 100 seeds (i.e. 100-seed weight)

[d] Seedcoat pattern as defined by Leaky (1988)

Table 2.1 CONT. Descriptions (cultivar name, seed origin, plant habit) of commercial and heirloom dry bean (*P. vulgaris*) cultivars included in the 2013-2014 yield trials. Seed descriptors (seed size, shape, weight, coat color, coat patter, corona color, length, width, and thickness) were evaluated from 2012 original seed stock (n=15) and 2013 yield trial stock (n=15).

Cultivar Code	**Cultivar Name**	**Seed Origin**	**Plant Habit**[a]	**Seed Size**[b]	**Seed Shape**	**100-Seed Weight**[c] (g)	**Seedcoat Color**	**Seedcoat Pattern**[d]	**Corona Color**	**Length** (mm)	**Width** (mm)	**Thickness** (mm)
KYE	**Kenearly Yellow Eye**	Seed Savers	B	L	round	41.7	white; gold	maximus virgarcus	brown	12.1	7.8	6.7
LAR	**Lariat**	NDSU	USV	M	oval	32.9	cream-beige; light brown	rhomboidius	beige	12.3	7.9	4.7
LC	**Low's Champion**	VT Bean Co.	B	M	truncate oval	38.9	red	uniform	grey	12.0	7.6	5.9
LS	**Lina Sisco's Bird Egg**	Seed Savers	B	L	round	40.6	cream-beige; red-purple	rhomboidius	pale beige	12.1	8.0	6.4
PER	**Peregion**	VT Bean Co.	V	S	oval	18.5	cream-beige; black; brown	striatus; uniform	beige	12.1	6.3	4.8
PP	**Painted Pony**	Seed Savers	B	S	kidney	24.7	brown; white	virgata minor	black	12.7	5.7	4.8
REX	**OAC Rex**	U. Guelph	USV	S	oval	19.3	white	uniform	white	8.9	5.9	5.2
SOL	**Soldier**	Osborne Family	B	M	cuboid	38.9	white; red	maximus virgarcus	white	13.8	7.0	6.5
SYE	**Steuben Yellow Eye**	Purcell Mt.	V	M	round	39.1	white; gold	stellatus majus	light grey	12.2	7.6	6.5
TE	**Tiger's Eye**	Seed Savers	USV	L	kidney	50.1	gold; red-purple	striatus	gold	16.5	7.7	5.7
YY	**Yin Yang**	VT Bean Co.	B	L	round	41.8	black; white	stellatus majus	black	11.5	8.1	6.9

[a] Plant Habit: V = Vine; UV = Upright Vine; USV = Upright Short Vine; B = Bush Seedcoat Color

[b] S = Small (<25g 100 seeds); M = Medium (25-40g per 100 seeds); L = Large (>40g per 100 seeds) as defined by Hidalgo (1988)

[c] Weight (g) of 100 seeds (i.e. 100-seed weight)

[d] Seedcoat pattern as defined by Leaky (1988)

Table 2.2 Description (site locations, geographic coordinates, elevation, rainfall, and growing degree days) of trial sites of the 2013-2014 dry bean (*P. vulgaris*) yield trials. National Weather Service (NOAA Online Weather Data, 2015) estimates represent accumulated rainfall rainfall (mm) and growing degree days ('GDD' calculated with base '50') during the Julian period 152-174 (June 1st - Oct. 1st).

Trial Site Location	Coordinates	Elevation	2013		2014	
		m	Rainfall (mm)	GDD	Rainfall (mm)	GDD
Afton, MN[a]	44°85'N, 92°82'W	269	298	2347	470	2266
Northfield, MN[a]	44°53'N, 93°18'W	289	274	2033	596	- - -
Osceola, WI[a]	45°23'N, 92°73'W	315	354	1467	559	1967
St. Paul, MN	44°98'N, 93°19'W	305	337	2288	438	2308

[a] Afton, Northfield, and Osceola estimates acquired from Hastings, Cannon Falls, and Amery stations, respectively.

- - - Data Missing from August-October

Table 2.3 Soil types, cropping history, and soil nutrient information for trial site locations included in the 2013-2014 heirloom dry bean (*P. vulgaris*) yield trials. Soil sampling was performed in the fall of 2013 and 2014 across all locations and replications. Soil tests were performed by University of Minnesota's Research Analytical Laboratory.

Site History				**Fall Soil Testing Results**					
Site	**Soil Type**	**Year**	**Previous Crop(s)**	**pH**	**OM (%)**	**BrayP (ppm)**	**NH_4OAc-K (ppm)**	**Ca (ppm)**	**Mg (ppm)**
Afton, MN	Baytown Silt Loam	**2013**	cover mixture of dikon radishes, annual rye, and crimson clover	**5.6**	**4.4**	**9**	**82**	**-**	**-**
		2014	cover mixture of dikon radishes, annual rye, and crimson clover	**5.5**	**4.2**	**11**	**105**	**1406**	**252**
Osceola, WI	Santiago Silt Loam	**2013**	onions	**6.9**	**2.4**	**25**	**60**	**-**	**-**
		2014	tomatoes	**7.3**	**2.2**	**50**	**77**	**1260**	**266**
Northfield, MN	Wadena Loam	**2013**	cover mixture of white clover and annual rye	**6.7**	**3.0**	**53**	**136**	**-**	**-**
		2014	lettuce followed by soybean/sorghum	**6.5**	**3.9**	**25**	**88**	**2158**	**312**
St. Paul, MN	Waukegan Silt Loam	**2013**	onions, broccoli, peppers, lettuce, cabbage, tomatoes	**6.7**	**3.6**	**143**	**296**	**-**	**-**
		2014	tomatoes, garlic, watermelons, winter squash eggplant	**7.4**	**3.8**	**174**	**444**	**2183**	**383**

Table 2.4 Random effect variance components and their respective contribution to the model's variance. Models were fit via maximum likelihood with and without the variance component in question and compared using a Chi-square (χ^2) distribution (df=1).

Effect[a]	**Variance**	**St. Dev.**	**Contribution**	χ^{2} [b]
$\sigma_{\beta(k)}^2$	3.248	1.802	9.2%	30.217***
σ_{β}^2	7.759	2.785	22.0%	87.016***
$\sigma_{\beta\alpha}^2$	7.58	2.753	21.5%	27.124***
σ_{ε}^{2}	16.649	4.08	47.2%	-

[a] where $\sigma_{\beta(k)}^2$, σ_{β}^2, $\sigma_{\beta\alpha}^2$, and σ_{ε}^{2} represent the variance associated with replication nested within environments, environment, cultivar-by-environment interaction, and random error, respectively. Note that cultivar effects are fixed.

[b] Statistical significance is indicated at probability levels 0.05, 0.01, and 0.001 by '*', **', '***', respectively.

Table 2.5 Ranked yield results (cultivar name, yield, seed weight, plant population, and stability analyses) from the 2013-2014 dry bean (*P. vulgaris*) yield trials. Trait averages for commercial check cultivars have been tabulated separately from heirloom cultivars.

	Cultivar Code	Cultivar Name	Yield (kg ha^{-1})	Yield (lb. acre^{-1})	Seed Weight g (100 seed)	Plant Popln[a] (plants ha^{-1})	Stability Analyses	
							CV (%)	b_i
Check Cultivars	LAR	Lariat	2617	2337	32.9	177922	20.1	-0.568
	ECL	Eclipse	2368	2115	18.4	202079	27.0	0.099
	REX	OAC Rex	2355	2103	19.3	219558	23.5	-0.220
		Mean	**2447**	**2185**	**23.5**	**199853**	**23.5**	**-**
Heirloom Cultivars	PER	Peregion	2127	1899	18.5	175680	15.4	-0.507
	LS	Lina Sisco's Bird Egg	1597	1426	40.6	-	23.1	0.310
	JCG	Jacob's Cattle Gold	1543	1377	41.0	189962	14.5	-0.001
	KP	Koronis Purple	1479	1321	48.0	171813	23.7	-0.359
	TE	Tiger's Eye	1448	1293	50.1	218554	21.1	0.008
	SYE	Steuben Yellow Eye	1421	1269	39.1	142753	22.3	-0.440
	YY	Yin Yang	1376	1229	41.8	161339	26.9	0.512
	HS	Hutterite Soup	1362	1216	28.1	197094	21.7	0.199
	ICA	Ireland Creek Annie	1347	1203	35.4	194260	20.2	0.137
	JC	Jacob's Cattle	1326	1184	52.0	205679	20.3	-0.006
	CAL	Calypso	1305	1166	38.3	164362	30.0	0.466
	BB	Bumble Bee	1221	1091	55.7	152732	26.0	-0.084
	PP	Painted Pony	1218	1088	24.7	155474	30.0	0.391
	KYE	Kenearly Yellow Eye	1168	1043	41.7	143845	19.9	-0.089
	SOL	Soldier	1150	1027	38.9	176684	26.0	0.358
	LC	Low's Champion	1143	1021	38.9	207902	21.7	-0.142
	DG	Dapple Grey	924	825	36.3	180960	36.6	0.361
		Mean[b]	**1362**	**1216**	**39.4**	**177443**	**23.5**	**-**

[a] Target Plant Population = 215,186 plants ha^{-1} (87,120 plants ac^{-1}) or approximately four plants per 0.304 meters (1foot).

Figure 2.1 Twenty dry bean (*P. vulgaris*) cultivars included in the 2013-2014 yield trials.

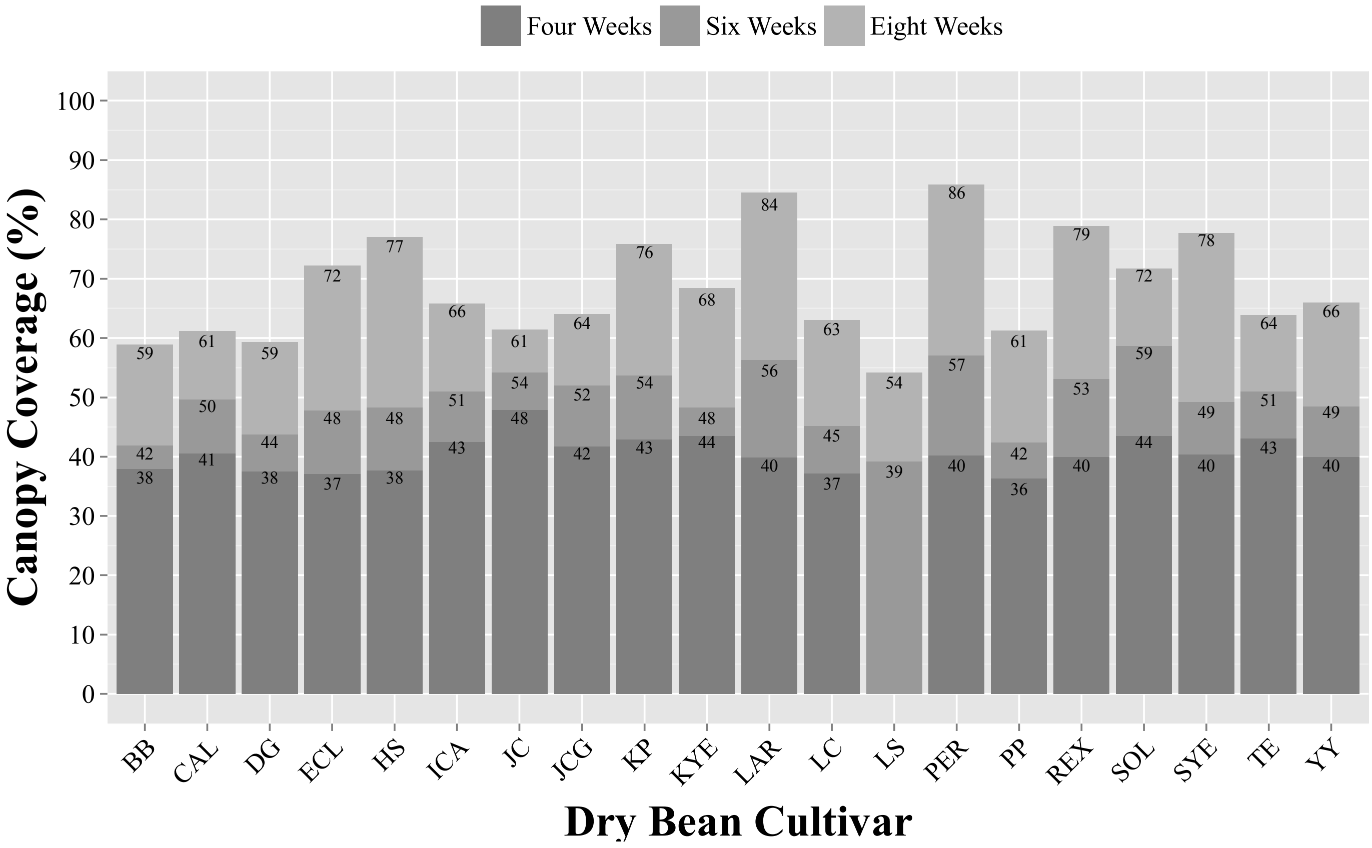

Figure 2.2 Canopy coverage progression of dry bean (*P. vulgaris*) cultivars in the 2013-2014 yield performance trials. Measurements were taken at three time points: four, six and eight weeks after planting. Canopy coverage at four weeks was only evaluated during the 2014 growing season.

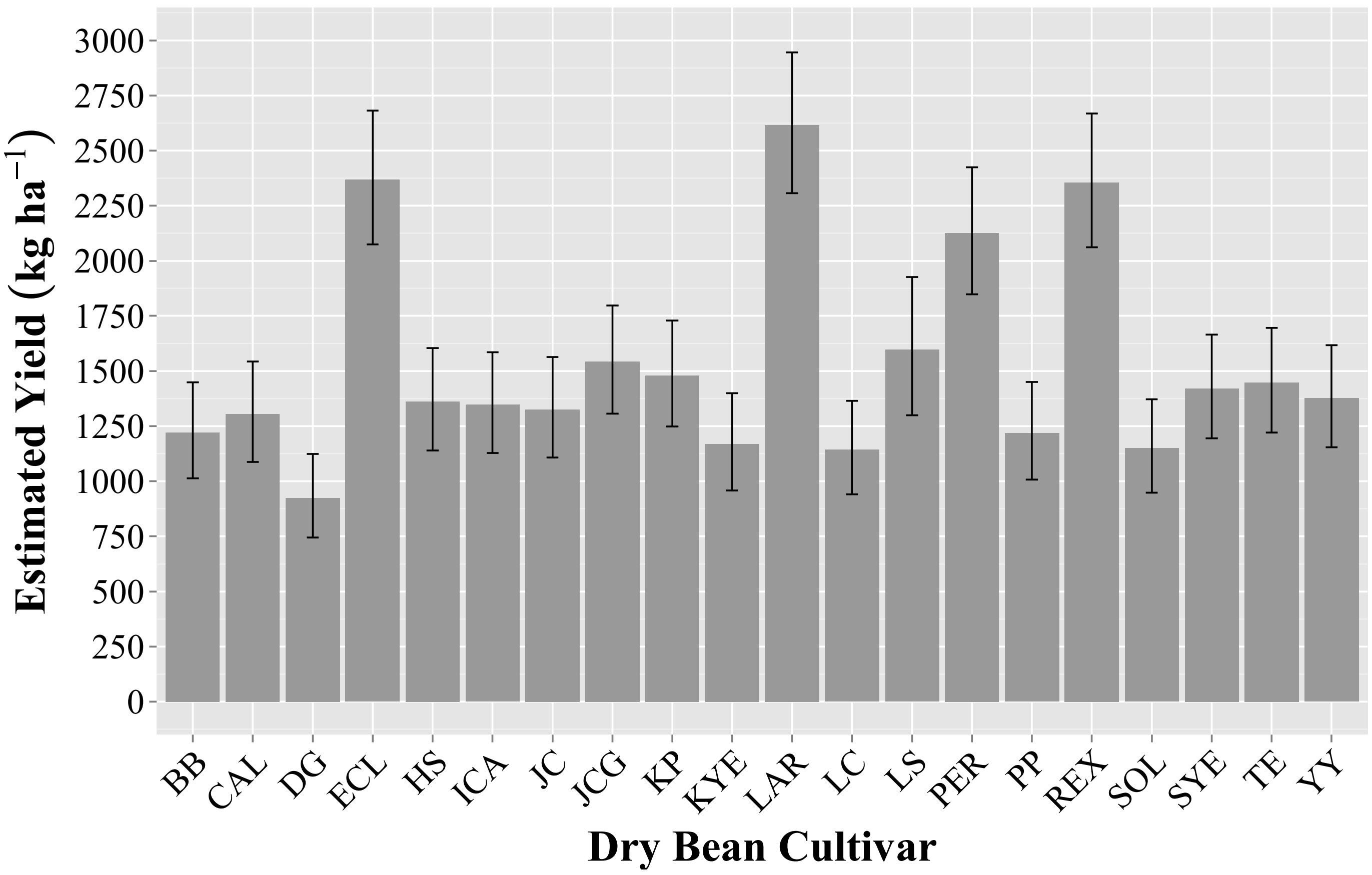

Figure 2.3 Yield estimates (kg ha^{-1}) of dry bean (*P. vulgaris*) cultivars in the 2013-2014 yield trials. Best linear unbiased estimates (BLUEs) are presented with profile confidence intervals ($\alpha = 0.05$).

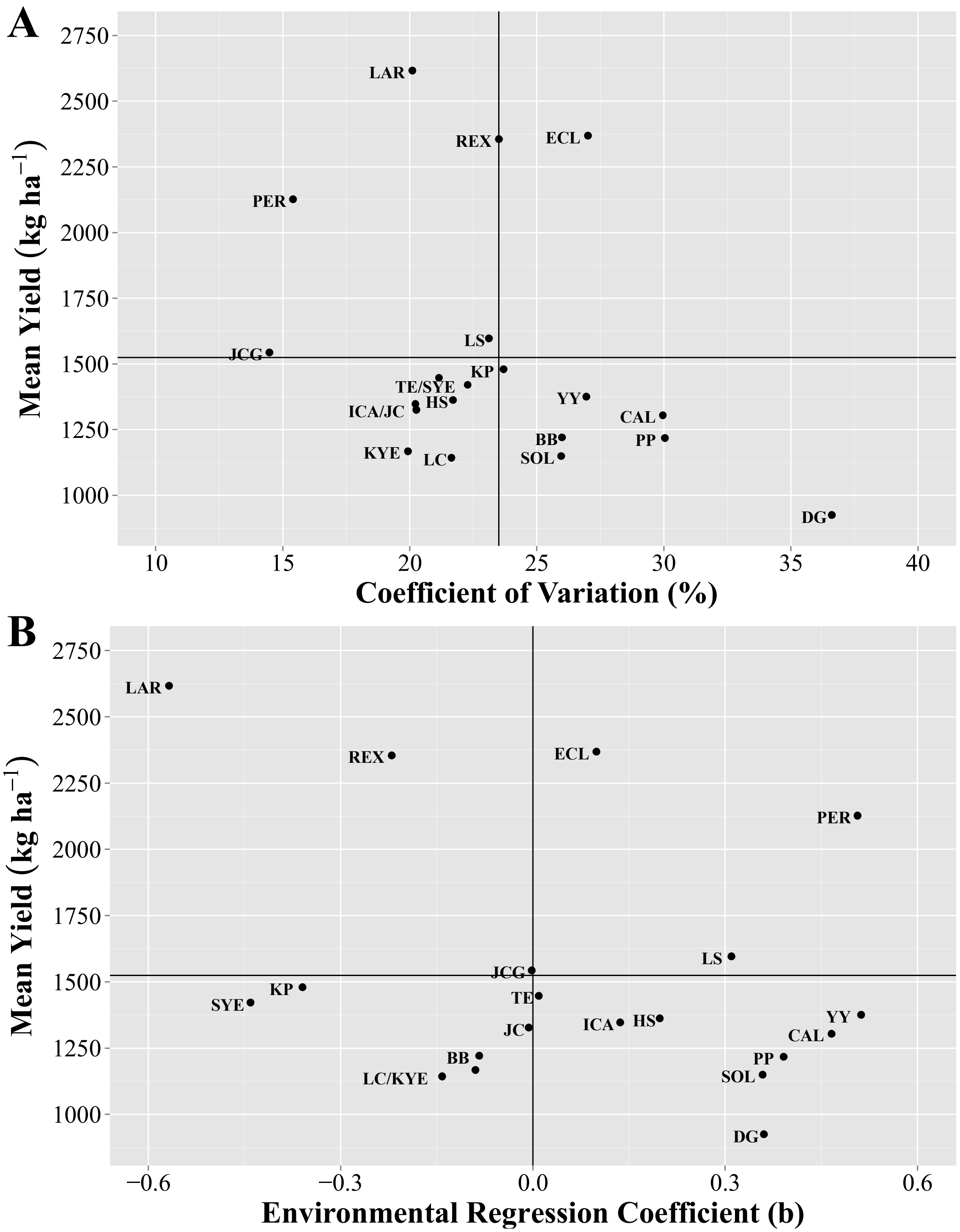

Figure 2.4 Yield stability of dry bean (*P. vulgaris*) cultivars in the 2013-2014 dry bean yield trials: A) Type I (static) stability is represented by cultivars in the upper left-hand quadrant and B) Type II (dynamic) stability is represented by cultivars with high yield and an environmental coefficient (b_i) near zero. Commercial check cultivars are denoted by cultivar codes 'ECL', 'LAR', and 'REX'; all others represent heirloom cultivars.

CHAPTER THREE

Potential for Gain from Selection Within Heirloom Dry Beans (*Phaseolus vulgaris*)

(Formatted for Publication in *HortTechnology)*

Summary

Though the yield and marketability of heirloom dry beans (*Phaseolus vulgaris* L.) advocates for their potential in local markets, heterogeneity of agronomic traits within an heirloom cultivar may not comply with established uniformity standards. The objectives of this project were to estimate genetic variation within a cultivar and, provided sufficient levels of variation, select for improved plants within an heirloom cultivar.

In conjunction with the University of Minnesota's organic heirloom dry bean yield trials, four cultivars, 'Jacob's Cattle Gold', 'Lina Sisco's Bird Egg', 'Peregion', and 'Tiger's Eye', were selected for pure line evaluation based on yield performance, yield stability across locations, and market potential. Sixty random plants were selected within each cultivar for "pure line" trialing in 2012. During the 2013 and 2014 growing seasons, bulked seed from each pure line was grown as a single plant row on certified organic land. Sampling of multiple plants within each plant row for eight morphological traits was used to estimate genetic variation within a cultivar. Estimates of genetic variation included standard deviation (*s*) of pure line means, coefficient of variation (CV) among pure lines, and broad-sense heritability (H^2) on an entry-mean basis.

Selection for six improved pure lines within 'Jacob's Cattle Gold', 'Lina Sisco's Bird Egg', 'Peregion', and 'Tiger's Eye' was performed after the 2013 season. A gain

from selection trial was established during the 2014 season on certified organic land. The objective of this trial was to compare the performance of the six pure lines selected within each cultivar in 2013 to the original heirloom population. Significant ($p < 0.05$) differences were detected among the improved pure lines and check treatments in the gain from selection trial despite the low genetic variation calculated in the pure line trial. Residual genetic variation within heirloom dry bean cultivars was sufficient to allow for positive selection of traits associated with improved maturity, yield, and architecture within an heirloom population.

Introduction

The common dry bean (*Phaseolus vulgaris* L.) was domesticated nearly 8,000 years ago as part of dual domestication events occurring in Mesoamerica and the Andean region of South America (Gepts, 1988; Singh et. al., 1988). *P. vulgaris* underwent significant morphological and biochemical changes during its domestication and is now one of the most widely consumed pulse crops in the world (Akibode and Maredia, 2011; Smartt, 1988; USDA, 2011e). Currently, diversity within the species today is primarily described one the basis of seed type (i.e. market classes) that can be further identified by gene pool and race (Kwak and Gepts, 2009; Singh et. al., 1991a).

The domestication of *P. vulgaris* coincided with a reduction in genetic variation as modern, elite cultivars diverged from wild ancestors (Gepts, 1998; Sonnante et. al., 1994). This reduction in genetic variation can be attributed to ecological constraints and direct human selection for favorable agronomic and horticultural traits, including plant architecture, yield, disease resistance, cooking/canning quality, and seed characteristics such as size, color, and shape (Evans, 1976; Gepts and Debouck, 1991). Direct selection occurred on many different scales and with variable intensity during the domestication process and, as such, there is a range of genetic variation that exists between wild and elite classes.

The term 'landrace' is commonly used to describe germplasm that exhibits intermediary levels of genetic variation between wild and elite germplasm (Sonnante et. al., 1994). Landrace dry beans are generally understood to be unimproved populations existing as heterogeneous mixtures of homozygous plants (Lioi et. al., 2005; Rodino et. al., 2009). Within the U.S. today, landrace populations represent both the Mesoamerican and Andean centers of origin. The majority of which were first introduced to the southwest U.S. by native communities and, to some extent, early European settlers in the northeast U.S. (Gepts and Debouck, 1991; Hendrick, 1931; Hidalgo, 1988; Silbernagel and Hannan, 1988). Little cultivar-specific information exists prior to the development of the seed industry in the U.S, though informal exchange of landrace populations and regional cultivation/selection for desired horticultural traits occurred for potentially thousands of years in native communities and settlements (Camacho Villa et. al., 2006; Gepts, 1988; Hendrick, 1931; Nazarea, 2005; Tiranti and Negri, 2007). Terms such as 'heirloom,' 'heritage,' 'folk,' or 'farmer-bred' are commonly used synonyms for 'landrace' within these informal settings.

The USDA's National Plant Germplasm System (NPGS) now maintains a large collection of landrace populations. In addition, global germplasm repositories, such as the International Center for Tropical Agriculture (CIAT), maintain the majority of *P.*

vulgaris accessions. (USDA, 2010; CIAT, 2013). On many occasions, preserved landraces have served as an “allele bank” for breeders looking to transfer disease or pest resistance, horticultural traits, and even abiotic stress resistance into elite germplasm (Cooper et. al., 2001; Singh et. al., 2003; Silbernagel and Hannan, 1988). Yet, due to generally unfavorable agronomic performance and the difficulty of regaining quantitative traits in crosses with elite cultivars, most modern breeding programs do not readily utilize landrace germplasm unless necessary for the transfer of beneficial alleles (Sullivan, 1988; Tanksley and Nelson, 1996).

It is difficult to define and characterize heirloom populations, as much of their preservation and sharing has occurred in informal, isolated settings (Gepts, 1988; Nazarea, 2005). The pedigree and selection history of these populations is often absent or anecdotal, recorded agronomic and horticultural information rarely exists, and landrace populations are typically named according to local preference (Tiranti and Negri, 2007). Thus, consistency in common name across regions is uncommon and populations maintained or sold through specialty seed companies may not coincide with the landrace population’s original region of adaptation (Harlan, 1975a). Researchers interested in utilizing heirloom germplasm in breeding efforts and growers interested in the production of heirloom cultivars are often discouraged by the lack of available background information and variability in the cultivar’s performance.

Relative to commercial dry bean cultivars adapted to the region, heirloom beans exhibited lower seed yield when grown under small-scale organic conditions (Swegarden, 2015, Table 2.5). Despite this yield differential, there is an expressed market demand from Minnesota food co-ops and restaurants for heirloom dry bean cultivars (RSDP, 2014, unpublished data). Growers interested in producing heirloom cultivars for local markets will need to be provided with not only stable and relatively-high yielding cultivars, but they will also require cultivars that exhibit acceptable levels of uniformity for agronomic traits.

Qualitative observations of phenotypic heterogeneity indicate that there is considerable genetic variability within heirloom dry beans for quantitative traits such as yield, maturity, and plant architecture. We hypothesize heirloom dry bean cultivars retain residual genetic variation for several key quantitative traits associated with maturity, yield, and architecture that allows for the selection of superior plants within an heirloom population. Further, pure line cultivars developed from these selections will exhibit performance superior to the original heirloom population. Trait-specific estimates of genetic variation within heirloom dry bean cultivars will help elucidate the potential for selection within a cultivar, identify target traits, and help guide future breeding efforts (Dudley and Moll, 1969). Selection of superior plants within an heirloom population will maintain desirable horticultural traits (i.e. identifiable seed coat and cooking quality) and may lead to the development of an improved cultivar for local production.

Materials and Methods

Plant Material and Seed Pretreatments

Seventeen heirloom dry bean cultivars were sourced from specialty seed companies (Osborne Family Farms, 2014; Purcell Mountain Farms, 2014; Seed Savers Exchange, 2014; Vermont Bean Company, 2014) in 2012 on the basis of identifiable seedcoats and bush-type architecture (Swegarden, 2015, Table 2.1). Heirloom cultivars were assumed to be heterogeneous populations of homozygous plants with low levels of outcrossing (Rodiño et. al., 2009). Cultivars were grown in plots of approximately 200 plants on the University of Minnesota's Agricultural Experiment Station in St. Paul, MN in summer of 2012. Plots were established on Waukegan silt loam soils with moderate-to-high fertility for dry bean production (data not shown) (Franzen, 2013). Sixty random plants were selected within each cultivar for "pure line" trialing (Johannsen, 1911; Acquaah, 2012). Seed from each plant was bulked and assigned an arbitrary pure line number designator between 1-60. Each pure line was subsequently identified as a combination of the cultivar's abbreviation (Swegarden, 2015, Table 2.1) and its number designator for the remainder of the experiment (i.e. 'PER14' is equivalent to the fourteenth random plant selected within the 'Peregion' cultivar).

"Pure Line" Field Design

During the 2013 growing season, bulked seed from each pure line (i.e. random plant selected in 2012) was planted as a single row on University of Minnesota (UMN) Agricultural Experiment Station's certified organic land in St. Paul, MN. Although sixty plants were selected within a cultivar, seed quality and quantity reduced the number of plant rows to 45-60 within a cultivar and prevented replication within years. Hand planting occurred on Julian day 158 (June 7th) in 2013. Single plant rows of 1.52 meters (5 feet) were completely randomized, with 0.61 meter (24 inch) row spacing, a plant density of 215,186 plants ha^{-1} (87,120 plants $acre^{-1}$) or approximately four plants per 0.304 meters (one foot), and a sowing depth between 2.54-3.81 centimeters (1-1.5

inches). Each plant row corresponded with a single random plant selection from 2012. Prior to planting, seed was inoculated with a commercial source (Novozymes, Franklinton, NC) of nitrogen-fixing Rhizobium bacteria (*Rhizobium leguminosarum* biovar *phaseoli*) in a peat-based suspension. The soil had been prepared by chisel plowing and then finished by field cultivating prior to seeding. Pure line plots followed an organic tomato (*Solanum lycopersicum*) crop, no additional inputs were used, and plots were certified organic by the National Organic Program standards (USDA, 2014a). The site's Waukegan silt loam soil was high fertility for dry bean production (Table 3.1) (Franzen, 2013). Weed-free plots were maintained using a wheel hoe (Valley Oak, Chico, CA) in the early season and bi-weekly hand cultivation mid-to-late season.

After the 2013 growing season, data collected from the concurrent UMN heirloom yield trials were used to select four cultivars to replicate in the 2014 growing season; 'Jacob's Cattle Gold', 'Lina Sisco's Bird Egg', 'Peregion', and 'Tiger's Eye', were selected for additional pure line evaluation in 2014 based on yield performance, stability across locations, and market potential (Swegarden, 2015, Figure 2.2). The growth habits of 'Jacob's Cattle Gold' and 'Lina Sisco's Bird Egg' were determinate bush (Type I); 'Peregion; and 'Tiger's Eye' exhibited an upright short vine (Type III and Type II, respectively) growth habit (Voysest and Dessert, 1991). It was also noted that 'Peregion' exhibited four distinct seedcoat phenotypes (dull black, shiny black, beige with black striping, and beige with brown striping) and two growth habits (Type II and III) during the 2013 pure line trial, indicating it was a genetic mixture. Pure lines within the selected subset of cultivars were again subject to the pure line field design during the 2014 growing season. Lack of quality seed and poor germination in 2013 reduced the number of plant rows contributing to the pure line trial: ‘Jacob’s Cattle Gold’ (n = 57), ‘Lina Sisco’s Bird Egg’ (n = 51), ‘Peregion’ (n = 59), and ‘Tiger’s Eye’ (n = 51). The 2014 field design differed from the 2013 trial in that it contained only four cultivars and was hand planted on the 153 day of the Julian calendar (June 2).

Trait Evaluation

Sampling of multiple plants within each plant row in 2013 and 2014 for eight morphological traits was used to estimate genetic variation within a cultivar. Measured traits contributing to estimates of variation included days to flowering (DAP), number of nodes, plant height (cm), pods in the upper 2/3 of the plant (%), yield per plant (g), 100-seed weight (g), and days to maturity (DAP) (Table 3.2). Total nodes and plant height were measured during the flowering time point; all other measured traits were evaluated at or after physiological maturity. Sampling of multiple plants within plant rows was not performed for maturity estimates (i.e. days to flowering and days to maturity), as these were measured on an entire plant row basis. For all other traits, plants were sampled from the inner 0.91 meters (3 feet) of each plant row. Three plants were sampled in 2013 and four plants in 2014. Single plants were hand harvested when all pods were beyond physiological maturity (Kandel et. al., 2013) in late August or early September.

Selection Procedures

Selection for superior pure lines within ‘Jacob’s Cattle Gold’, ‘Lina Sisco’s Bird Egg’, ‘Peregion’, and ‘Tiger’s Eye’ was performed after the 2013 season. Standardized z-scores (i.e. standard normal scores) were calculated for within each trait mean. Z-scores were then summed across all traits, except for days to flowering and days to maturity, to obtain a "total z-score." These two traits were omitted on the basis that favorable z-scores, indicating earlier flowering and maturity, would be negative. The total z-score was used as an index to equally weigh each trait and allow for simultaneous selection of multiple traits.

Six pure lines (i.e. plant rows) within each cultivar were selected on the basis of total z-score (i.e. largest), visual inspection in the field, and seedcoat quality; these six lines will herein be described as "improved pure lines." These selections corresponded to approximately 10% selection intensity. Selections performed within 'Peregion' were purposefully aimed at those seed types with a beige color coat and striped coat pattern, thus eliminating the completely black seedcoats. Total z-scores were again calculated using combined data from 2013 and 2014. Combined total z-scores of selected improved pure lines within a cultivar were plotted alongside the total z-scores of all remaining pure lines that were not selected in 2013 to illustrate the variation in pure line performance and the relative performance of improved pure line selections (Figure 3.2).

Gain From Selection Trial

A gain from selection trial was established during the 2014 season on the University of Minnesota Agricultural Experiment Station's certified organic land in St. Paul, MN. The objective of this trial was to determine if gain from selection was made within each of the four cultivars selected for continued trialing: 'Jacob's Cattle Gold', 'Lina Sisco's Bird Egg', 'Peregion', and 'Tiger's Eye'. The performance of the six improved pure lines selected within each cultivar in 2013 was compared to both the original heirloom population and an equal mixture of the sixty random plant rows subject to pure line evaluation. Seed of each selected pure line was increased in the Plant Growth Facility on the University of Minnesota during the winter of 2013-2014. Each selected pure line used in the gain from selection trial retained its combination of the cultivar's abbreviation and its number designator used in the pure line trial. The original heirloom population was designated as a combination of the cultivar's abbreviation and an "OP" (i.e. "JCGOP" was the original population of 'Jacob's Cattle Gold'); the equal mixture of sixty random plant rows was designated as a combination of the cultivar's abbreviation and "CK" (i.e. "JCGCK" was the equal mixture of sixty random plant rows from 'Jacob's Cattle Gold').

The gain from selection experiment consisted of a randomized complete block design with four replications. A block was comprised of thirty-two treatment entries, with each treatment corresponding to an improved pure line, original heirloom population ("-OP"), or an heirloom check ("-CK") that was an equal mixture of all sixty random plant rows within a cultivar. An experimental plot consisted of a treatment planted in a single-row plot that was 2.14 meters (7 feet) long with 0.61 meter (24 inches) row spacing. Seeding occurred on Julian day 153 (June 2) of 2014. Seeding rates, crop history, soil preparation, soil fertility, *Rhizobium* inoculation, and weed control methods were identical to the pure line trial (see above) conducted during the 2014 season.

Morphological trait evaluations outlined in the pure line trial were again used to measure performance of the selected pure lines in the gain from selection trial (Table 3.1). Aside from days to flower and days to maturity, which were measured on a plot basis, four random plants were sampled for morphological trait evaluation from the inner 1.22 meters (4 feet) of each plot. Trait data were subject to visual assessment of normality and homogeneity of variances prior to statistical analyses. The following model was fit using maximum likelihood and subject to analysis of variance (ANOVA):

$$y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\,\beta)_{ij} + \varepsilon_{ijk} \qquad \text{(Eq. 3.1)}$$

where y_{ijk} is the measured trait observation of the i^{th} pure line in the j^{th} replication ($i = 1, 2, \ldots, g$; $j = 1, 2, 3, 4$), μ is the overall mean, α_i is the effect of the i^{th} pure line, β_j is the effect of the j^{th} replication, $(\alpha\beta)_{ij}$ is the effect of the i^{th} pure line with the j^{th} replication, and ε_{ijk} is the random sampling error term.

F-tests of significance compared the effects of replication (β_j) and pure line (α_i) to the experimental error term (i.e. the interaction term: $\alpha_i\beta_j$). For each significant pure line effect, a protected Fisher's least significant difference (LSD) with sampling was calculated, where $t_{0.05/2;DFerror}\sqrt{\frac{2s^2}{rs}}$. Pureline trait means and LSDs (where applicable) were reported and compared to the performance of the "OP" and "CK" lines in each cultivar (Tables 3.3-3.6). Trait z-scores were again calculated within each trait and then summed across traits to obtain a total z-score for each pure line treatment (Figure 3.3).

Statistical Analysis of Genetic Variation

Statistical analysis was focused on only the four cultivars ('Jacob's Cattle Gold', 'Lina Sisco's Bird Egg', 'Peregion', and 'Tiger's Eye') that were evaluated in the 2013-2014 pure line trials. Trait data were individually analyzed within each cultivar and subject to transformation if residuals displayed heterogeneity among pure lines.

Transformations were selected on the basis of a Box-Cox test and implemented only if residuals were appropriately adjusted after transformation (Box and Cox, 1964). All data analyses were performed using R-software (version 3.1.2); models were executed and evaluated using the 'lme4' package (Bates et. al., 2014; R Core Team, 2014). The following random effects model was fit and variances estimated with restricted maximum likelihood (REML):

$$y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk} \qquad \text{(Eq. 3.2)}$$

where y_{ij} is the measured trait observation of the i^{th} pure line in the j^{th} year ($i = 1, 2, \ldots, g$; $j = 1, 2$), μ is the overall mean, α_i is the effect of the i^{th} pure line, β_j is the effect of the j^{th} year, $(\alpha\beta)_{ij}$ is the effect of the i^{th} pure line with the j^{th} year, and ε_{ijk} is the random sampling error term. Variables α_i, β_j, $(\alpha\beta)_{ij}$, were fit as random effects. As such, all effects were declared independent, normally distributed and scaled toward zero with a variance of σ^2_α, σ^2_β, $\sigma^2_{\alpha\beta}$, $\sigma_\varepsilon{}^2$, respectively (Yang, 2007). The significance of each random effect variance component was determined using the log-likelihood ratio test statistic. Models were fit and compared via maximum likelihood, with and without the variance component in question, to a Chi-square distribution with one degree of freedom.

Results from the test of significant associated with random effects of pure line are displayed along with estimates of variation (Table 3.3). Trait mean ($\bar{x}$), standard deviation (s) and the coefficient of variation (CV = $\sigma/\bar{x}$) were calculated among pure lines for all cultivars and traits. Variance estimates from the random effects model were used to calculate broad-sense heritability (H^2) on an entry-mean basis according to the

Fehr (1991):

$$\frac{\sigma_g^2}{\frac{\sigma_\omega^2}{n}+\frac{\sigma_{ge}^2}{2}+\sigma_g^2} \qquad \text{(Eq. 3.3)}$$

where σ_g^2 is the variance associated with purelines, σ_{ge}^2 is the variance associated with the pure line x year interaction, and σ_ω^2 is the variance among plant samples within a plot. The harmonic mean number of samples across years was used for *n* in Eq. 3.3.

Results

Selection for Improved Pure Lines

Year effects on the performance of pure lines within a cultivar altered the relative ranking of combined total z-scores for pure lines between the 2013 and 2014. That is, improved pure line selections (noted by dark triangles in Figure 3.2) did not always have the highest total z-scores once the 2013 and 2014 data were combined. Despite the relative rank changes between the two years of the pure line trial, improved pure lines consistently appeared among those pure lines with the highest combined total z-score within each cultivar (Figure 3.2). In only two instances were combined total z-scores of improved pure lines below the mean of the cultivar (i.e. $z < 0.0$): JCG05 ($z = -1.18$) and PER52 ($z = -3.41$). Four 'Tiger's Eye' improved pure lines appeared in the top five performing pure lines. Two improved pure lines in 'Jacob's Cattle Gold' appeared in the top five. Within the top ten performing pure lines in 'Lina Sisco's Bird Egg', four represented improved pure line selections made in 2013. Though only one improved pure line was seen in the top ten pure lines within 'Peregion', selections within 'Peregion' were made for only those seed types with a beige color coat and striped coat pattern. As a result, several of the improved pure line selections within 'Peregion' did not exhibit the highest total z-scores. The top performing pure line (i.e. the pure line with the highest combined total z-score) in all four cultivars, however, was also an improved pure line selection made in 2013. Further investigation indicated that the top performing pure line within each cultivar was relatively balanced in its distribution of z-scores across traits (i.e. bias was not induced by superior performance within one or two traits alone).

Estimates of Variation

Estimates of genetic variation (standard deviation (s) of pure line means, CV among pure lines, and broad-sense heritability (H^2) on an entry-mean basis) were used to identify cultivars with sizeable genetic variation and traits that exhibited variation across cultivars (Table 3.3). Traits such as days to flowering, total nodes, number of pods, and percent upper pods exhibited a highly positive Pearson's correlation ($r > 0.5$) among all three statistics. A small negative correlation was observed only between the σ and H^2 correlation for plant height and the σ and CV correlation for hundred-seed weight.

Standard deviation (s) was most informative for comparisons made among cultivars for the same trait, as the s between traits was strongly biased by the magnitude of the trait mean. 'Peregion' demonstrated the highest s across the four cultivars for all traits except hundred-seed weight ('Lina Sisco's Bird Egg'), percent upper pods ('Tiger's Eye'), and days to maturity ('Tiger's Eye'). 'Jacob's Cattle Gold' exhibited the lowest s for six out of the eight measured traits.

Estimation of the CV allowed for the comparison of the relative magnitude of standard deviations among traits. Highest average CV was calculated for yield per plant

(CV = 23.8%) and number of pods (CV = 19.9%). Maturity estimates, including days to flower (CV = 3.15%) and days to maturity (CV = 2.5%), exhibited the lowest CV estimates of variation. A slight negative correlation (r = -0.25) occurred between s and CV for the hundred-seed weight trait. This was attributed to the small seed size, and therefore small trait mean, for hundred-seed weight observed in 'Peregion'. Across all traits, 'Peregion' and 'Tiger's Eye' consistently exhibited the highest CV estimates.

Estimated heritabilities were low-to-moderate for the eight traits measured in the pure line variation trial, ranging from H^2=0.0 to H^2=0.79. Average H^2 across cultivars was highest for hundred-seed weight (H^2 = 0.36) and lowest for percent upper pods (H^2 = 0.04), though many average H^2 estimates across cultivars were biased upwards by the moderate heritability estimates of 'Peregion' and 'Tiger's Eye'. Cultivar 'Jacob's Cattle Gold' exhibited a positive H^2 for only two traits: yield per plant (H^2 = 0.36) and number of pods (H^2 = 0.02). 'Lina Sisco's Bird Egg' did not exhibit positive heritability for total nodes and percent upper pods, and the estimated heritabilities of number of pods and yield per plant were very low (H^2 = 0.07 and H^2 = 0.04, respectively). Greatest heritability within 'Lina Sisco's Bird Egg' was estimated for hundred-seed weight (H^2 = 0.67). Aside from percent upper pods and plant height, 'Peregion' exhibited moderate-to-high heritability (i.e. $0.28 < H^2 < 0.79$) across all traits. Heritability estimates across all traits for 'Tiger's Eye' were greater than zero, though they were low-to-moderate in magnitude ($0.05 < H^2 > 0.40$). Inconsistencies in variance estimates of percent upper pods and plant height suggest imperfections in trait evaluation methodology.

The effect of pure line significantly contributed to the model's variation (Eq. 3.2) for several traits. ‘Peregion’ exhibited significant ($p < 0.05$) pure line effects for days to flower, total nodes, yield per plant, and hundred-seed weight. Hundred-seed weight was significantly ($p < 0.05$) affected by variation among pure lines within (Lina Sisco’s Bird Egg’. Finally, ‘Tiger’s Eye’ exhibited significant ($p < 0.05$) pure line effects for total nodes. Pure line effects did not significantly contribute to the variance of any measured trait in ‘Jacob’s Cattle Gold’. In almost all traits significant ($p < 0.05$) year effects and pure line x year effects were observed across cultivars.

Gain from Selection Trial

The 2014 gain from selection trial illustrated differences between the improved pure line selections and original heirloom populations. Analysis of variance (ANOVA) of trait data indicated significant ($p < 0.05$) effects of pure lines for multiple traits within each cultivar (Tables 3.4 - 3.7). The effects of pure line were not significant for any trait across all four cultivars. In no cultivar was the effect of pure line significant for plant height. Significant differences for number of pods (LSD = 1.81) and days to maturity (LSD = 1.72d) were established in ‘Jacob’s Cattle Gold’. The mean number of pods among improved pure lines, however, did not exceed the JCGOP treatment, and there was a significant difference between the JCGCK and JCGOP trait means. Number of pods (LSD = 2.64), percent upper pods (LSD = 11.9%), and yield per plant (LSD = 2.66g) exhibited significant differences among ‘Lina Sisco’s Bird Egg’ pure lines in the gain from selection trial. Again, however, the mean number of pods and yield per plant for the improved pure line selections did not exceed the LSCK or LSOP treatment entries, respectively. Within ‘Tiger’s Eye’, significant differences for total nodes (LSD =

0.76), yield per plant (LSD = 3.02g) and days to maturity (LSD = 1.3d) were established. Mean days to maturity for the improved pure lines did not exceed the TECK or TEOP trait mean. Four traits within ‘Peregion’ displayed significant differences between entry means: days to flower (LSD = 1.31d), total nodes (LSD = 1.36), hundred seed weight (LSD = 1.62g), and days to maturity (LSD = 2.10). Improved pure line mean for days to flower and total nodes did not exceed either the PERCK or PEROP trait means.

Inconsistencies in the performance of the original heirloom population entry (“OP”) and the entry comprised of an equal mixture of sixty plant rows (“CK”) were noted for several traits (Figure 3.3). These inconsistencies, however, were significant ($p < 0.05$) for only four trait-cultivar combinations: number of pods (‘Jacob’s Cattle Gold’), percent upper pods (‘Lina Sisco’s Bird Egg’), yield per plant (‘Lina Sisco’s Bird Egg’), and days to maturity (‘Tiger’s Eye’) (Tables 3.4, 3.5, 3.7). Total z-scores of all six improved pure lines for ‘Jacob’s Cattle Gold’, ‘Lina Siso’s Bird Egg’, and ‘Tiger’s Eye’ exceeded both the “CK” and “OP” treatment entries. Improved pure lines within ‘Peregion’ exhibited total z-scores above, between, and below the “CK” and “OP” treatment entries, suggesting that the total z-score method of selection was not necessarily effective in this genetic mixture. In no instance did the mean of improved pure lines outperform both of the “CK” or “OP” treatments for a single trait across all four cultivars. That is, gain from selection for a particular trait varied according to the heirloom population in which selection was performed.

Discussion

Collective information gathered from both the estimates of variation in the pure line trial and results from the gain from selection trial support the original claim that heirloom cultivars exhibit genetic variation for quantitative traits. Further, the evaluated heirloom cultivars exhibited enough genetic variation to permit selection within a cultivar. These two trials, however, highlight the difference between estimated and functional genetic variation.

In theory, heritability is calculated as a means of predicting response to selection within a population (Dudley and Moll, 1969). Given that heritability was calculated within populations that were not developed with the sole purpose of introducing new genetic variation, estimated heritability was expected to be low (Fehr, 1987). It was surprising to find, however, that significant differences were detected among the improved pure lines and check treatments in the gain from selection trial despite the low heritabilities ($H^2 < 0.10$) calculated in the pure line trial. This trend was readily apparent in the 'Lina Sisco's Bird Egg' and 'Jacob's Cattle Gold' cultivars. It was also interesting to note that significant pure line effects in the pure line trial did not necessarily lead to significant differences among the improved pure lines and check treatments in the gain from selection trial, as was the case for hundred-seed weight in 'Lina Sisco's Bird Egg' and yield per plant in 'Peregion'.

One possible explanation for these occurrences may be the experimental design used to calculate heritability on an entry-mean basis; ideally replication within a year and across locations would help estimate variance components and provide a more accurate estimate of heritability. The significant effect of year and, occasionally, a pure line x year interaction in the pure line trial may have contributed to the small estimated variance component attributed to pure line. In addition to year effects and the genetics underlying quantitative traits, the inability to account for spatial variation via replication within a year may have also influenced the estimates of heritability. Regardless of their inability to adequately predict gains within a cultivar, however, the estimates of heritability were useful for describing the inherent levels of genetic variation among cultivars.

Results from the 2013-2014 pure line trial can be used to inform data collected from the 2013-2014 heirloom dry bean yield trials (Swegarden, 2015). In the heirloom yield trials, ‘Jacob’s Cattle Gold’ and ‘Tiger’s Eye’ exhibited similar yield and yield stability, yet the data presented herein describes low-to-moderate heritability exhibited for all traits within ‘Tiger’s Eye’ and heritability estimates near or equal to zero for traits in ‘Jacob’s Cattle Gold’. It is possible that the inherent upright short vine growth habit of ‘Tiger’s Eye’ may have played a role in the yield stability observed in the 2013-2014 yield trials (Kelly et. al., 1987; Swegarden, 2015). The pure line trial data, however, suggested that the observed stability might have resulted from either the lack of variation for traits within a cultivar (‘Jacob’s Cattle Gold’) or the inherent low-to-moderate variability within a cultivar that became undetectable when the cultivar was grown in a large population (‘Tiger’s Eye’). It remains to be seen if selection within either of these cultivars, both of which exhibited significant trait differences in the gain from selection trial, improves yield stability across locations.

Information from the pure line trial and the gain from selection trial can now be used to a) reduce the number of pure lines evaluated b) introduce pure lines that exhibited a positive rank change in the combined pure line trial data from 2013-2014 or c) adjust selection strategies according to estimated trait heritabilities, breeder's trait preference, and the cultivar in which selection will be performed. It may also be advantageous to perform selection among the pure lines that incorporates traits associated with maturity, such as days to flowering and days to maturity. To focus on the improvement of yield and architecture, these traits, though they were measured throughout the experiment, were not incorporated into the total z-score selection index. It is important, however, to monitor these traits alongside yield components, given negative association between maturity and yield (Kelly et. al., 1987). The observed maturation period (~70-85 DAP) of these heirlooms is advantageous to growers and a balance between yield and maturity must be considered.

The inherent variability within heirloom cultivars may, in fact, provide a buffer against abiotic stressors and prove to be useful when selecting for horticultural traits such as cooking quality or seedcoat color (Harlan, 1975a; Nazarea, 2005; Tiranti and Negri, 2007). If such is the case, it may be advantageous to select the top two or three pure lines within each cultivar and evaluate their performance as a mixture. Care must be taken to not, however, return levels of variability to that of the original heirloom population. This is particularly important for hundred seed weight and total nodes; variation in hundred seed weight may lead to issues with cooking quality or processing and variation for total nodes may significantly influence architecture and harvestability.

Without adequate anthropological background or pedigree history, the observed residual variation within these four cultivars could not be attributed to past selection pressures, such as a genetic bottleneck, drift, or selection. It can be assumed, however, that most heirloom dry bean cultivars available through specialty seed companies that do not enforce stringent regeneration policies or breeding methods upon their dry bean material may also exhibit variability in performance.

While promising, the apparent gains in performance for quantitative traits associated with maturity, yield, and plant architecture need to be reevaluated across both years and locations. The organic field site on which these pure lines were evaluated and selected is notorious for high soil fertility (Table 3.1) and the gain from selection trial was only performed in one year. Expansion of the gain from selection trial to multiple locations will further evaluate differential performance among improved pure lines, allow for the estimation of realized heritability, and help establish the pure line's potential as a regionally adapted cultivar.

The methods employed herein preserved the horticultural integrity of each heirloom cultivar while generating pure lines that may result in an improved heirloom cultivar for regional small-scale production. More importantly, the heirloom pure line trial illustrated that residual genetic variation within heirloom dry bean cultivars was sufficient to establish the potential for gain from selection and allow for the successful selection within an heirloom population.

Table 3.1 Rainfall, growing degree days (GDD), cropping history, and soil nutrient information (pH, OM, P, K, Ca, and Mg) for the heirloom dry bean (*P. vulgaris*) pure line evaluation trials conducted on the University of Minnesota's St. Paul campus (44°98'N, 93°19'W). Soil sampling was performed in the fall of 2013 and 2014. Soil tests were performed by the University of Minnesota's Research Analytical Laboratory. National Weather Service (NOAA Online Weather Data, 2015) estimates represent accumulated rainfall precipitation (mm) and GDD (base '50') during the Julian period 152-174 (June 1st - Oct. 1st).

Year	Rainfall[a] (mm)	GDD[b]	Previous Crop	pH	OM (%)	BrayP (ppm)	NH_4OAc-K (ppm)	Ca (ppm)	Mg (ppm)
2013	337	2288	onions, broccoli, peppers, lettuce, cabbage, tomatoes	6.7	3.6	143	296	-	-
2014	438	2308	tomatoes, garlic, watermelons, winter squash eggplant, peppers	7.4	3.8	174	444	2183	383

Table 3.2 Eight morphological traits associated with maturity, yield, and architecture measured to estimate residual genetic variation within heirloom dry bean (*P. vulgaris*) cultivars.

Trait	Definition	Units
Days to Flower	Days after planting (DAP) to 50% of plants within a plant row containing one open flower.	DAP
Plant Height	Vertical (90°) height of plant from soil surface to the terminal trifoliate along main stem; measured at flowering.	cm
Nodes	Number of nodes along the plant's main stem, including hypocotyl scar; measured at flowering.	Integer
Days to Maturity	Days from planting (DAP) to 50% of the plants reaching physiological mature of both the seed and pod. Visually estimated by pod color.	DAP
Pods Per Plant	Total number of pods on an individual plant at harvest.	Integer
Upper Pods	Percentage of pods that sit in the upper two-thirds of the plant's main stem. Visually estimated "upper two-thirds" at the time of harvest.	%
Yield per Plant	Total seed yield per plant at harvest. Diseased, immature, and split seed was cleaned prior to weighing. Expressed as dry matter yield per plant.	grams
Hundred Seed Weight	Estimated weight of one hundred seeds. Ten seeds per plant were weighed and multiplied by a factor of ten. Expressed as dry matter weight.	grams

Table 3.3 Estimates of genetic variation (standard deviation (s) of pure line means, CV among pure lines, broad-sense heritability (H^2) on an entry-mean basis, and pure line effect) as calculated among pure lines of four heirloom dry bean (*P. vulgaris*) cultivars in the 2013-2014 pure line trial. Number of pure lines contributing to the estimates of variation is noted below cultivar name. Statistical significance of the pure line effect is indicated at probability levels 0.05, 0.01, and 0.001 by ‘*’, **’, ‘***’, respectively.

	‘Jacob’s Cattle Gold’ (n=57)	**‘Lina Sisco’s Bird Egg’** (n=51)	**‘Peregion’** (n=59)	**‘Tiger’s Eye’** (n=51)	**Avg.**
Days to Flower (DAP)					
$\bar{x}$	34.5	36.1	43.4	34.3	
s	0.74	1.03	2.06	0.93	**1.19**
CV (%)	2.2	2.9	4.8	2.7	**3.15**
H^2	0.00	0.27	0.76	0.25	**0.32**
Pure Line	$p > 0.05$	$p > 0.05$	$p < 0.001$***	$p > 0.05$	
Total Nodes (#)					
$\bar{x}$	7.0	7.2	14.9	13.5	
s	0.52	0.38	2.35	0.80	**1.01**
CV (%)	7.5	5.3	15.8	5.9	**8.63**
H^2	0.00	0.00	0.79	0.40	**0.30**
Pure Line	$p > 0.05$	$p > 0.05$	$p < 0.001$***	$p < 0.05$*	
Plant Height (cm)					
$\bar{x}$	39.5	38.7	50.8	36.7	
s	2.24	2.41	3.33	2.98	**2.74**
CV (%)	5.7	6.0	6.6	8.1	**6.6**
H^2	0.00	0.36	0.08	0.19	**0.16**
Pure Line	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	
Number of Pods (#)					
$\bar{x}$	10.4	13.1	22.8	8.2	
s	1.61	2.3	5.64	1.77	**2.83**
CV (%)	15.5	17.6	24.8	21.5	**19.9**
H^2	0.02	0.07	0.30	0.34	**0.18**
Pure Line	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	

Table 3.3 CONT. Estimates of genetic variation (standard deviation (s) of pure line means, CV among pure lines, broad-sense heritability (H^2) on an entry-mean basis, and pure line effect) as calculated among pure lines of four heirloom dry bean (*P. vulgaris*) cultivars in the 2013-2014 pure line trial. Number of pure lines contributing to the estimates of variation is noted below cultivar name. Statistical significance of the pure line effect is indicated at probability levels 0.05, 0.01, and 0.001 by ‘*’, **’, ‘***’, respectively.

	‘Jacob’s Cattle Gold’ (n=57)	**‘Lina Sisco’s Bird Egg’** (n=51)	**‘Peregion’** (n=59)	**‘Tiger’s Eye’** (n=51)	**Avg.**
Upper Pods (%)					
$\bar{x}$	82.3	86.3	74.6	80.2	
s	6.91	5.35	7.18	9.72	**7.29**
CV (%)	8.4	6.2	9.6	12.1	**9.1**
H^2	0.00	0.00	0.00	0.15	**0.04**
Pure Line	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	
Yield per Plant (g)					
$\bar{x}$	14.7	13.7	20.0	12.5	
s	2.86	2.96	5.46	3.33	**3.65**
CV (%)	19.44	21.7	27.3	26.6	**23.8**
H^2	0.36	0.04	0.28	0.34	**0.26**
Pure Line	$p > 0.05$	$p > 0.05$	$p < 0.001$***	$p > 0.05$	
100 Seed Weight (g)					
$\bar{x}$	46.3	41.0	20.2	52.0	
s	2.57	3.21	2.41	2.67	**2.71**
CV (%)	5.6	7.8	11.9	5.1	**7.6**
H^2	0.00	0.67	0.73	0.05	**0.36**
Pure Line	$p > 0.05$	$p < 0.001$***	$p < 0.001$***	$p > 0.05$	
Days to Maturity. (DAP)					
$\bar{x}$	83.3	84.7	86.9	77.0	
s	1.49	1.99	2.27	2.53	**2.07**
CV (%)	1.8	2.4	2.6	3.3	**2.5**
H^2	0.00	0.45	0.28	0.33	**0.27**
Pure Line	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	

Table 3.4 Gain from selection trial results from pure line selections made within dry bean (*P. vulgaris*) cultivar 'Jacob's Cattle Gold'. Calculated "Total Z-score" does not include days to flowering and days to maturity. LSD groupings are denoted by subscripts for traits that exhibited significant pure line effects ($p < 0.05$). Fisher's LSD calculated with $t_{0.025,21} = 2.0796$; means with the same letter are not significantly different. $\text{Mean}_{\text{improved}}$ in parentheses indicates higher mean of all improved pure lines than "CK" and "OP" entries.

Pure Line	Flower (DAP)	Nodes (#)	Height (cm)	Pods (#)	Upper Pods (%)	Yield (g/plant)	100-Seed (g)	Maturity (DAP)	Total Z-Score
JCG05	32.8	6.5	45.1	8.1_{bc}	86.2	**12.92**	45.52	76.0_{b}	0.29
JCG07	32.8	**6.4**	46.1	8.8_{b}	84.7	12.14	**46.33**	**75_{ab}**	**0.60**
JCG26	32.5	**6.3**	45.8	7.9_{bc}	79.5	11.30	**46.68**	**75_{ab}**	-0.42
JCG27	**32.0**	**6.6**	43.4	8.9_{ab}	**90.5**	12.80	44.01	**74.3_{a}**	0.21
JCG29	33.5	**6.6**	**46.5**	**9.1_{ab}**	74.6	**13.19**	45.51	**75.0_{ab}**	0.31
JCG37	33.0	6.4	**47.8**	8.4_{b}	**88.9**	12.76	45.88	76.5_{b}	**1.16**
JCGCK	33.0	**6.6**	44.8	6.4_{c}	83.0	8.47	44.50	76.5_{b}	-1.60
JCGOP	**32.3**	6.4	43.6	**10.6_{a}**	79.0	12.34	43.43	77.0_{b}	-0.53
$\text{mean}_{\text{improved}}$	32.8	6.5	-45.8	8.53	-84.1	-12.51	-45.66	-75.3	
$\text{sd}_{\text{improved}}$	0.5	0.12	1.44	0.48	6	0.69	0.93	0.8	
Fisher's LSD	-	-	-	1.81	-	-	-	1.72	-

Table 3.5 Gain from selection trial results from pure line selections made within dry bean (*P. vulgaris*) cultivar 'Lina Sisco's Bird Egg'. Calculated "Total Z-score" does not include days to flowering and days to maturity. LSD groupings are denoted by subscripts for traits that exhibited significant pure line effects ($p < 0.05$). Fisher's LSD calculated with $t_{0.025,20} = 2.0859$; means with the same letter are not significantly different. $\text{Mean}_{\text{improved}}$ in parentheses indicates higher mean of all improved pure lines than "CK" and "OP" entries.

Pure Line	Flower (DAP)	Nodes (#)	Height (cm)	Pods (#)	Upper Pods (%)	Yield (g/plant)	100-Seed (g)	Maturity (DAP)	Total Z-Score
LS01	34.3_{b}	**7.4**	47.4	11.4_{a}	93.0_{ab}	10.64_{a}	**43.28**	77.8	**1.69**
LS17	**33.5_{ab}**	**7.4**	**47.9**	12.3_{a}	82.2_{bc}	11.85_{a}	41.58	**76.3**	0.99
LS20	**33.5_{ab}**	6.9	45	10.8_{a}	90.4_{ab}	10.03_{ab}	42.77	79	-0.14
LS39	**33.3_{a}**	7.3	44.6	11.6_{a}	**96.8_{a}**	**12.48_{a}**	42.84	77.5	1.27
LS48	33.8_{ab}	7.1	**47.6**	**12.4_{a}**	95.9_{a}	11.50_{a}	42.12	76.8	1.43
LS53	34.3_{b}	**7.4**	47.5	**12.4_{a}**	91.4_{ab}	**12.18_{a}**	41.99	**76.5**	**1.57**
LSCK	35.5_{c}	6.9	43.3	8.1_{b}	**96.9_{a}**	7.63_{b}	40.76	**76.5**	-1.52
LSOP	34.7_{c}	7	44.6	10.4_{ab}	75.5_{c}	11.95_{a}	**43.79**	76.7	-0.71
$\text{mean}_{\text{improved}}$	(33.8)	(7.25)	(46.7)	(11.8)	91.6	11.45	42.43	77.3	
$\text{sd}_{\text{improved}}$	0.43	0.21	1.46	0.66	5.24	0.94	0.64	1.01	
Fisher's LSD	0.92	-	-	2.64	11.9	2.66	-	-	

Table 3.6 Gain from selection trial results from pure line selections made within dry bean (*P. vulgaris*) cultivar 'Peregion'. Calculated "Total Z-score" does not include days to flowering and days to maturity. LSD groupings are denoted by subscripts for traits that exhibited significant pure line effects ($p < 0.05$). Fisher's LSD calculated with $t_{0.025,21} = 2.0796$; means with the same letter are not significantly different. Mean$_{improved}$ in parentheses indicates higher mean of all improved pure lines than "CK" and "OP" entries.

Pure Line	Flower (DAP)	Nodes (#)	Height (cm)	Pods (#)	Upper Pods (%)	Yield (g/plant)	100-Seed (g)	Maturity (DAP)	Total Z-Score
PER07	42.3_{ab}	10.1_{c}	**52.3**	16.8	**84.5**	13.78	18.99_{bc}	83.5_{b}	-1.27
PER17	41.5_{a}	15.0_{b}	49.6	18.9	69.8	15.18	21.25_{a}	**81.8_{a}**	0.37
PER26	**41.3_{a}**	15.6_{b}	49.1	**20.1**	74.5	**18.88**	20.41_{b}	**81.8_{a}**	**1.11**
PER52	**41.3_{a}**	10.4_{c}	46.8	15.6	77.1	14.48	**21.33_{a}**	83.5_{b}	-2.01
PER55	43.0_{bc}	**17.1_{a}**	49.3	**21.5**	76.8	**16.55**	18.20_{c}	82.0_{a}	**0.9**
PER59	44.0_{c}	**15.9_{ab}**	50.0	15.6	64.9	14.54	**22.51_{a}**	85.3_{b}	0.41
PERCK	**41.25_{a}**	14.7_{b}	**51.4**	17.9	74.2	16.27	18.24_{c}	83.8_{b}	-0.21
PEROP	42.0_{ab}	15.7_{b}	50.1	18.1	**84.3**	14.28	19.66_{bc}	85.0_{b}	0.69
***mean**$_{improved}$*	42.2	14.0	49.5	(18.1)	74.6	15.57	(20.5)	(83.0)	
***sd**$_{improved}$*	1.11	3	1.76	2.47	6.73	1.87	1.6	1.39	
Fisher's LSD	**1.31**	**1.36**	**-**	**-**	**-**	**-**	**1.62**	**2.1**	

Table 3.7 Gain from selection trial results from pure line selections made within dry bean (*P. vulgaris*) cultivar 'Tiger's Eye'. Calculated "Total Z-score" does not include days to flowering and days to maturity. LSD groupings are denoted by subscripts for traits that exhibited significant pure line effects ($p < 0.05$). Fisher's LSD calculated with $t_{0.025,21} = 2.0796$; means with the same letter are not significantly different. Mean$_{improved}$ in parentheses indicates higher mean of all improved pure lines than "CK" and "OP" entries.

Pure Line	Flower (DAP)	Nodes (#)	Height (cm)	Pods (#)	Upper Pods (#)	Yield (g/plant)	100-Seed (g)	Maturity (DAP)	Total Z-Score
TE09	**31.5**	12.6_{bcd}	43.8	8.8	**86.8**	12.65_{bc}	54.49	72.8_{bc}	-0.15
TE18	31.8	**13.4_{a}**	44.4	**9.4**	79.8	**14.01_{ab}**	55.81	**71.3_{a}**	1.11
TE27	**31.5**	**13.3_{ab}**	**45.6**	8.6	83.2	12.76_{bc}	56.4	**71.3_{a}**	1.0
TE33	32.3	12.9_{abc}	43.1	**9.4**	80.2	13.24_{abc}	54.29	72.3_{abc}	-0.05
TE42	32.3	12.8_{abc}	**45.6**	7.9	**92.1**	12.61_{bc}	**59.35**	72.8_{bc}	**1.46**
TE47	32.3	12.8_{abc}	44.9	9.3	73.6	**15.98_{a}**	57.02	71.8_{ab}	**1.09**
TECK	32.5	11.9_{d}	42.4	7.9	66.8	11.23_{bc}	**57.2**	73.3_{c}	-2.11
TEOP	31.8	12.3_{cd}	42.5	7.1	75.9	10.66_{c}	54.66	71.8_{ab}	-2.3
***mean**$_{improved}$*	32.0	(13.0)	(44.6)	(8.9)	(82.6)	(13.54)	56.23	72.0	
***sd**$_{improved}$*	0.4	0.31	1	0.59	6.37	1.31	1.86	0.69	
Fisher's LSD	-	0.76	-	-	-	3.02	-	1.3	

Figure 3.1 Four heirloom dry bean (*P. vulgaris*) cultivars selected for evaluation in the 2013-2014 pure line trial. From top-left to bottom-right: 'Jacob's Cattle Gold' (JCG), 'Lina Sisco's Bird Egg' (LSBE), 'Peregion' (PER), and 'Tiger's Eye' (TE).

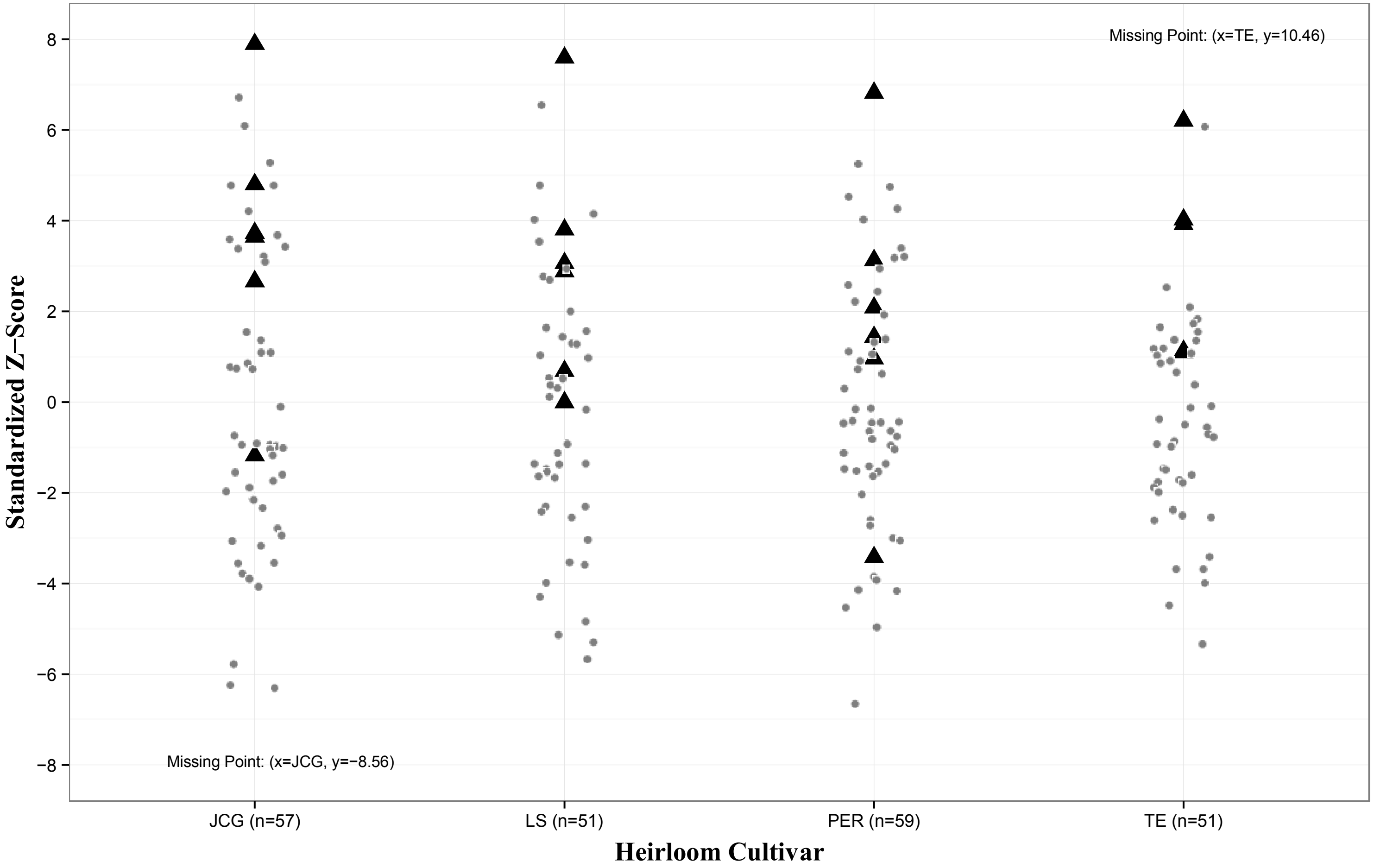

Figure 3.2 Heirloom dry bean (*P. vulgaris*) cultivar pure line performance from 'Jacob's Cattle Gold' (JCG), 'Lina Sisco's Bird Egg' (LS), 'Tiger's Eye' (TE), and 'Peregion' (PER) from the pure line variation trial conducted in 2013-2014. Z-scores were calculated and summed across six measured traits; pure lines with a higher Total Z-Scores were interpreted as 'improved' lines. Improved pure line selections are represented by solid triangles. Small circles indicate the remaining pure lines that were not selected after the 2013 growing season.

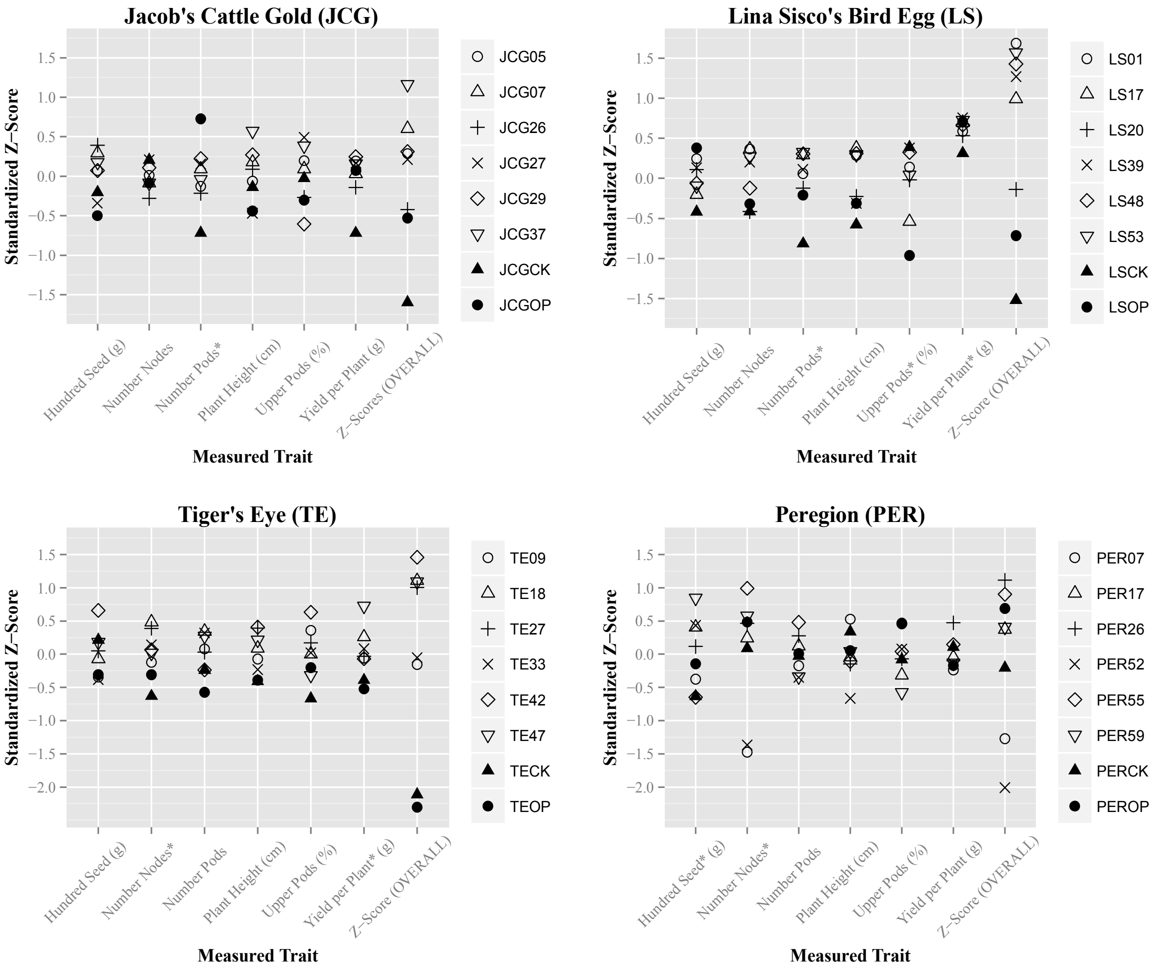

Figure 3.3 Heirloom dry bean (*P. vulgaris*) performance of entries in the 2014 gain from selection trial; entries represent pure lines selected within 'Jacob's Cattle Gold' (JCG), 'Lina Sisco's Bird Egg' (LS), 'Tiger's Eye' (TE), and 'Peregion' (PER). Z-scores were calculated within each trait and summed across six measured traits to obtain 'Z-Score (OVERALL).' Open shapes represent improved pure line selections. Black filled circles indicate the original heirloom population ("OP") entry, and black filled triangles denote the entry comprised of an equal mixture of the sixty plant rows ("CK") in the pure line trial. Traits exhibiting significant differences among pure lines are noted by '*' along x-axis labels.

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