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EVALUATION OF DRY BEAN GENOTYPES FOR PERFORMANCE UNDER ORGANIC PRODUCTION SYSTEMS; EVALUATION OF EARLY NITROGEN FIXATION IN DRY BEAN

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EVALUATION OF DRY BEAN GENOTYPES FOR PERFORMANCE UNDER ORGANIC PRODUCTION SYSTEMS; EVALUATION OF EARLY NITROGEN FIXATION IN DRY BEAN

By

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ABSTRACT

EVALUATION OF DRY BEAN GENOTYPES FOR PERFORMANCE UNDER ORGANIC PRODUCTION SYSTEMS; EVALUATION OF EARLY NITROGEN FIXATION IN DRY BEAN

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The performance of 32 diverse dry bean (*Phaseolus vulgaris* L.) genotypes, including one non nodulating check, was evaluated under organic and conventional management systems, in side by side plots. Research was conducted at multiple locations: Kellogg Biological Station, Gull Lake, MI in 2007-2009, and in Gratiot County, MI in 2007 and 2008 and in Tuscola County in 2009. The conventional treatment was managed using recommended practices, while organic plots were managed with approved methods for certified organic production. The best performing bean genotypes were generally from the Middle American gene pool. The black bean genotype 'Zorro' performed well at all locations and years, and across both management practices systems.

Recognizing nitrogen as a limiting factor in organic production, the same 32 diverse genotypes plus a high N-fixing check and a non nodulating check, were screened under greenhouse conditions for their ability to fix nitrogen. Seed was sterilized, inoculated with *Rhizobium etli* UMR 1597, and fertilized with a nitrogen free solution. Plants were harvested at initial flowering, weighed for root mass and biomass measured for height and root length, rated for nodule growth and nitrogen content of biomass. Genotypes of Middle American origin were superior to those of Andean origin for nitrogen fixation and biomass yield at the early establishment growth stage. Black, red, and pink seed classes ranked highest in terms of both biomass and nitrogen accumulated.

Dedication: To my Grandmother, Theresa Wik and my Mother, Patricia Stier, for their love, support, and encouragement.

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CHAPTER 1

HISTORY AND BACKGROUND

The common bean, *Phaseolus vulgaris* L., is indigenous to the Americas. The center of origin for this species is in the Andes region of South America (Kami et al., 1995), from where it spread south into South America and north into Central America and Mexico and eventually was carried into North America through trade (Gepts, 1998). The common bean was domesticated in two major geographic regions; the Andean gene pool and the Middle American gene pool (Gepts, 1988). Within these regions there is evidence of multiple domestication events. The two major gene pools are divided further into races which are based on their agro-ecological adaptation (Singh et al., 1991). The Andean gene pool is comprised of three races, Nueva Granada, Peru, and Chile whereas the Middle American gene pool comprises the races Mesoamerica, Durango, and Jalisco. Based on seed morphology, plant architecture, and RAPD analysis Beebe et al. (2000) identified a fourth group of beans in the Middle American gene pool which was designated as Race Guatemala due to its presence primarily in that country. Crossing between both gene pools is made difficult by the presence of post zygotic fertility barriers which result in hybrid seedlings that are weak and may die due to the combination lethal genes present in both gene pools (Beaver and Osorno, 2009). The common bean is inbreeding with a low level of out crossing, which can increase in certain tropical regions in its various places of origin (Graham and Ranalli, 1997). In California Ibarra-Perez et al. (1997) found a 6.9% rate of outcrossing between a black seeded genotype with purple hypocotyl and a white seeded genotype with green hypocotyl.

According to Gepts (1998), common beans from Middle American Race Mesoamerica migrated from Central America into the Caribbean and from there north into the Southeastern region of North America; these types generally consisted of small black or white seeded beans. Medium seed sized beans belonging to Race Durango comprise both white seed coat, or Great Northern, and mottled seed coat, or Pinto bean. Race Durango beans moved north through Central America into Mexico and Southwestern North America and north into the Great Plains region as far north as modern day Saskatchewan (Gepts, 1998). Seed of the common bean from the Andean gene pool was taken to the Iberian Peninsula by Spanish and Portuguese sailors where it subsequently spread largely to highland regions of East Africa and into Europe. European settlers brought common bean cultivars to North America, introducing the Andean gene pool into Northeast North America. The diversity of bean seed types currently grown in the U.S. is classified into the same races based on local adaptation and market opportunities. Kidney (Nueva Granada race), cranberry (Chile race), and yellow (Peru race) beans are market classes commonly grown in North America and are members of the Andean gene pool. Race Mesoamerica is represented by the small seeded navy and black beans. The medium-sized pinto and great northern beans are both of race Durango, and small reds and pinks belong to race Jalisco. Race Guatemala types are climbing beans from Southern Mexico and Guatemala are not grown in North America.

Currently, the United States is the sixth leading producer of dry beans in the world, after Brazil, India, Myanmar, and Mexico. One fifth of the dry beans produced in the U.S. are exported. Michigan and North Dakota together produce half of the beans grown in the U.S. Michigan is the number one producer of black beans, small reds, and cranberry beans while ranking second in production of navy beans and kidney beans. The largest single seed class produced in the United States is pinto bean. (http://www.ers.usda.gov/Briefing/drybeans/, accessed December 2009)

The major production area in Michigan is the central Saginaw valley and thumb, consisting of Huron, Sanilac, Tuscola, Saginaw and Bay counties where primarily small sized seed classes, such as black and navy beans are grown. Further west, there is significant production in Gratiot, Isabella, and Montcalm counties. In the sandy soils of Montcalm and Isabella County large-seeded types such as kidney and cranberry are the primary commercial classes produced. The majority (96%) of beans in Michigan are produced under rain fed conditions though irrigation is employed in areas with light and sandy soils such as Montcalm County. The majority of Andean beans possess an upright determinate type I-growth habit, which produces a uniform large seeded bean. Producers of small-seeded beans prefer the upright type II indeterminate upright architecture for direct harvest as opposed to type III prostrate vine growth habit that favors development of diseases such as white mold (*Sclerotinia sclerotiorum*). Type IV climbing types, such as those of Race Guatemala, are not grown commercially in Michigan. Many producers direct harvest the bean crops which is facilitated by taller, upright plants with concentrated pod set in the middle of the canopy and a thicker stem that resists lodging. Growers continue to windrow and thrash large seeded bush types to ensure seed quality, though production trends toward direct harvest especially in the smaller seed classes such as black and navy beans. Factors reducing productivity include drought, disease, and insect pests in combination with insufficient nutrient levels and low or high pH soils (Kelly et al., 1999). In Michigan, precipitation can be erratic leading to periods of water stress that reduce crop productivity. Growers typically band a side dress of fertilizer at a rate of 40 to 55 kg ha^{-1} at planting time whereas, soybean, (*Glycine max)*, is not usually fertilized since it is capable of fixing sufficient nitrogen to produce an acceptable yield.

CURRENT TRENDS IN ORGANIC PRODUCTION

Organic production is increasing in Michigan, which consistently ranks in the top five states for organic production of dry beans (ERS, http://www.ers.usda.gov/data /organic/ accessed December 2009). In the period from 1997 to 2005, area planted to organic beans has increased nearly threefold, from 334 ha to 968 ha in Michigan (table 1-1). In the same period production of organic soy increased from 2,470 ha to 5,850 ha. In 1997, there were fewer than 6,070 ha of certified organic crop land in Michigan. By 2005 that figure had increased to over 17,401 ha (fig 1-2). Production of organic dry beans is being consolidated into fewer states as the top five states producing organic dry beans are increasing in production area; the number of states producing dry beans is decreasing (ERS-NOP, Organic Briefing Room, http://www.ers.usda.gov/briefing/organic/ accessed December 2009). Nationally organic crop production has experienced considerable growth recently. From a national value of $3.6 billion in 1997, organic crop value has grown to $21.1 billion in 2008 (http://www.ers.usda.gov/Briefing/drybeans/, accessed December 2009). Though there is an increase in certified organic acreage, supply still does not meet demand (Dimitri and Oberholtzer, 2009). In 2002 the National Organic Program (NOP) began regulating organic production by setting standards and certifying third parties who certify farms and production facilities. The Organic Materials Review Institute (OMRI) reviews products and practices and approves/disapproves for use in organic production. Artificially produced chemicals and fertilizers are generally not approved for application in organic production. Insects and disease must be controlled with approved, naturally occurring pesticides. Herbicides are not approved, necessitating weed control by mechanical means such as cultivation and hand pulling. The use of a flame to kill young weeds and sterilize the surface of the soil to prevent future germination has gained attention in recent years. Soil nutrient levels are managed through crop rotations, often involving a cover crop which may be a legume or other crop, and application of manures and compost. (USDA-ERS, http://www.ers.usda.gov/ accessed December 2009)

Changes in weather patterns and movement of dry bean production to more marginal crop lands has resulted in a need for dry beans with tolerance to drought stress and to soils of marginal fertility. Different production systems such as organically grown dry beans present their own unique challenges. In searching for germplasm useful in developing dry bean genotypes adapted to drought stress in Idaho, Singh et al. (2009) evaluated genotypes grown in Southwest North America where beans were cultivated for thousands of years under drought stress in marginal soils. Singh et al. (2009) conducted field evaluations to find genetic variation in landraces adapted to the Southwest for use in other areas of production. The common Red Mexican bean was identified as tolerant of such limiting conditions as drought and weeds following evaluation under seven production systems, including on farm and on station systems as well as organic and conventional with high and low inputs. Production systems affected crop characteristics such as days to maturity was increased in the stressed environments (Singh et al., 2009). Stressful growing conditions were also correlated with a reduction in seed weight. There were also large differences in seed yield among the landraces and genotypes evaluated across different production systems. Greatest yields were observed under the conventional system with higher levels of inputs on research stations and the lowest yields were produced under on-farm low input organic systems. The increased inputs of the on farm system yielded nearly three times the on farm low input organic system. This suggests that some cultivars are better adapted to different production systems than other genotypes. Singh et al. (2009) concluded that testing dry bean genotypes in organic systems would be useful in identifying genotypes best suited to that system and better control of environmental conditions could be achieved by having certified organic ground available for research on research stations.

Costs of supplying nutrients are increasing on a wide scale as the cost of energy increases. Crops that are better able to acquire more nutrients from the soil, or fix nitrogen from the atmosphere will help to relieve costs to producers in both developed and developing countries. A reduction in the application of nutrients to fields may also have the added benefit of reducing the amount of pollution due to run off and ground water contamination. Another major concern is the impact of agriculture on global warming. Nitrous gases and nitrous oxides are more effective at trapping heat in the atmosphere than CO_2. N "oxide" emissions have been associated with the application of nitrogen containing fertilizers to agriculture systems, specifically the over application of N to the soil (Peoples et al., 2009).

Crop legumes are often used in crop rotations to help increase the nitrogen level available in the soils. Alternating nitrogen fixing crops with non fixing cereal crops which require addition of nitrogen to the soil has been a means to improve yields of the non fixing crops. Those crops able to fix nitrogen may be split into two groups based on harvested product. One group is forages which are grown for biomass either for high-N feed for animals or for integration into the soil as green manure in order to improve soil quality. The other group is grain legumes which are annuals cultivated for the high value protein rich seed they produce as well as for the residual nitrogen fixed by the crop (Buttery et al., 1992).

CROP SYSTEMS AND NITROGEN FIXATION

Forages consist of both perennial and annual plants such as alfalfa (*Medicago sativa*) and clover (*Trifolium* spp.) and are harvested for their biomass to feed animals or are incorporated into the soil to contribute to nutrient levels, especially nitrogen (Peoples et al., 1995). Breeding of forage legumes focuses on production of quality biomass. Forages are often cited as being quite efficient in fixing large quantities of nitrogen per year. Estimates range up to 500 kg N ha^{-1} per year fixed for perennial forages such as alfalfa (Lindemann and Glover, 2003). This large amount of nitrogen fixed is attributable to the length of time the perennial forages are established in the ground compared to annual legume crops. According to Bliss (1993b) there is a relationship between maturity and nitrogen fixed with later maturing dry bean genotypes fixing more nitrogen owing to the fact that they are in the ground longer than shorter season cultivars providing them more time to fix nitrogen.

Grain legumes, such as soybean, dry bean, and cowpea (*Vigna unguiculata*) are selected to partition resources into the seed. This seed is then harvested, with the residual straw typically left in the field. These residues may contribute to increased nitrogen levels in the soil; however, the plant is selected to translocate most of the nitrogen into the seed (as reviewed in Buttery et al., 1992). As a result, the crop residue from grain legumes often contributes little nitrogen to the following crop. Beckie and Brandt (1997) calculated that integration of field pea (*Pisum sativum*) residue contributed 15 kg N ha^{-1} for each 1000 kg of grain yield. Soon and Arshad (2002) estimated that pea straw contributed up to 41kg N ha^{-1} with an additional 2-3 kg N ha^{-1} in the root system indicating that the roots may contribute a small percentage to the total nitrogen left for the next crop.

Some grain legume crops are more efficient at fixing nitrogen. Soybean has the ability to fix enough nitrogen through biological nitrogen fixation (BNF) to achieve acceptable yields without the need for additional fertilizer. Piha and Munns, (1997a), reported soybean fixing up to 189 kg N ha^{-1}. This was approximately two-fold higher than their findings for common beans, where early maturing genotypes fixed 35 kg N ha^{-1} as compared to the 109 kg N ha^{-1} fixed by later maturing genotypes.

Nitrogen fixing plants form a symbiotic relationship with various soil bacteria such as *Rhizboium* and *Bradyrhizobium*. Formation of nodules is preceded by the plant exuding flavonoides into the root zone which stimulates the *Rhizobium* to release nodulation factors, initiating the process of infection. A root hair is modified by the legume plant into an infection thread, or hook, through which the bacteria enters the plant cell. The bacteria then move into the cortex of the root and begin to multiply. Through signaling between host and bacteria, the cortex cells divide and enlarge to form a nodule. The bacteria differentiate into bacteroids which is the phase of life that fixes nitrogen. This nodule serves as the housing for the nitrogen fixation process. While Rhizobia are aerobic bacteria, nitrogen fixation requires the absence of oxygen, thus oxygen is limited in the center of the nodule through the action of leghemoglobin, which is similar to hemoglobin in the blood of animals and binds to oxygen. Leghemoglobin controls the levels and movement of oxygen in the plant cells of the nodule and provides the bacteria with oxygen for respiration but not enough to reduce the function of nitrogenase. The presence of leghemoglobin results in the red, pink or orange color of a functioning nodule. Other colors, such as cream, green or gray indicate the nodule is not fixing nitrogen either due to age or the symbiosis of a non-fixing, or poorly fixing *Rhizobium*. Plants likely sanction non performing nodules by restricting carbohydrate flow to those nodules. Once the nodule has formed however, no other bacteria can colonize that infection site. Inside the nodule N^2 is combined with H^2 to form ammonia, through the activity of nitrate reductase, which can then be utilized by the plant. This process requires an investment from the plant in the form of protection, carbohydrates, and proteins for sustenance of the bacteria and energy for fixation while the bacteria provide nitrogen in a form usable by the plant. Disease, abiotic stress and developing seed may all compete with the nodules for resources and thus reduce fixation. (Maiti, 1997; http://en.wikipedia.org/wiki/Rhizobia, accessed November 2009).

Figure 1-1. Simplified equation of the process of nitrogen fixation.

$$\begin{array}{c}\text{Energy}\\ \downarrow \\ N^2+3H^2 \rightarrow 2NH^{3-}\end{array}$$

NITROGEN FIXATION AND THE COMMON BEAN

Like most members of Fabaceae, the common bean *P. vulgaris*, has the ability to fix nitrogen from the air through a symbiotic relationship with *Rhizobia* spp. Unlike many crop legumes, however, the ability to fix a sufficient amount of nitrogen to affect competitive yields through this symbiosis is lacking in dry bean. Estimates of 50 kg N ha^{-1} fixed are reported as common by Bliss (1993a). Since it is unlikely that 50% of the nitrogen applied as fertilizer is assimilated by bean plants, Bliss (1993a) points out that 50 kg N ha^{-1} fixed is equivalent to applying nitrogen at a rate of 100 kg ha^{-1}. Typical nitrogen application rates are closer to 50-60 kg N ha^{-1} with recommendations up to 100 kg n ha^{-1} when high yields (2,000 kg ha^{-1} or greater) are desired, and with certain determinate seed classes such as kidney and cranberry beans that respond to N (http://www.ag.ndsu.nodak.edu/plantsci/breeding/drybean, accessed December 2009). Accordingly, beans would have to fix between 50 and 100 kg N ha^{-1} in order to achieve competitive yields. Dry bean breeding programs typically supplement soil nitrogen with fertilizer during the process of selection and yield evaluation. As a result, genotypes are not selected for their ability to fix nitrogen as they are being primarily selected for disease resistance, maturity, yield, and quality traits.

METHODS TO STUDY NITROGEN FIXATION IN COMMON BEAN

Within the primary gene pool of *P. vulgaris*, there is variation for improved nitrogen fixation (Graham, 1981; Park and Buttery, 1990; Rennie and Kemp, 1983; Bliss, 1993). In reviewing studies conducted at CIAT, Graham (1990) reports fixation rates of bean genotypes ranging from 3 kg N ha^{-1} to 125 kg N ha^{-1}. There is a considerable variation suggesting the opportunity for potential improvement in BNF. Graham states that nitrogen fixation is a quantitative trait, suggesting that improvements can be made by cyclic or recurrent breeding methods to further improve nitrogen fixation. Utilizing three common bean genotypes identified in previous studies, Elizondo-Barron et al. (1999) demonstrated that following two generations of selection gain for seed yield was 10.2% while there was an increase of 8.1% for seed nitrogen. Significant increases were seen between the average values of the original parent lines (C0 in their study) and the selected C2 plants. And increase in nitrogen from selection was seen with the original parent lines was 5.0 g N fixed per four plants whereas the C2 selected lines achieved 6.2 g N fixed per four plants. Seed yield for every four plants was 137 g for the parental lines and averaged 180 g per four plants in the selected C2 lines. Elizondo-Barron et al., (1999) selected solely on seed nitrogen and shoot biomass to achieve these increases. St. Clair et al. (1988) measured gains over the recurrent parent, Sanilac, in population 24 which utilized Puebla 152 as the donor parent for nitrogen fixation. One line in particular, 24-17, fixed 1,110 mg N $plant^{-1}$, while Puebla 152 fixed an average of 1,053 mg N $plant^{-1}$ and Sanilac fixed 629 mg N $plant^{-1}$. Both Elizondo-Barron et al. (1999) and St. Clair et al. (1988) found progeny with significant increases in nitrogen fixed over one or both parents used in their respective studies.

Bliss (1993b) suggested that there is a need to combine this variation into a single agronomic package-that is a single genotype which has the appropriate agronomic characteristics, adaptation to day length, and disease resistance-to be usable in commercial situations. One obstacle to selecting genotypes for their BNF ability is the difficulty in observing and measuring root traits, especially of field grown plants. Methods to measure the amount of nitrogen fixed such as acetylene reduction analysis, ^{15}N dilution, and total nitrogen content have been developed. Each method utilized in studying BNF characteristics has advantages, but also has limitations and as such no single method is adequate.

A once popular method to measure nitrogen fixation is acetylene reduction analysis (ARA) which allows the indirect measure of nitrogen fixation by measuring the evolution of ethylene from the process in the presence of acetylene. The level of ethylene is then measured through gas chromatography. The level of ethylene is then interpreted as a measure of nitrogenase activity, and thus nitrogen fixation (Hardarson and Danso, 1993; Herridge and Danso, 1995). ARA offers measurements at a very specific point in time, however does not account for the level of fixation during the entire growing season. Also, nodule activity is dependent on time of day as well as soil moisture and temperature. In addition nodules or roots containing nodules are typically detached from the plant. The limitations to fixation are not known when these tissues are removed from their source of carbohydrates. Hardarson and Danso (1993) also suggest that there is the potential of the presence of the acetylene to reduce the activity of nitrate reductase which is an enzyme critical to nitrogen fixation. All these factors might result in invalid estimates of BNF. None the less, St. Clair and Bliss (1991) utilized ARA to identify the high fixing genotype Puebla 152. They also found that differences in nitrogen fixation as determined by ARA were a moderately heritable quantitative trait.

Tropical legumes transport nitrogen from fixation within the plant in the form of ureides, allontoin, and allontoic acid. Cool season legumes transport nitrogen from fixation as amides. Nitrogen derived from soil or fertilizer is transported in the form of nitrates (Hardarson and Danso, 1993). For crops such as soybean, dry bean, and cowpea, the amount of ureide in the sap bleeding from a cut stem has been used as a means to measure nitrogen fixation (Hardarson and Danso, 1993; Thomas et al., 1984). Thomas et al. (1984) utilized a subset of the Puebla 152 x Sanilac population developed by St. Clair and Bliss (1991) and discovered that Puebla 152 transported a greater amount of N than Sanilac. Also, during vegetative growth of both parents most nitrogen transported was in the form of nitrate, though Puebla 152 began transporting more ureides during late vegetative growth. This changed, however during blooming and pod fill when ureide levels increased in both parents. Thomas et al. (1984) concluded that percent of nitrogen in the form of ureides in both high fixing genotypes and low fixing genotypes of those studied was not necessarily different, though the rate of translocation was higher in those that fixed larger amounts of nitrogen. Rate of nitrogen fixation in this study was determined by ARA.

Nodule number or mass could also provide an estimate of the potential for BNF, but this does not take into account the activity of the nodules themselves. Also, edaphic and weather conditions interact to effect this characteristic resulting in a reduced ability of researchers to notice and document differences based on observing nodules (Herridge and Danso, 1995). Measuring nodule characteristics is destructive and is only useful in breeding programs practicing selection at the genetic family level where some plants may be sacrificed to measure nodule characteristics while others are left to produce seed (Wolyn et al., 1991). Hardarson and Danso (1993) indicate that nodule number or mass is best when used together with other measurements of nitrogen fixation in order to be useful in interpretation of data. This would help to determine if nodules present were actually fixing nitrogen while still giving an idea of potential based on the number and condition of nodules present.

Other indirect methods include shoot dry weight and the total N of the shoots. According to Hardarson and Danso (1993) measuring the biomass of the crop is a simple and relatively accurate method to estimate nitrogen fixation when comparing different genotypes. Shoot dry weight can only be used to compare genotypes in the same study or planting, as no value is given to the actual nitrogen fixed thus comparisons with the work of other researchers is limited. The primary assumption in this strategy is that nitrogen is the limiting factor; if other limitations exist the comparisons between genotypes may not be related to nitrogen fixation. Differences in nitrogen fixation between genotypes may only be apparent when plants are grown in low N or no N soil conditions (St. Clair and Bliss, 1991; Bliss, 1993b; Graham and Vance, 2000; Rennie, 1983) Evaluation is facilitated by the visual appearance of the plants (Pereira et al., 1989). Plant type may confound the results, however, as some growth habits in and of themselves may limit nitrogen uptake by reduced soil mining, susceptibility of roots to pathogens, or availability of *Rhizobium* infection sites.

Nitrogen difference methods rely on the availability of a reference crop. This reference crop must acquire soil nitrogen in a similar manner to the crop being grown, but is not able to fix nitrogen itself. The amount of nitrogen in the reference crop represents the available N, from both the soil and any nitrogen fertilizer applied. The difference between a nitrogen fixing genotype and the amount of nitrogen in the non-fixing reference crop represents the nitrogen fixed by the fixing genotype. This method has been shown to be a good predictor of N fixation and produces similar results to the ^{15}N dilution method, discussed below (Patterson and La Rue, 1983; Talbott et al., 1982; Witty, 1983).

The ^{15}N Dilution method requires the addition of fertilizer enhanced in the level of ^{15}N, or alternatively having a reduction of ^{15}N contained in the fertilizer. Plants are fertilized with the modified fertilizer and the percent N in the plant derived from the atmosphere (%Ndfa) is determined by comparing the level of ^{15}N in the plant tissue to determine how much nitrogen came from the fertilizer. The proportion which did not originate from the fertilizer as determined by the percent of ^{15}N is the nitrogen that was fixed. St. Clair and Bliss (1991) evaluated a population of inbred back cross lines (IB) using a ^{15}N depleted fertilizer. Lines from an IB cross were identified as high potential fixers using ARA. These high fixing lines were then planted in the field and fertilized with a solution containing 0.01 atom % ^{15}N (compared to a natural abundance of ^{15}N in the atmosphere of 0.386%). The lines planted included the parental checks, Puebla 152, donor parent and Sanilac, the low fixing recurrent parent along with the IB lines and a non fixing soybean cultivar. They sampled plants at mid-pod fill and at maturity. At each harvest, plants were dried and ground and digested using the Kjeldahl method. The distillate was then analyzed by converting to N_2 by LiOBr oxidation (Ross and Martin, 1970). The ratio of ^{15}N to ^{14}N was then determined and used to determine the percent nitrogen derived from the atmosphere (%Ndfa). The purpose of this study was to identify lines with the agronomic traits of Sanilac with the enhanced BNF of Puebla 152. They found that several of the F_3 families studied were superior to Sanilac in BNF, and four lines fixed nitrogen at levels similar to Puebla 152. These plants had acceptable agronomic characteristics which demonstrate that BNF ability is not necessarily linked to late maturity or indeterminate growth.

In working with *Lotus sp.*, *Medicago sativa*, and *Trifolium repens*, Steele et al. (1983) found that the concentration of ^{15}N was dependent on the strain of Rhizobia colonizing the root nodule. Differences in the accumulation of ^{15}N during nitrogen fixation would be one disadvantage to estimating nitrogen fixation with ^{15}N. Another disadvantage of the ^{15}N dilution method is the cost of the fertilizer. There may be difficulties in applying the fertilizer in an even and consistent manner to ensure that all treatments and plants within each treatment have the same access to the enriched or depleted fertilizer. Choice of a reference crop can also affect the outcomes of the study. Danzo et al. (1993) indicate that choice of the reference crop is the greatest obstacle to accurate nitrogen fixation measurement. Both the non fixing reference crop and the fixing crop must assimilate nitrogen from the soil in the same way. Graham (1990) also attributes error in measuring nitrogen fixation due to the fact that applying nitrogen fertilizer has been associated with the reduction or even inhibition of nodulation and nodule activity.

Reference crops are often grass species which have different root systems than legumes. Roots are typically more fibrous and are less deeply rooted than grain legumes (Danso et al., 1993; Herridge and Danso, 1995; Rennie, 1983). This can influence the layers of soil that the reference crop assimilates nitrogen from, either from the upper layer where the ^{15}N enriched/depleted fertilizer is most likely to be, or perhaps deeper where the applied fertilizer is less likely to have reached. An alternative reference crop is a non-nodulating grain legume. St. Clair and Bliss (1991) used the non nodulating soybean genotype "Clay" as their non-fixing reference crop. Utilizing soybean allays some of the concerns regarding problems of using a different species for a reference crop having a similar growth habit and belonging to the same family (Fabaceae) as bean with similar growth habits and root architecture, though with differences such as longer maturity and higher yield than common bean. Henson (1993) utilized dwarf sorghum line BR005 and wheat line BR10 as reference crops for a study of 17 dry bean genotypes to compare results of nitrogen fixation estimates with different reference crops. The ^{15}N dilution method was used. Warm temperatures during reproductive stage of the beans inhibited fixation, and resulted in senescence of the nodules. The wheat assimilated nitrogen in a similar manner to beans at most sampling dates while the sorghum assimilated nitrogen at a much higher rate than the dry beans. This experiment underscores the importance of 1) multiple seasons might be necessary to satisfactorily evaluate nitrogen fixation and 2) reference crop behavior influences estimates of nitrogen fixation. Henson (1993) concludes that the bean genotype with the lowest amount of ^{15}N in its tissue is the most efficient nitrogen fixer, and the one with the greatest amount of ^{15}N is the least efficient fixer. Using this reasoning a reference crop is not needed.

Common bean lines unable to fix nitrogen have been developed through mutation breeding. These mutated genotypes either do not produce nodules or produce nodules that do not possess the ability to fix nitrogen. Park and Buttery (1992) utilized chemical ethyl methyl sulfonate (EMS) mutagen to generate non-fixing lines from common bean genotype OAC Rico, belonging to the navy market class. Shirtliffe et al. (1995) were able to identify R69 and R99 as being unable to fix nitrogen by evaluating the growth of the plants in *Rhizobium*-inoculated, nitrogen limiting conditions. R69 produced small nodules which were pale in color and did not appear to be fixing nitrogen. R99 produced no nodules except in the presence of one strain of *Rhizobium*. These nodules were similar to those in R69, though less than one per plant developed. R99 is considered a non-nodulating mutant. Including a non-fixing reference crop that is of the same species addresses some of the limitations such as different root growth characteristics or nitrogen assimilation differences since the non-fixing reference crop has the same growth characteristics as the crop being evaluated. Since there are differences in biomass or seed yield potential between genotypes, care must be taken when making comparisons between the non fixing genotype and normal N-fixing genotypes.

St. Clair and Bliss (1991) studied the potential to increase BNF by crossing a widely recognized high fixing line, Puebla 152 (Bliss 1993b; Bliss et al., 1989; Pereira and Bliss, 1987; St. Clair and Bliss, 1991; Thomas et al., 1984; Wolyn et al., 1991) with a poor fixing line, Sanilac. The purpose of the project was to produce a genotype with high BNF potential with the agronomic traits necessary in the Midwestern United States. An inbred backcross population was developed and the ^{15}N dilution method was utilized to screen the resulting lines. They discovered that some lines fixed a high percentage of plant nitrogen; this nitrogen did not always result in increased seed yield or seed nitrogen which demonstrates that partitioning and efficiency are still important agronomic traits when considering enhanced BNF. They also noted that if the plants being studied were of the same growth type, shoot N was a good measure of nitrogen fixation (St Clair and Bliss, 1991).

The importance of the environment and other factors cannot be ignored (Buttery et al., 1992). The interaction of the host plant and the *Rhizobium* strain infecting the roots affect the rate of nitrogen fixation. In their review, Buttery et al. (1992) found that nitrogen fixation can be inhibited or reduced by drought, excess soil moisture, and disease. In addition, an excessive level of nitrate in the soil, either from a rich soil or that applied through fertilizer may inhibit nitrogen fixation. Any factor that reduces the plant's ability to provide carbohydrates may reduce the nitrogen fixation which is dependent on that source of energy. Plants supplied with combined nitrogen from the soil or fertilizer exhibit more stability in terms of yield than those relying on nitrogen fixation alone. In comparing fixation of common bean to other legumes, Buttery et al. (1992) mention that the timing of peak N fixation and the duration of N fixation differs. Dry beans do not begin substantial fixation until early reproductive phase and nitrogen fixation peaks during pod fill, dropping off rapidly as carbohydrates are preferentially translocated to the seed and no longer to the roots. It should be noted that as mentioned elsewhere in this literature review that Puebla 152 begins to fix nitrogen in late vegetative phase and continues beyond stages when declines are seen in other genotypes that fix less nitrogen. Extending the period of nitrogen fixation might be an effective means to improving the total nitrogen fixed by commercial dry bean genotypes. Pena-Cabriales et al. (1993) discovered that genotypes did differ in the timing of peak nitrogen fixation and also in partitioning of that fixed nitrogen to pods and seeds. Evaluating several genotypes in the field and greenhouse they discovered that most nitrogen fixation did not occur until reproductive growth began. Common bean genotypes 'Flor De Mayo' and 'Kallmet' did not assimilate nitrogen during maturity, however remobilized nitrogen from elsewhere in the plant into the pods. Kallmet partitioned less than half of its total N into the pods, with the remainder retained in the straw. Nodule number and mass decreased during maturity, with evidence that Flor De Mayo experienced nodule senescence. This may be misleading, however, in that it is suspected that nodules on lateral roots are likely still functioning at this stage and contributing to an increase in fixed nitrogen (Hardarson et al., 1989). These findings reflect what has been reported in soybean where similar periods of fixation were reported (Zapata, 1987). Park and Buttery (1989) showed that variation existed in dry beans for tolerance to high soil nitrates and nodule development. Some genotypes were completely inhibited in nodule development under nitrogen levels of 10.5 mM nitrogen, their highest nitrogen treatment. Nodule dry weight increased from the 0 N treatments to the 3.5 mM nitrogen treatment suggesting that some nitrogen early in the development of the plant may help to increase later nitrogen fixation. Since earlier nodulation may be important to later total nitrogen fixed, genotypes capable of forming nodules under higher initial levels of nitrogen should be selected. Application of urea to the foliage at flowering resulted in an increase in seed yield while not reducing nodule activity and consequently nitrogen fixed (Da Silva et al., 1993). Foliar application might not inhibit the function of nitrogenase in the nodules, which is an integral component of nitrogen fixation. Applying urea to the soil at the same rate as that applied to the foliage did not result in the same increases in seed yield. Treatments receiving no fertilizer fixed the greatest amount of nitrogen, nearly 50 kg N ha^{-1}, compared to the treatment receiving 50 kg N ha^{-1} applied as urea to the soil which fixed just over 10 kg N ha^{-1} (Da Silva et al., 1993). Working with common bean genotypes Puebla 152 and 'Negro Argel,' Muller et al. (1993) found that nitrogen level in the soil did not have a significant effect on total nitrogen fixed. Both Puebla 152 and Negro Argel were identified as having superior BNF in this greenhouse study.

While considerable focus has been on nitrogen levels, either applied as fertilizer or already present in the soil, Tsai et al. (1993) looked at the levels of other major nutrients: P, K, and S. These nutrients are evidently important to both the proper development and subsequent function of nodules on common bean. Highest nitrogen fixation for bean genotype Carioca was observed at medium soil fertility levels of 50 mg P kg^{-1} 1.63 mg K kg^{-1}, and 10 mg S kg^{-1}, at four different nitrogen levels. Greatest levels of fixation occurred at the lower soil nitrogen levels; 5 mg N kg^{-1} soil and 15 mg N kg^{-1}. These findings have implications as to where dependence on nitrogen fixation might be most applicable. In soils generally deficient in these nutrients, maximum nitrogen fixation may not be achieved. In regions with adequate levels of soil fertility nitrogen fixation may be sufficient to provide nitrogen needed to produce acceptable yields.

Moisture levels have also been implicated in reducing nitrogen fixation. Pena-Cabriales and Castellanos (1993) showed that percent nitrogen fixed was not affected as much by drought stress at either vegetative growth or reproductive stage as was the grain yield. Plants subjected to reproductive water stress likely had already fixed a large portion of the nitrogen they would have fixed. Nodulation and BNF must have the ability to recover from such stresses when water becomes available again.

The above findings demonstrate that there is an opportunity for improvement in BNF of common bean. Identifying genotypes able to nodulate earlier in their life cycle would expand the time (duration) during which N fixation is occurring. Similarly, genotypes that continue to fix N up to physiological maturity would extend the period of nitrogen fixation. Coupling earlier nodulation with a greater ability to nodulate at high soil nitrate levels would improve genotype N fixation capacity considerably. Enhancing nitrogen uptake during the vegetative and early reproductive growth stages would enhance nitrogen available for remobilization to support the developing seed.

THE ROLE OF *RHIZOBIUM*

The other partner in the symbiosis needs to be addressed in discussing improving nitrogen fixation in grain legumes. Various *Rhizobium* strains have been shown to have different abilities to fix nitrogen as well as compete with other strains for infection sites on the plant roots. Where beans have been grown historically, there is likely an existing population of *Rhizobium*. The indigenous strains may not be the best adapted to fix nitrogen with the genotype planted but may out compete those inoculant strains which are superior in nitrogen fixation (Alvaro et al., 1989; Dubois and Burris, 1986; Deoliveira and Graham, 1990; Perret and Broughton, 1998; Rosas et al., 1998; Vasquez-Arroyo et al., 1998; Weiser et al., 1985).

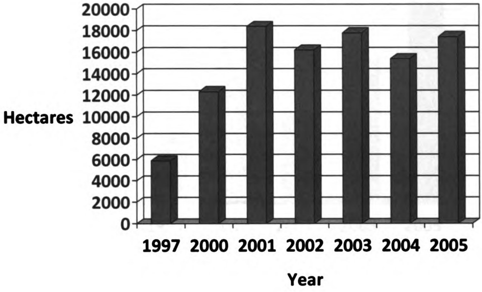
Vasquez-Arroyo et al. (1998) studied the occupancy of the nodules of three field grown common bean genotypes. They discovered that there was considerable variability in the ARA values of the different strains isolated and that they had different abilities to compete for nodulation sites. In addition there was a strain:genotype interaction. For example strain N4, as identified in the study had poor ARA values with common bean genotype FM-M-38, while the same bean genotype had high ARA values with strain Q21. In the same study 64% of nodules on the roots of common bean genotype Negro Queretaro were inhabited by *Rhizobium* strain Q21 (Vasquez-Arroyo et al., 1998). Rosas et al. (1998) performed competition studies by mutating *Rhizobium etli* strain KIM5s, creating a non-fixing strain. Plants were planted in the greenhouse in a low nitrogen soil mix containing indigenous *Rhizobium* sp. Pots were inoculated with the mutated strain, called KM6001. Plants were later evaluated visually for color of their foliage. Plants that formed nodules with the non-fixing mutant *Rhizobium* strain would be lighter green since they were fixing less nitrogen, while those that were dark green were nodulated with indigenous strains able to fix nitrogen. Of the 820 genotypes screened, two did not nodulate normally, the navy bean Sanilac and a non-nodulating line NOD125 developed at CIAT. Those common bean genotypes showing N deficiency yellow color preferentially selected in some manner the non-fixing *Rhizobium etli* strain KM6001. By extension the researchers identified common bean genotypes that preferentially nodulated with *Rhizobium etli* strain KIM5s, which is a strain superior at fixing nitrogen. Identifying common bean genotypes which may form associations with specific applied inoculant strains would circumvent the problem of forming nodules with inefficient indigenous strains of *Rhizobium*.

Bliss (1993a) encourages the utilization of indirect methods to measure nitrogen fixation. The nitrogen difference method, seed nitrogen content, and shoot mass are credited with being quick and accurate in predicting nitrogen content as well as cost effective when considering the size of many breeding programs where hundreds of lines need to be evaluated. Elizondo-Barron et al. (1999) crossed common bean genotypes Puebla 152, RIZ 21, and BAT271 in all possible combinations, bulking the reciprocals. These three genotypes were originally identified in a previous study as having superior nitrogen fixation, though possessing different nitrogen fixation traits. Seventeen lines were identified from $F_{2.3}$ lines which were originally derived from Puebla 152 x BAT 271 and Puebla 152 x RIZ 21. After two cycles of recurrent selection these lines were evaluated in the field where soil conditions were low in nitrogen and seed was inoculated with a single strain, *Rhizobium etli* UMR1632. Field data collected included seed yield and total seed nitrogen. Selection was based on family means and appeared to be an effective means of achieving increased seed yield and seed nitrogen. While methods such as total seed nitrogen or those measuring nitrogen in biomass late in the crop cycle may provide an accurate measure of total nitrogen fixed, they may not identify critical events in the plant's life cycle where attention needs to be given to increase nitrogen fixation. For example, earlier nodulation and fixation or the extension of fixation late into physiological maturity may have direct impact on total nitrogen fixed. BNF is a complex system and improvements made at defined steps in the process may be the most efficient means to achieve results in a timelier manner.

OBJECTIVES

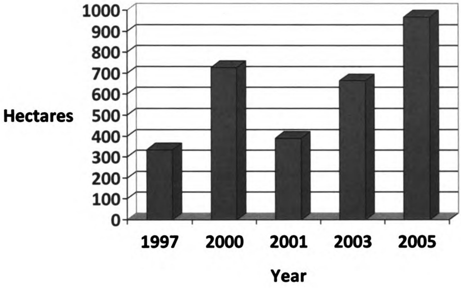
The objective of this research was to evaluate dry bean genotypes under an organic production system and compare with conventional production to identify genotypes best suited for organic production. Identifying characteristics that enhance the suitability of a genotype to perform in organic production systems was investigated. Nitrogen availability in organic systems appears to be a limiting factor thus a second objective was to identify cost effective and accurate means of evaluating the BNF potential of bean genotypes both for elite line screening and investigation of the genetic characteristics influencing BNF for use in future breeding programs. Having a simple and efficient protocol to evaluate BNF would facilitate routine screening of bean breeding lines during the breeding process to increase the BNF potential of future variety releases.

Figure 1-2. Hectares of certified organic cropland in Michigan, 1997 through 2005.



Data from USDA-ERS.

Figure 1-3. Hectares Organic Dry beans (*Phaseolus vulgaris* L.) produced in Michigan.



Data from USDA-ERS.

Table 1-1. U.S. organic dry bean (*Phaseolus vulgaris* L.) hectares by state, 1997 through 2005.

State	1997	2000	2001	2003	2005
	-------------------		-Hectares	----------------	
California	449	373	259	263	186
Michigan	334	728	390	664	968
Colorado	225	1525	2213	1409	1442
North Dakota	177	501	1126	692	409
Idaho	176	233	184		
Wisconsin	97			110	
Kansas	96				
Texas	77				432
Oklahoma	61				
Minnesota		432		139	192
Nebraska		347		104	
Missouri		256	402		
Utah		255	226		
Iowa			243	362	
Illinois			236		
Arizona				57	
Washington					226
Other	199	2468	826	183	126
Total	1891	7118	6105	3983	3981

Data from USDA-ERS.

CHAPTER 2

COMPARISON OF DRY BEAN GENOTYPES UNDER ORGANIC AND CONVENTIONAL PRODUCTION SYSTEMS

ABSTRACT

Thirty two diverse dry bean genotypes were evaluated side by side under organic and conventional production systems. Trial sites were located in grower fields in Gratiot County, MI, in 2007 and 2008 and in Tuscola County in 2009. Trials were also conducted at Kellogg Biological Station in Kalamazoo County, MI, in all three years. The conventional plots were treated following standard acceptable management practices including application of granular fertilizer at planting and use of chemical seed treatments and foliar sprays to control pests. For the organic treatments certified organic land was used and only approved methods for organic production were followed. *Rhizobium* inoculant was applied to seeds in the organic treatment prior to planting. Higher yields were observed in conventional treatments than in the organic treatment. Seed classes that yielded well in the organic system included pink, red, and black seeded genotypes. These groups also had the highest accumulation of nitrogen of the seed classes under organic production. The black bean genotype Zorro was among the five highest five yielding genotypes at all sites under both organic and conventional treatments. The small red germplasm line TARS-SR05 accumulated the highest nitrogen yield (> 100 kg ha^{-1}) under low soil nitrogen conditions. Some genotypes appear better suited to organic production than others; however, those genotypes performing poorly under the organic treatment also performed poorly under conventional treatment. Genotypes of Andean

origin did not perform as well as genotypes of Middle American origin in either organic or conventional systems. Older cultivars, such as the heirloom navy bean 'Michelite, commonly believed to be better suited to organic production, did not perform as well as modern commercial cultivars.

INTRODUCTION

The increased interest in organic production of dry beans has emphasized the need to identify dry bean genotypes that will perform successfully in an organic system. Modern breeding programs utilize conventional production systems during the breeding process to develop commercial cultivars. Application of fertilizer and chemical pesticides are normally utilized to minimize pests, disease, and nutrient deficiencies in order to maximize yield, eliminate variability, and provide a more uniform environment for selection. With the low cost of nitrogen fertilizers, breeders paid little attention to biological nitrogen-fixation (BNF) so in the absence of direct selection for N-fixing ability this valuable characteristic may have been lost in current bean cultivars.

The area planted to organic dry beans has seen a considerable increase in recent years. In the period from 1997 to 2005 the number of acres planted in Michigan expanded from 334 to 968 ha (ERS-NOP, Organic Briefing Room, http://www.ers.usda.gov/briefing/organic/ accessed December 2009). Challenges encountered in conventional production also affect the production of dry beans in an organic system. While insects may be controlled by insecticides in both systems, only approved natural pesticides may be applied in organic systems. Nutrient levels are also addressed differently between the two systems. Nutrients are typically applied in the form of fertilizers in conventional production systems. Organic production systems rely on the application of manures and compost as well as crop rotation to maintain nutrient levels in soils, and forage legumes are often included in many crop rotations as they contribute to soil fertility by fixing nitrogen.

Studies have been conducted to compare the performance of different dry bean genotypes in contrasting production systems. Singh et al. (2009) discovered that there was considerable interaction between production system and genotype. Commercial dry bean genotypes and land races were compared under seven different production systems involving organic and conventional practices, high input and low input, as well as on farm and on station treatments. Genotypes such as pinto bean 'Othello' and great northern 'Matterhorn' were more stable across production systems. Others, such as pinto bean 'Buster' and pinto bean 'Bill Z' were more responsive to high inputs and may be better suited to systems where fertilizer and supplemental irrigation are utilized to maximize yield.

Comparing yield of conventional and organic production systems in developed and developing countries of the world, Badgley et al. (2007) showed that on average, the ratio of the yield between organically produced and conventionally produced pulse crops was 1.86 and reached 3.99 in some locations. For the developed world the ratio was 0.86. The differences observed between the developed world and the developing world between organic and conventional production systems likely rely on the availability of inputs in the developing world. Fertilizers and pesticides may not be readily available in developing countries nor the technologies to utilize these inputs effectively, bringing productivity in both conventional and organic systems into a similar yield potential range.

Anecdotal evidence suggests that there is an expectation for organic systems to yield less than conventional systems due to the reduced inputs. There seems to be potential to increase yields in organic systems to be on parity, at least, with conventional system in the developed world. Breeding programs typically select for disease resistant cultivars which would be beneficial in both conventional and organic production systems. Also, plant architecture and other agronomic and phenological characteristics such as maturity would likely be advantageous under both organic and conventional systems. However, a more vigorous vegetative growth that is somewhat more open may help to reduce weed competition by shading the ground more quickly, perhaps reducing the need to cultivate as the plant canopy closes to control weeds. A canopy that closes more quickly may also make it impossible to cultivate without damage to the bean plants. Thus, dry bean canopy would need to be dense enough to eliminate the need for cultivation after canopy closure.

One area, however, that is generally ignored in conventional breeding programs is plant nutrition. Since conventional production systems rely on the addition of synthetic fertilizers to compensate for soil lacking in proper nutrient levels little effort has been made to select for genotypes able to resist nutrient deficiencies or are better able to utilize nutrients, or even provide their own nutrients through BNF. As discussed elsewhere, many researchers have discovered variability in dry beans for their ability to fix nitrogen. Bliss (1993b) and Graham (1981) noted considerable variability in BNF between dry bean genotypes, and suggested that there was potential to breed dry beans capable of producing sufficient nitrogen through BNF to meet production goals.

Ability of dry bean genotypes to fix nitrogen may result in increased yield under conditions of environmental stress. In two years of trials in Staples, MN, De Jensen et al.

(2004) found that inoculation of seed with *Rhizobium* as well as a biocontrol agent, *Bacillus subtilis*, increased yield of dry beans and soybeans which had been planted in soils heavily infected with root rot causing pathogens *Fusarium solani*, *F. oxysporum*, and *Rhizoctonia solani*. In addition to the inoculant, tillage resulting in the break up of compacted soil also increased yield. Superior nitrogen fixing dry bean genotypes such as TARS-SR05 which have been developed to tolerate poor soil conditions such as low fertility, compaction, and the negative effects of root rot organisms (Smith et al., 2007) may alleviate the need for application of nitrogen and thus help to reduce the negative effects of root rot pathogens.

Soils in Michigan are often adequate in levels of phosphorus, potassium, and other nutrients considered limiting in BNF (http://ipmnews.msu.edu, accessed December 2009). While external factors such as erratic rainfall limit BNF, the lack of ability of some genotypes to fix sufficient nitrogen to achieve a competitive yield without addition of exogenous nitrogen is a major factor limiting BNF.

Since BNF ability has not been a selection criterion in modern breeding programs, the potential of elite breeding lines and commercial bean cultivars to fix nitrogen may be limited. Graham (1981) and Bliss (1993a) have suggested that selection based on yield indirectly selects for improved BNF. Yield is a major component of modern breeding programs, and perhaps a primary factor affecting the success of a genotype in being considered for commercial release. How will selection based on conventional production methods translate to cultivars adapted to organic production systems?

The objectives of the present study were to compare response of diverse dry bean genotypes to organic production systems. In addition, the identification of key traits that contribute to the success of dry bean genotype(s) in organic production systems was studied. The study was also intended to understand if dry bean genotype(s) developed for conventional production systems would be competitive in an organic production system.

MATERIALS AND METHODS

Thirty two diverse dry bean genotypes, including one non-nodulating check, R99, were selected for side by side comparison of performance in organic and conventional production systems. These genotypes (table 2-1) were chosen to represent the market classes grown in Michigan as well as representing the greatest diversity available among modern dry bean genotypes. Important commercial cultivars were chosen in order to be able to offer recommendations to growers wishing to produce beans organically. Elite breeding lines were also chosen to evaluate them in an organic production system prior to potential release. Field tests were conducted on both experiment station land and farmers fields where conventional land and organically certified land was located adjacent to each other. Kellogg Biological Station (KBS) is located in northern Kalamazoo County, Ross Township MI, and has both conventionally maintained experimental fields as well as organically certified fields. Soil type at the KBS sites is Kalamazoo loam, see table 2-2 for soil characteristics. Trial plots in Gratiot County in 2007 were planted on a Metamora-Capac Loam; in 2008 the soil was a Selfridge loam (see table 2-2). The fields in Gratiot County in both 2007-near Ithaca, MI and 2008-in St. Louis, MI, were cultivated by the organic grower. Tests in 2009 were located in Tuscola County and were planted on an alkaline Tappan Loam. Corn was the previous crop at all locations and all years except at KBS in 2009 where pumpkins and squash (*Cucurbita* spp.) were planted the previous season. Precipitation was variable from year to year and throughout each field season (Table 2-3). Below normal rainfall, 217 mm at KBS in 2007 resulted in severe drought stress.

Experimental design was a side by side RCB design. Genotypes were separated according to seed size to facilitate planting, with 16 small seeded lines planted in one half of the field with 16 large and medium seeded planted in adjacent plots. The same planting design was used in all fields in each season. Each location had one conventional field and one organic field side by side. The conventional plot was lost to flooding in Tuscola County in 2009. Four reps were planted in each treatment.

Each plot at KBS consisted of four-6m rows spaced 0.5m apart. Seeds were planted at a density of 267,000 plants ha^{-1}. The two outer rows served as guard rows to limit border affects on the research station. The center 4.6m of the two center rows was harvested to estimate seed yield. Plots on farmer's fields in Gratiot County and Tuscola County consisted of two-6m rows relying on the variety in the adjacent plot to limit border affects. Plants were harvested by pulling and then thrashing mechanically.

Seed to be planted in the conventional treatment was coated with seed treatment containing an insecticide-Cruiser, fungicide-ApronMax, and bactericide-Streptomycin as is typically used on seed beans in Michigan. Weeds were controlled through chemical means prior to planting, applying the preemergent herbicides Sonalan, Eptam, and Dual in a single application prior to planting. Plots were mechanically cultivated prior to canopy closure. Under conventional management, granular fertilizer (19-19-19) was applied at planting beneath the seed at a rate to provide 45 kg N ha^{-1}. Urea was applied at 50% bloom at a rate of approximately 34 kg ha^{-1} at KBS. Insects, specifically potato leaf hopper, *Empoasca fabae*, were controlled with Asana (Esfenvalerate (S)-cyano (3-phenoxyphenyl methyl (S)-4-chloro-alpha-(1-methylethyl) Benzeneacetate) at a rate of 190 ml ha^{-1} as needed according to current recommendations.

Seed for the organic treatment was coated with a commercial preparation of *Rhizobium leguminosarum* bv *phaseoli* (Nitrastik-D, BMD Crop Bioscience, Brookfield, WI) by swirling seed in the powdered peat preparation with a small amount of water. No fertilizer was applied. Weed control consisted of hand pulling and cultivation, including a rotary hoe used to control early weeds just after seedling emergence. Insects, specifically potato leaf hopper, were controlled with an OMRI approved insecticide, Pyganic EC 5.0, labeled for potato leaf hopper applied at a rate of 95 ml ha^{-1} resulting in 90.25 ml active ingredient ha^{-1}.

Days to 50% bloom, maturity, and stand counts were recorded. After harvest seed was air dried and cleaned to remove field debris. Samples were measured for moisture content and weighed. A sub sample of 30g was taken from each plot and ground to 40 mesh in a Christy-Turner Lab Mill (Ipswitch, Suffolk, UK) to pass through a 1 mm screen. Samples were sent to A&L Great Lakes Lab, (Fort Wayne, IN) for Kjeldahl analysis to determine total seed nitrogen content of three reps of organic and convention treatments from the KBS.

RESULTS

Yields varied substantially between years on both organic and conventional treatments at KBS (table 2-4). In 2007 yield in both organic and conventional was similar, with conventional plots yielding slightly higher (1934 kg ha^{-1}) than organic treatment, (1862 kg ha^{-1}). In 2008 the difference between organic and conventional treatments was greater with conventional yielding 1967 kg ha^{-1} and organic yielding 1432 kg ha^{-1}. The highest yields were recorded in 2009 with conventional yielding an average of 3627 kg ha^{-1} and the organic treatment averaging 2877 kg ha^{-1}. The three year mean yield of the organic treatment at KBS was 2058 kg ha^{-1} compared to three year mean yield of 2507 kg ha^{-1} for the conventional treatment.

In Gratiot County in 2007 the difference in average yield between organic and conventional was not significant, with conventional yielding 1840 kg ha^{-1} and the organic treatment averaging 1797 kg ha^{-1} (table 2-8). The difference in average yield between organic and conventional increased substantially in 2008 with average yield 2493 kg ha^{-1} in conventional while the organic treatment yielded 1575 kg ha^{-1}. In 2009 the on farm trial was moved to Tuscola County. Organic yield was higher in Tuscola County than the previous two years in Gratiot County, with an average of 2675 kg ha^{-1} (Table 2-9). The conventional treatment in Tuscola County was lost due to heavy rain the week after planting that reduced plant emergence. The same reduction in emergence was not seen in the side by side organic plot which emerged and grew normally the remainder of the season. Though the fields were adjacent, with the required buffer strip separating the certifi

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certified organic and conventional fields, the soils in each treatment were significantly different in friability. The conventional soil was more compacted either due to differences in management or the use of heavy machinery on the conventional plot when the soil was wet. Nutrient stress was observed in the Tuscola County organic trial. The pH of the soil was high, 7.7, which may have resulted in Zinc and Manganese deficiencies. Not all genotypes had symptoms of nutrient deficiency and deficiency ratings were made on all entries. Air pollution damage was also evident at the Tuscola County plots in 2009, which are located in the Wisner Oil Fields with active oil wells surrounding the vicinity. In particular, 'Jaguar', B05039, B04431, and 'Bunsi' showed typical bronzing on the upper leaves due to ozone air pollution.

At the Gratiot County site in 2007 halo blight (*Pseudomonas phaseolicola*) was a problem on 'CELRK' genotype causing early plant death in both organic and conventional treatments. Symptoms were generally limited to CELRK and showed only minor infection on neighboring plots, but did not result in early death of those plants. In 2007 and 2008 white mold (*Sclerotinia sclerotiorum*) was not observed on either organic or conventional treatments. Increased precipitation, cool nights, and the resulting heavy vegetative growth lead to a low level of white mold infection in 2009 in Tuscola County on the organic treatment.

Total seed nitrogen was analyzed for all three years at KBS for both conventional and organic treatments. Significant differences were seen between years and treatments in total nitrogen content of the seed. In 2007 the organic treatment averaged 63 kg N ha^{-1} while the conventional treatment averaged 73 kg N ha^{-1}. Average nitrogen levels for organic were 52 kg ha^{-1} and 93 kg ha^{-1} for conventional in 2008. Average nitrogen levels increased significantly in 2009 for both treatments to 98 kg ha^{-1} in organic and 139 kg ha^{-1} in conventional (table 2-13).

Climatic as well as pest factors resulted in increased levels of stress at KBS in both 2007 and 2008. Precipitation in 2007 at KBS was less than normal, especially early in the season and during vegetative growth (table 2-3) resulting in drought conditions which reduced yields in both conventional and organic treatments. After 6 weeks with little rain 37.5 mm of supplemental overhead irrigation was applied, with an additional application of 37.5 mm applied later in the summer. The drought stress delayed maturity causing re-growth in many of the genotypes. Re-growth was more prevalent in the conventional treatment than the organic treatment. Neither the organic nor the conventional treatments were irrigated at KBS in 2008. Irrigation was applied in 2009 to supplement natural precipitation at a rate of 12.5 mm per week for 5 weeks in July and August.

Weed pressure was minor in the organic and conventional treatments in 2007. Higher weed pressure in the organic treatment in 2008 may have resulted in reduced yield and reduced total nitrogen values. Grass weeds, including crabgrass, *Digitaria* spp. and foxtail, *Setaria* spp., were the primary component of the weed pressure in the organic plot. Early cultivation and hand hoeing was inadequate to reduce weed pressure. None of the bean genotypes seemed able to effectively compete with the weeds resulting in small plants and reduced yields. Weed growth was a general problem in the organic plots when compared to the conventional plots; however at all years and sites other than KBS organic in 2008, cultivation and hand weeding reduced weed pressure to minor levels comparable to that observed in conventional treatments.

The pink and red seed classes were the highest yielding group over all years for both organic and conventional treatments, 2562 kg ha^{-1} and 2829 kg ha^{-1} respectively (table 2-7). Genotypes of Andean origin-kidney and cranberry, grouped due to their relatedness, had the lowest yield in the organic treatment at 1618 kg ha^{-1} while the average for the conventional treatment was slightly higher 1928 kg ha^{-1}. In both organic and conventional treatments the kidney and cranberry market class were statistically similar to the non nodulating check, R99. Pink, red, and black seed classes produced the highest yields at KBS. However, the pink and red seed class ranked third in the conventional system with the pinto seed class producing more than the pink and red class. Pink and red bean genotypes produced the highest nitrogen yield in organic while the black seed class ranked second in nitrogen accumulated. When nitrogen fertilizer was provided there was little variation in the nitrogen content of the seed. Most seed classes at KBS in the conventional system had a nitrogen yield of 100 kg ha^{-1} or greater (table 2-12). The lowest nitrogen yield was observed in the kidney seed class at 89.5 kg N ha^{-1}. The navy seed class also tended toward the low end on nitrogen yield with Michelite, Bunsi, and Vista possessing less than 70 kg N ha^{-1}. The only kidney genotypes having nitrogen yield greater than the mean was Montcalm and Chinook Select in the organic treatment at KBS. Chinook Select was the only kidney bean in the conventional treatment at KBS to have seed nitrogen content greater than the mean.

The genotype with the lowest N yield was R99 in the organic treatment (table 2-13). Nutrient levels on organic fields at KBS are controlled through the use of cover crops and crop rotation including wheat (*Triticum* spp), corn (*Zea mays*), and a grain legume such as soybean with occasional inclusion of other crops such as pumpkin and squash, *Cucurbita* spp which were planted in 2008 at KBS in the field used in this study in 2009. It is expected that nitrogen levels are relatively low in such a treatment as no manures are applied. The genotype with the highest N yield was the black bean cultivar Zorro in the conventional treatment, yielding 133 kg N ha^{-1}. Zorro also had a high nitrogen yield in the organic treatment, producing 97 kg N ha^{-1}.

At KBS three genotypes 'Zorro', Buster, and the navy breeding line N05324 were in the top five yielding cultivars for both conventional and organic treatments. All genotypes yielding higher than the mean in both conventional and organic treatments at KBS were of the Middle American gene pool. Except for the kidney bean USDK-CBB-15 the same trend was observed among genotypes yielding above the mean in both years in Gratiot County. Andean genotypes were heavily represented in the group of genotypes producing below average yields.

No genotype yielded significantly better in organic production than in conventional production, whereas some genotypes yielded better in both treatments. The black bean genotype Zorro was in the top five yielding genotypes in both organic and conventional systems and at both KBS and Gratiot County. Except for the R99, the non nodulating check, the bottom five yielders at all sites were all Andean kidney beans. Plant stand showed a slight negative correlation with both seed yield (r^2=-0.27, p$\leq$0.0001) and N yield (r^2=-0.23, p$\leq$0.001), respectively (table 2-14a).

DISCUSSION

Dry bean genotypes generally yielded more in the conventional than the organic plots. Middle American genotypes such as Zorro showed competitive yields in organic production, and Zorro was consistently represented in the five highest yielding genotypes under both treatments.

Soils were generally adequate in nutrients such as P and K. The 2009 Tuscola County site had a high pH, 7.7, resulting in typical Zn and Mn deficiencies. This site would be useful in screening genotypes for nutrient deficiency. Symptoms of Zn and Mn deficiency were visible on some genotypes and navy bean genotypes were particularly susceptible to micronutrient deficiency. Navy beans N05324, N05311, Bunsi and R99 and the black bean cultivar Condor showed severe symptoms of Zn and Mn deficiency. Neither TARS-SR05 nor Zorro seemed to be affected by nutrient deficiencies.

Despite soil conditions resulting in nutrient deficiencies such as those encountered in Gratiot County in 2007 and 2008 and Tuscola County in 2009 resulting from elevated pH, the black bean genotype Zorro was in the group of 5 highest yielding genotypes. The limited number of elite cultivars as well as the large number of black seeded genotypes likely increased the range in yields reducing the mean for the seed class. Overall pinks and reds as a seed class have the highest yield. The small red bean 'Merlot' as well as the pink bean 'Sedona' produced the highest yields in the organic treatments. These two genotypes remain in the group of 5 highest yielding genotypes in the conventional system. It is possible that these representatives from race Jalisco are better adapted than other genotypes to high pH soils. Singh et al. (2007) cites the origin of the Common Red Mexican bean as the arid highlands of Mexico. It is likely that selection under the historically drought stressed and alkaline conditions have resulted in red and pink market classes being generally better adapted to growth under high pH and low soil moisture. As a class the pinks and reds had the highest nitrogen yield in the organic plot at KBS supporting the suggestion that pink and red beans have characteristics, such as increased drought tolerance, lending them to production in diverse production systems and varying levels of stress. Singh and Westermann (2002) studied the inheritance of zinc deficiency and found that the black bean 'T39' was susceptible to zinc deficiency while the great northern Matterhorn was resistant to zinc deficiency. This trait was controlled by a single dominant gene.

White mold was not observed on either organic or conventional treatments in 2007 and 2008. Increased precipitation, cool nights, and the resulting heavy vegetative growth lead to a low level of white mold pressure in 2009. Only Zorro appeared to be affected in the organic treatment in Tuscola County, likely due to its increased plant vigor and susceptibility to white mold. Both organic and conventional treatments had slight white mold pressure at KBS in both large and small seeded genotypes. Genotypes with type III growth habit such as the navy bean 'Michelite' were more susceptible to white mold infection while growth types I and II seemed to avoid infection. The occurrence of white mold in Michelite likely contributed to its poor performance.

Stand counts were taken to investigate potential damage from the use of the rotary hoe shortly after emergence to control weeds and break the crust formed after rain. Plant stand was slightly correlated with flowering time and had a slight significant negative correlation with both seed yield and N yield (r^2=-0.27, $p \leq 0.0001$, and r^2=-0.23, $p \leq 0.001$, respectively, Table 2-14). This suggests that any reduction in stand due to the use of the rotary hoe was minor. Further reductions in stand may be detrimental as the seeding rate for dry beans is lower than other crops such as soybean. In addition dry bean seedlings are not as resilient to the practice of rotary hoeing; especially larger seeded genotypes that produce bigger seedlings which may experience more plant damage due to the mechanical damage caused by the rotary hoe. The strong negative correlation, (r^2=-0.7, $p \leq 0.0001$) observed between 100 seed weight and maturity is explained by the fact that the determinate large seeded kidneys and cranberry genotypes, are earlier maturing than the navy and black seed classes. Days to flower were also negatively correlated with 100 seed weight. Other slight, non-significant correlations were observed between plant height at flowering and seed yield, nitrogen yield, and 100 seed weight. Increased plant height may be regarded as an indirect measure of plant vigor, and perhaps root growth in certain genotypes.

At KBS, 2007 and 2008 were stressful years due to lack of precipitation (table 2-3). The soil, a Kalamazoo Loam, at KBS is light and sandy and drains quickly. The organic field at KBS in 2008 provided extreme weed pressure. Crabgrass, *Digitaria* spp. and foxtail, *Setaria* spp. were the dominant weed species and grew thickly. These grasses were difficult to control with cultivation and hand weeding. Weeds were a concern in all organic treatments, though were better controlled in other years and locations. Weed pressu

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pressure in the conventional plots was non existent due to the use of preemergence herbicides. Late season weeds in conventional plots likely did not affect yield since the plants had reached physiological maturity by the time the weeds had begun to compete.

Bliss (1993b) indicated that a fixation rate of 100 kg N ha^{-1} would be enough to achieve competitive yields of approximately 2000 kg ha^{-1}. Zorro yielded nearly 100 kg N ha^{-1} and TARS-SR05 yielded considerably more than 100 kg N ha^{-1} in the organic treatment. In the low nitrogen levels at KBS, these two genotypes fixed sufficient nitrogen (100 kg N ha^{-1}) to achieve competitive yields. The kidney seed class acquired the lowest nitrogen yield, 89.5 kg N ha^{-1} in the conventional treatment. In the organic treatment the kidney seed class yielded slightly better than the non nodulating check with the kidney seed class having a nitrogen yield of 64.6 kg ha^{-1}. This suggests that the plants are not as efficient at extracting nitrogen from the soil, or that they are inefficient at partitioning nitrogen from the roots or stems into the seed. Nodule evaluations from the previous study on screening for nitrogen fixation in the greenhouse suggests that Andean genotypes have well developed nodules apparently capable nitrogen fixation based on their size and red or orange color.

In a scatter diagram of seed yield in the organic system versus the conventional system over 3-years at KBS only (Figure 2-1.), Middle American gene pool genotypes grouped in the upper right hand corner. These genotypes yielded well under both conventional and organic production systems indicating that they were responsive to the application of fertilizer but also fixed sufficient nitrogen to produce a competitive yield in the absence of applied fertilizer. Characteristics that this group may have include roots better able to extract soil nitrogen as well as the ability to partition acquired nitrogen into the seed. Genotypes in the bottom right, however, would be less responsive to fertilizer. They may be less able to extract soil nitrogen than the genotypes in the upper right quadrant. The TARS-SR05 genotype fixed more nitrogen than Zorro, almost 112 kg N ha^{-1}, and produced similar seed yields. TARS-SR05 was developed by Smith et al. (2007) in an effort to produce a small red bean with resistance to multiple root diseases and stress. Selection was carried out on $F_{4:5}$ lines in compacted and waterlogged fields with high pressure from *Rhizoctonia solani*, *Xanthomonas axonopodis* pv. *phaseoli*, and *Fusarium solani*. TARS-SR05 had an average yield higher in the organic treatment, 2588 kg ha^{-1}, than in the conventional treatment, 2390 kg $ha,^{-1}$at KBS. Higher performance under organic treatments demonstrates that this genotype may be tolerant of stress and performs better under such conditions. Selection under low fertility, compaction and pathogen pressure helped to produce a line that is stress tolerant which might prove useful in breeding genotypes designed for organic production systems.

The presence of the non nodulating R99 in this quadrant causes some concern, though, as the only nitrogen it should be able to acquire is that in the soil. One explanation could be that the amount of fertilizer applied at planting and the additional nitrogen applied at flowering are leached from the soil or utilized early leaving the plant deficient in nitrogen later. The nitrogen in the organic system, however, may be more sustainable, since it is derived from the decomposition of organic matter from previous crops and has a longer release period than the nitrogen applied in more soluble fertilizer. Moreover, the nitrogen may still be at an acceptable level in the soil at pod fill to produce a yield greater than is expected from a non nodulating dry bean.

Genotypes falling in the upper left quadrant may be responsive to the application of nitrogen but not able to fix nitrogen efficiently. The non nodulating R99 would be expected to be present in this quadrant. These genotypes would be best suited to soils rich in nutrients and where application of nitrogen in the form of fertilizer is possible. In the group of 32 genotypes studied, however, only one genotype, the black seeded breeding line B05039 was placed in the upper left quadrant.

The lower left quadrant represents genotypes that do not respond to application of nitrogen by increasing yield, nor are these genotypes the most efficient nitrogen fixers of this group of genotypes evaluated. The genotypes in the lower left quadrant may not be as efficient at partitioning nitrogen to the seed as those in the upper right quadrant. The genotypes in the lower left quadrant are dominated by the low N-fixers in the kidney-cranberry and navy seed classes implying lower overall yield.

There is a preconception that cultivars developed prior to the advent of the extensive use of synthetic pesticides and fertilizers are better adapted to organic production. Organic production is similar to conventional production prior to the advent of modern technologies as both rely on composts, manure, and legumes and other cover crops to improve soil fertility. Also, prior to application of pesticides, cultivars resisting disease would be selected over those that were susceptible. However, these older cultivars have not undergone the rigorous selection in breeding programs that modern genotypes have for traits such as disease resistance, plant architecture and yield. Carr, et al. (2006)

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investigated this supposition in wheat genotypes by comparing yields in organic production to conventional production. They found that modern genotypes were much better adapted to modern organic production than the older genotypes pre-dating common use of synthetic fertilizers and pesticides. In this study, the navy bean Michelite was included since it was released in 1938 thus was developed prior to the advent of synthetic fertilizers and pesticides. T39, a selection from the landrace Black Turtle Soup, was pure-line selected in the 1970s. Michelite and T39 did not produce high yields and were surpassed in organic production yield by many modern genotypes which had been selected for yield under conventional systems. Those genotypes with the highest yield in both conventional and organic production systems were generally those genotypes more recently developed through modern breeding under conventional production systems. These genotypes seem to be better suited to organic production than those genotypes developed prior to the use of chemical pesticides and fertilizers.

Variability exists in the 32 dry bean genotypes studied in yield potential in both organic and conventional systems. In addition to yield, there are differences in the nitrogen content of the seeds between organic and conventional production systems and also between genotypes. Genotypes showing the greatest yield potential in organic systems belong to the Middle American gene pool represented by Zorro, a consistently high yielding genotype in multiple environments and different production systems. Small reds and pinks such as Merlot and Sedona, respectively, yield competitively and appear to tolerate high pH conditions where micronutrients may be limiting as well as fixing sufficient nitrogen when grown under nitrogen limited conditions in organic systems.

Table 2-1. Seed class, seed size, gene pool, race, and growth habit for 32 dry bean (*Phaseolus vulgaris* L.) genotypes evaluated in organic and conventional production systems at Kellogg Biological Station and Gratiot County in 2007, 2008, and 2009.

Genotype	Seed class	Seed Size[1]	Gene Pool[2]	Race[3]	Growth Habit[4]
B05039	Black	Small	M.A.	M	II
Vista	Navy	Small	M.A.	M	II
B04431	Black	Small	M.A.	M	II
T39	Black	Small	M.A.	M	II
Condor	Black	Small	M.A.	M	II
R99	Navy/No Nod[5]	Small	M.A.	M	III
N05324	Navy	Small	M.A.	M	II
Michelite	Navy	Small	M.A.	M	III
B05055	Black	Small	M.A.	M	II
Bunsi	Navy	Small	M.A.	M	III
115-11M	Black	Small	M.A.	M	II
N05311	Navy	Small	M.A.	M	II
Jaguar	Black	Small	M.A.	M	II
Seahawk	Navy	Small	M.A.	M	II
Zorro	Black	Small	M.A.	M	II
TARS SR05	Small Red	Small	M.A.	Jalisco	II
Sedona	Pink	Medium	M.A.	Jalisco	II
Chinook Select	Kidney	Large	Andean	N.G.	I
Red Hawk	Kidney	Large	Andean	N.G.	I
K05604	Kidney	Large	Andean	N.G.	I
Matterhorn	Great Northern	Medium	M.A.	Durango	II
USDK-CBB-15	Kidney	Large	Andean	N.G.	I
Buster	Pinto	Medium	M.A.	Durango	II
P06131	Pinto	Medium	M.A.	Durango	II
Santa Fe	Pinto	Medium	M.A.	Durango	II
CELRK	Kidney	Large	Andean	N.G.	I
Capri	Cranberry	Large	Andean	Chile	I
Montcalm	Kidney	Large	Andean	N.G.	I
K03240	Kidney	Large	Andean	N.G.	I
Merlot	Small Red	Medium	M.A.	Jalisco	II
Beluga	Kidney	Large	Andean	N.G.	I
Othello	Pinto	Medium	M.A.	Durango	III

[1]Small=18-29 g 100 seeds^{-1}; medium=30-45 g 100 seeds^{-1}; large=46-60 g 100 seeds^{-1}
[2]Gene pool according to Gepts (1988) M.A.=Middle American; [3]Race according to Singh et al. (1991) N.G.=Race Nueva Granada; M=Race Mesoamerica; [4]Growth habit according to Singh, (1982). [5]No Nod=non nodulating.

Table 2-2. Soil analysis and soil type of Kellogg Biological Station, Gratiot County, and Tuscola County research sites for 2007-2009.

	Kellogg Biological Station						Gratiot Co.				Tuscola Co.
	Organic			Conventional			Organic		Conventional		Organic
Year	2007	2008	2009	2007	2008	2009	2007	2008	2007	2008	2009
Organic matter%		1.9	1.9		1.8	1.8		1.4		2.1	4.5
Phosphorus(ppm)	42	54	57	62	45	70	100	32	124	56	38
Potassium (ppm)	89	70	131	76	54	112	288	116	333	151	97
Magnesium(ppm)	284	197	195	180	183	136	259	191	238	211	376
Soil Type[1]	---Kalamazoo Loam---			------Kalamazoo Loam-----			MCSL[2]	SLS[3]	MCSL[2]	SLS[3]	TL[4]

[1] Soil classifications from Web Soil Survey at http://www.nrcs.usda.gov/, accessed December 2009.

[2]Metamora-Capac Sandy Loam

[3]Selfridge Loamy Sand

[4]Tappan Loam

Table 2-3. Precipitation (mm) from June to September at study sites where 32 dry bean (*Phaseolus vulgaris* L.) genotypes were evaluated for production in organic systems in 2007, 2008, and 2009.

Year and Month	Kellogg Biological Station[1]	Gratiot County	Tuscola County
2007	------------mm ---------		
June	37	41	
July	24	67	
August	88	99	
September	68	49	
Total	217	256	
2008			
June	26	90	
July	131	40	
August	16	36	
September	333	82	
Total	506	248	
2009			
June	96		110
July	5		69
August	174		64
September	32		32
Total	307		275

[1]Precipitation data for KBS in 2007 was from records at Ceresco, MI, the closest monitoring site with functioning equipment that year. Data from 2008 and 2009 were collected at KBS.

Table 2-4. Mean yield of 32 dry bean (*Phaseolus vulgaris* L.) genotypes grown in organic and conventional systems at Kellogg Biological Station 2007-2009.[1]

Organic		Conventional	
Genotype	Yield kg ha^{-1}	Genotype	Yield kg ha^{-1}
Zorro	2598 a	N05324	3385 a
TARS-SR05	2588 a	Buster	3239 ab
B04431	2553 a	Zorro	3203 ab
Buster	2463 ab	B04431	3020 abc
N05324	2439 ab	Condor	2953 abc
N05311	2435 ab	Merlot	2844 abcd
Seahawk	2380 abc	Sedona	2831 abcd
Jaguar	2333 abc	115-11M	2756 abcde
Merlot	2310 abc	B05039	2752 abcde
115-11M	2284 abcd	Jaguar	2676 abcde
Condor	2221 abcd	N05311	2660 abcde
R99	2213 abcd	Seahawk	2564 abcde
Santa Fe	2191 abcd	Bunsi	2493 abcde
B05055	2189 abcd	Santa Fe	2479 abcde
P06131	2173 abcd	B05055	2424 abcde
Sedona	2162 abcd	Matterhorn	2407 abcde
Bunsi	2068 abcd	Beluga	2406 abcde
Chinook Select	1994 abcd	K03240	2403 abcde
T39	1966 abcd	Chinook Select	2402 abcde
B05039	1957 abcd	TARS-S05	2390 abcde
Vista	1927 abcd	T39	2353 abcde
Othello	1909 abcd	Montcalm	2302 abcde
Matterhorn	1901 abcd	Vista	2269 abcde
Red Hawk	1795 abcd	Othello	2260 abcde
Capri	1747 abcd	P06131	2253 abcde
USDK-CBB-15	1728 abcd	Michelite	2198 bcde
Montcalm	1703 abcd	Red Hawk	2179 bcde
Beluga	1695 abcd	USDK-CBB-15	2140 bcde
K03240	1686 abcd	Capri	2130 bcde
Michelite	1604 bcd	CELRK	1946 cde
CELRK	1455 cd	K05604	1811 de
K05604	1377 d	R99	1641 e
Mean	2064	Mean	2507
LSD	931.2	LSD	1142

[1]Genotypes followed by the same letter are not significantly different, Tukey's HSD p=0.05.

Table 2-5. Mean yield of 9 Andean dry bean (*Phaseolus vulgaris* L.) genotypes and non nodulating R99 check grown in organic and conventional systems at Kellogg Biological Station 2007-2009.

Organic		Conventional	
Genotype	Yield kg ha^{-1}	Genotype	Yield kg ha^{-1}
R99	2213 a	Beluga	2406 a[1]
Chinook Select	1994 ab	K03240	2403 a
Red Hawk	1795 bc	Chinook Select	2402 a
Capri	1747 bcd	Montcalm	2302 a
USDK-CBB-15	1728 bcd	Red Hawk	2179 ab
Montcalm	1703 bcd	USDK-CBB-15	2140 ab
Beluga	1695 bcd	Capri	2130 ab
K03240	1686 bcd	CELRK	1946 b
CELRK	1455 cd	K05604	1811 b
K05604	1377 d	R99	1641 b
Mean	1739	Mean	2136
LSD	398	LSD	537

[1]Genotypes followed by the same letter are not significantly different, Tukey's HSD p=0.05.

Table 2-6. Mean yield of 22 Middle American dry bean (*Phaseolus vulgaris* L.) genotypes grown in organic and conventional systems at Kellogg Biological Station 2007-2009.[1]

Organic		Conventional	
Genotype	Yield kg ha^{-1}	Genotype	Yield kg ha^{-1}
Zorro	2598 a	N05324	3385 a
TARS-SR05	2588 a	Buster	3239 ab
B04431	2553 a	Zorro	3203 ab
Buster	2463 ab	B04431	3020 abc
N05324	2439 abc	Condor	2953 abcd
N05311	2435 abc	Merlot	2844 abcde
Seahawk	2380 abcd	Sedona	2831 abcde
Jaguar	2333 abcd	115-11M	2756 bcde
Merlot	2310 abcd	B05039	2752 bcdef
115-11M	2284 abcd	Jaguar	2676 bcdef
Condor	2221 abcd	N05311	2660 bcdef
R99	2213 abcd	Seahawk	2564 cdef
Santa Fe	2191 abcd	Bunsi	2493 cdef
B05055	2189 abcd	Santa Fe	2479 cdef
P06131	2173 abcd	B05055	2424 cdef
Sedona	2162 abcd	Matterhorn	2407 def
Bunsi	2068 bcde	TARS-S05	2390 def
T39	1966 bcde	T39	2353 def
B05039	1957 bcde	Vista	2269 ef
Vista	1927 cde	Othello	2260 ef
Othello	1909 cde	P06131	2253 ef
Matterhorn	1901 de	Michelite	2198 fg
Michelite	1604 e	R99	1641 g
Mean	2212	Mean	2611
LSD	530	LSD	612

[1]Genotypes followed by the same letter are not significantly different, Tukey's HSD p=0.05.

Table 2-7. Mean yield of 32 dry bean (*Phaseolus vulgaris* L.) by seed class grown in organic and conventional production systems at Kellogg Biological Station, 2007 to 2009

Organic		Conventional	Difference[1]
Seed Class	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}
Pink/Red	2353	2688	-335
Black	2262	2767	-505
Navy	2142	2595	-453
Pinto	2127	2528	-401
Kidney	1687	2191	-504
No Nod	2213	1641	572
Mean	2064	2507	-443
LSD	273.8	442.2	

[1]Difference=organic-conventional

Table 2-8. Mean yield of 32 dry bean (*Phaseolus vulgaris* L.) genotypes Gratiot County in organic and conventional production systems, 2007-2008.[1]

Organic		Conventional	
Genotype	Yield kg ha^{-1}	Genotype	Yield kg ha^{-1}
Merlot	2817 a	Buster	3075 a
Sedona	2537 ab	Zorro	3019 a
T39	2533 ab	N05324	2949 a
N05311	2504 ab	Sedona	2938 a
Zorro	2469 ab	Merlot	2901 ab
P06131	2463 ab	B04431	2830 abc
115-11M	2408 abc	Condor	2721 abcd
Condor	2397 abc	115-11M	2697 abcd
Santa Fe	2354 abc	P06131	2628 abcde
TARS-SR05	2214 abcd	N05311	2545 abcde
Jaguar	2170 abcd	TARS-SR05	2530 abcde
B04431	2137 abcd	B05039	2520 abcde
Buster	2135 abcd	Jaguar	2513 abcdef
B05055	2110 abcd	Santa Fe	2499 abcdefg
Matterhorn	2098 abcd	Seahawk	2444 abcdefg
N05324	2075 abcd	Matterhorn	2444 abcdefg
USDK-CBB-15	2057 abcd	T39	2375 abcdefg
Othello	2002 abcd	B05055	2364 abcdefg
Seahawk	1983 abcd	Othello	2361 abcdefg
B05039	1975 abcd	Vista	2319 abcdefg
Vista	1922 abcd	Chinook Select	2132 bcdefg
Capri	1905 abcd	Bunsi	2112 cdefg
Sanilac	1611 abcd	Capri	2087 cdefg
Bunsi	1608 abcd	Michelite	2051 cdefg
Red Hawk	1603 abcd	Montcalm	2027 defg
Montcalm	1504 abcd	R99	1997 defg
Chinook Select	1482 abcd	K03240	1962 defg
K03240	1441 bcd	USDK-CBB-15	1956 defg
Beluga	1286 bcd	Beluga	1935 defg
K05604	1241 bcd	Red Hawk	1871 efg
CELRK	1105 cd	K05604	1699 fg
R99	896 d	CELRK	1687 g
Mean	1910	Mean	2234
LSD	1332	LSD	826

[1]Genotypes followed by the same letter are not significantly different, Tukey's HSD p=0.05.

Table 2-9. Yield of 32 dry bean (*Phaseolus vulgaris* L.) genotypes grown in Tuscola County in an organic production system in 2009.

Genotype	Yield ($kg\ ha^{-1}$)
Zorro	3846 a[1]
TARS-SR05	3583 ab
Merlot	3402 abc
P06131	3373 abc
Vista	3348 abc
N05311	3309 abc
B05055	3256 abc
Seahawk	3157 abcd
115-11M	3118 abcde
Condor	3083 abcde
B05039	2937 abcde
Jaguar	2892 abcde
Santa Fe	2868 abcde
T39	2817 abcde
Sedona	2791 abcde
Matterhorn	2743 abcde
N05324	2728 abcde
Montcalm	2603 abcde
B04431	2568 abcde
Sanilac	2565 abcde
Bunsi	2532 abcde
Capri	2516 abcde
R99	2467 abcde
Buster	2384 abcde
Othello	2327 abcde
Red Hawk	1990 bcde
USDK-CBB-15	1947 bcde
K03240	1937 bcde
Chinook Select	1810 cde
K05640	1796 cde
Beluga	1523 de
CELRK	1398 e
Mean	2675
LSD	1724

[1]Genotypes followed by the same letter are not significantly different, Tukey's HSD p=0.05.

Table 2-10. Mean yield by 32 dry bean (*Phaseolus vulgaris* L.) genotypes grown in organic and conventional production systems at Kellogg Biological Station from 2007 to 2009.

	Organic	Conventional	Difference[1]
Seed Class	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}
Pink/Red	2324	2726	-402
Black	2263	2767	-504
Navy	2142	2595	-453
Pinto	2127	2528	-401
Kidney	1687	2191	-504
No Nod	2213	2170	43
Mean	2126	2505	-379
LSD	273.8	442.2	

[1]Difference=organic-conventional

Table 2-11. Mean nitrogen accumulated by 32 dry bean (*Phaseolus vulgaris* L.) genotypes at Kellogg Biological Station in organic and conventional production systems from 2007 to 2009.

	Organic	Conventional	Difference[1]
Year	kg N ha^{-1}	kg N ha^{-1}	kg N ha^{-1}
2007	63.0	73.2	-10.2
2008	52.1	93.2	-41.1
2009	98.4	139.3	-40.9
Mean	71.2	101.9	-30.7
LSD	9.2	9.6	

[1]difference=organic-conventional

Table 2-12. Mean Nitrogen Yield of 32 dry bean (*Phaseolus vulgaris* L.) genotypes grown in organic and conventional production systems at Kellogg Biological Station, years 2007-2009.

Organic Genotype	N-Yield kg ha^{-1}	Organic Genotype	N-Yield kg ha^{-1}
TARS-SR05	111.9a	Zorro	133.0 a
Zorro	96.7 ab	TARS-SR05	127.7 ab
N05311	89.4 ab	R99	119.6 abc
B05055	88.2 ab	Jaguar	118.4 abc
Jaguar	87.1 ab	Condor	118.2 abc
B05039	84.3 ab	Matterhorn	115.8 abc
B04431	80.5 ab	P06131	115.5 abc
Santa Fe	78.6 ab	N05311	114.5 abc
Seahawk	77.1 ab	Santa Fe	114.3 abc
Merlot	75.9 ab	Buster	112.7 abc
Sedona	75.8 ab	B04431	110.6 abc
Condor	74.5 ab	Seahawk	110.6 abc
Montcalm	73.9 ab	B05039	108.2 abc
Chinook Select	73.4 ab	B05055	106.6 abc
P06131	72.7 ab	N05324	104.6 abc
115-11M	71.9 ab	Merlot	104.0 abc
N05324	70.7 ab	Sedona	103.8 abc
Buster	70.5 ab	Chinook Select	103.4 abc
Beluga	69.8 ab	T39	101.5 abc
T29	69.1 ab	Beluga	99.4 abc
Vista	68.4 ab	USDK-CBB-15	95.8 abc
Capri	66.8 ab	Othello	93.4 abc
Othello	66.7 ab	Montcalm	93.1 abc
Red Hawk	65.0 ab	Capri	91.9 abc
K03240	63.9 ab	K03240	89.6 abc
Matterhorn	62.6 b	115-11M	89.3 abc
USDK-CBB-15	61.4 b	Bunsi	89.0 abc
Bunsi	57.9 b	Red Hawk	85.5 abc
Michelite	52.8 b	Michelite	83.5 abc
CELRK	52.7 b	Vista	82.3 abc
R99	52.6 b	CELRK	78.0 bc
K05604	52.4 b	K05604	68.0 c
Mean	71.7	Mean	101.9
LSD	49.1	LSD	53.9

[1]Genotypes followed by the same letter are not significantly different, Tukey's HSD p=0.05.

Table 2-13. Yield by seed class of 32 dry bean (*Phaseolus vulgaris* L.) genotypes grown in Tuscola County in an organic production system in 2009.

Seed Class	Yield ($kg\ ha^{-1}$)
Red	3142a[1]
Black	3065a
Navy	2940a
Pinto	2741ab
No Nod	2467ab
Kidney	2196b
Average	2759
LSD	1063

[1]Seed classes followed by the same letter are not significantly different, Tukey's HSD p=0.05.

Table 2-14. Pearson correlations for maturity, plant stand, days to flower, height at flowering, 100 seed weight, yield and nitrogen yield for 32 dry bean (*Phaseolus vulgaris* L.) genotypes grown in organic and conventional Trials[1].

Table 2-14a. All treatments.

	Maturity	Plant Stand	Days to Flower	Height at Flowering	100 seed Weight	Yield
Maturity	1.00					
Plant stand		1.00				
Days to Flower	0.55***	0.27***	1.00			
Height at Flowering	-0.12	-0.08	-0.22***	1.00		
100 Seed Weight	-0.7***	-0.42***	-0.60***	0.23***	1.00	
Yield	-0.01	-0.27***	0.16***	0.16***	-0.08*	1.00
N yield	0.11	-0.23**	0.11*	0.29***	-0.02	0.89***

[1]* sig at 0.05, ** at 0.01, *** at 0.001 or less

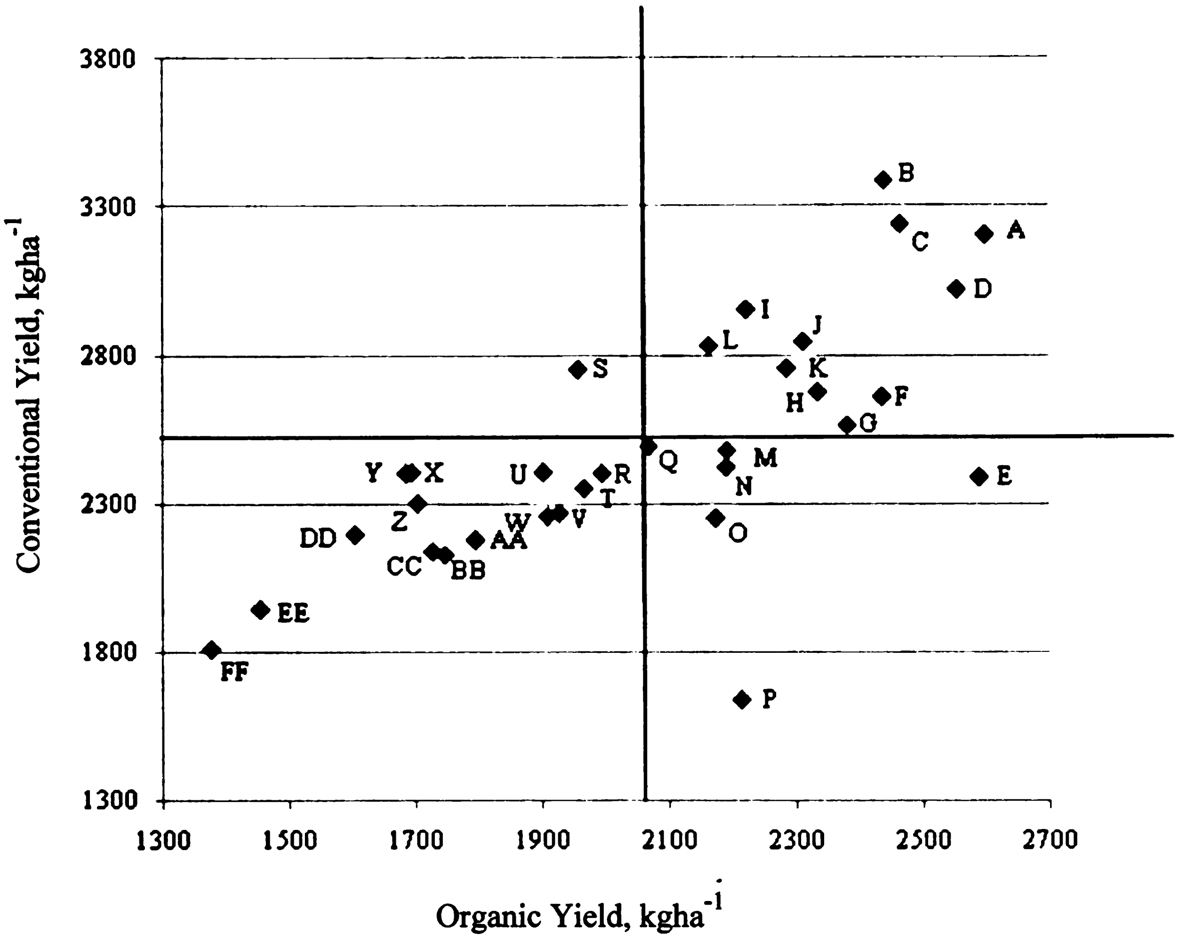
Table 2-14b. Organic treatment.

Organic	Maturity	Plant Stand	Days to Flower	Height at Flowering	100 Seed Weight	Yield
Maturity	1.00					
Plant stand	0.25*	1.00				
Days to Flower	0.66***	0.2**	1.00			
Height at Flowering	0.18*	-0.28***	-0.01	1.00		
100 Seed Weight	-0.33***	-0.47***	-0.57***	-0.07	1.00	
Yield	0.47***	-0.22***	0.37***	0.49***	-0.06	1.00
N yield	0.43*	-0.16*	0.36***	0.44***	0.01	0.75***

Table 2-14c. Conventional treatment

Conventional	Maturity	Plant Stand	Days to Flower	Height at flowering	100 Seed Weight	Yield
Maturity	1.00					
Plant stand	0.27*	1.00				
Days to Flower	0.76***	0.16*	1.00			
Height at Flowering	0.29***	-0.7***	-0.02	1.00		
100 Seed Weight	-0.48***	-0.6***	-0.53***	0.26***	1.00	
Yield	0.34***	-0.44***	0.32***	0.4***	0.07	1.00
N yield	0.44***	-0.49***	0.25***	0.51***	0.05	0.74***

Figure 2-1. Scatter plot of yield of 32 dry bean (*Phaseolus vulgaris* L.) genotypes grown in organic and conventional treatments at Kellogg Biological Station. Conventional yield is on the Y axis and Organic yield on the X axis.[1]



[1]The horizontal line represents the average yield for the conventional treatment and the vertical line represents the average yield for the organic treatment from 2007 to 2009.

A	Zorro	I	Condor	Q	Bunsi	Y	K03240
B	N05324	J	Merlot	R	Chinook	Z	Montcalm
C	Buster	K	115-11M	S	B05039	A	Red Hawk
D	B04431	L	Sedona	T	T39	B	Capri
E	TARS	M	Santa Fe	U	Matterhorn	C	I05101
F	N05311	N	B05055	V	Othello	D	Michelite
G	Seahawk	O	P06131	W	Vista	E	CELRK
H	Jaguar	P	R99	X	Beluga	F	K05604

CHAPTER 3

EVALUATION OF DRY BEAN GENOTYPES FOR EARLY ESTABLISHMENT OF BIOLOGICAL NITROGEN-FIXATION

ABSTRACT

Bean genotypes with higher potential for nitrogen fixation need to be identified to support vigorous growth, competitive yields, and reduced reliance on external nitrogen fertilizer. Thirty-three diverse dry bean genotypes representing seven commercial market classes were evaluated for N fixation at the early flowering growth stage in a greenhouse pot study. One high nitrogen fixing check and one non-nodulating, non fixing check were included for comparison, and to determine N fixed by the difference method. Plants were grown in an inert medium with nutrients supplied as a modified nutrient solution lacking nitrogen. Seed was inoculated with *Rhizobium etli* strain UMR1597 at planting and again 10 days later. Plants were harvested at first bloom, rated for nodule growth, measured for plant height, root length, and biomass and root mass. Nitrogen levels were determined in plant biomass. Dry beans of Middle American origin with indeterminate growth habit appeared to acquire greater nitrogen than those genotypes of Andean origin with determinate growth habit. Little variation was seen among elite breeding lines and commercial genotypes. The high nitrogen fixer, Puebla 152 accumulated nearly 60% more nitrogen than genotypes that fixed the next highest amount of nitrogen, the pinto bean 'Santa Fe' and the black seeded 'Zorro'. Selection criteria based on biomass, total nitrogen accumulated in the biomass, and root mass of nodulating dry bean genotypes

grown in low nitrogen conditions are suitable measurements to identify bean genotypes with superior biological nitrogen fixation.

INTRODUCTION

Finding a method to efficiently evaluate dry bean genotypes for their ability to fix nitrogen has led researchers to utilize several methods such as acetylene reduction analysis (ARA; St. Clair and Bliss, 1991), total biomass or total seed nitrogen (Wolyn et al., 1989), ^{15}N methods (Kipe-Nolt and Giller, 1993), nitrogen difference method (Rennie, 1983) as well as nodule and root measurements (Wolyn et al., 1989). Each method offers different advantages and costs but breeders need a methodology to screen a large number of genotypes in the most efficient manner with the minimum of cost.

St. Clair and Bliss (1991) used the ARA method to identify high and low fixing lines in a population derived from cross of the high N-fixing black bean 'Puebla 152' and the low N-fixing navy cultivar 'Sanilac'. St. Clair and Bliss (1991) asserted that significant differences in BNF between genotypes could be detected in a single growing season and that the ranking of those genotypes would likely remain constant though levels of nitrogen fixed could change with changing environmental parameters. St. Clair and Bliss (1991) found that ARA was highly correlated with simpler, indirect measures of nitrogen fixation. If genotypes are grown in nitrogen limited conditions shoot biomass was reported to be strongly tied to the amount of nitrogen fixed. In the absence of ARA, however, St. Clair and Bliss (1991) would not have discovered that some dry bean genotypes were superior nitrogen fixers based on the activity of their nodules. Since nitrogen in the biomass is not completely translocated to seed yield, nitrogen partitioning is as important a characteristic as is nodule activity. The cost associated with ARA as well as calibrating issues and the fact that ARA only provides a measurement for a specific time point during the season are major limitations to its use for assessing N-fixation. Another disadvantage to the ARA system of measuring nitrogen fixation is that ARA may falsely identify high nitrogen fixers i.e. those individuals with high nodule activity which did not translate N into increased yield (St. Clair and Bliss, 1991).

^{15}N methods have been widely used to measure nitrogen fixation. This method relies on the fact that the natural abundance of ^{15}N in the atmosphere is about 0.37% of nitrogen in the atmosphere, with the rest of the nitrogen in the atmosphere being ^{14}N. Kipe-Nolt and Giller (1993) applied an isotope labeled fertilizer containing 29.3% ^{15}N to one plot prior to planting and the equivalent amount of unlabelled ammonium sulfate to another plot. Seed was planted after allowing the fertilizer to distribute equally in the soil for one week. Plants were harvested at 56 days in both treatments with an effort made to recover the entire root system and nodules, along with the biomass including the stems, leaves and pods. Plants in the unlabelled plot were sampled beginning at 13 days and weekly there after to 56 days in the same manner as the isotope labeled plot with enough plants left to grow to maturity and sampled for yield (Kipe-Nolt and Giller, 1993). At pod fill, the pods contained over half of the total shoot nitrogen. In the genotypes studied the roots accounted for a minor amount of the nitrogen in the plant. Analysis of the total nitrogen content in the seed, or biomass, at sampling time will give a ratio of ^{15}N to ^{14}N

in the tissue. This information can then be utilized to determine which percent of the nitrogen was acquired from the fertilizer, the rest from nitrogen fixation. Kipe-Nolt and Giller (1993) discovered that less than 11% of the fertilizer N applied was recovered by the plant noting that the nitrogen sources were soluble and easily leached from the soil before plants were able to access them. A reference crop was utilized as a check to measure the availability of nitrogen from the soil. Sorghum was chosen as the non fixing reference crop in the study of Kipe-Nolt and Giller (1993) though the plant growth rate was slower and resulted in different nitrogen uptake rates from that of the bean genotypes studied.

Kipe-Nolt and Giller (1993) observed results similar to St. Clair and Bliss (1991) in the variability in partitioning of nitrogen in dry bean genotypes. Some high fixing genotypes as determined by total biomass nitrogen did not translocate the nitrogen into the pods or seed as compared to other high fixing lines which more effectively mobilized nitrogen into the seed resulting in improved yield. Kipe-Nolt and Giller (1993) also discovered that some of the higher fixing genotypes also began nodulation earlier than those genotypes with lower nitrogen fixing ability. They attributed the increase in nitrogen fixation of certain lines to the earlier nodulation instead of increased or extended fixation at or beyond pod fill due to the senescence of nodules at the 56 day sampling date when all genotypes were in the pod filling stage. Kipe-Nolt and Giller (1993) associated increased biomass accumulation with improved BNF as well as enhanced development of nodules. However, some lines that were better able to acquire nitrogen from the soil may be vigorous due to nitrogen from the soil instead of nodules thus evaluation of nodules and biomass together would be useful in evaluating bean genotypes.

One limitation of ^{15}N methods suggested by Kipe-Nolt and Giller (1993) is the mobility of nitrogen in the soil. Irrigation and precipitation gradually remove the fertilizer from the soil profile reducing its availability to the plants. Costs of purchasing fertilizer enriched with ^{15}N as well as difficulties incorporating the fertilizer into the soil in a consistent manner are limitations of this method. Other indirect measures, such as nodulation and biomass were correlated with results from ^{15}N method (Kipe-Nolt and Giller, 1993). In reviewing methods used to measure nitrogen fixation, Wolyn et al. (1989) point out that application of nitrogen in any form may decrease nodule formation and subsequent activity since nitrogen availability in the soil may inhibit nitrogen fixation.

Instead of applying a fertilizer enriched in ^{15}N, a fertilizer depleted in ^{15}N could be used, or an alternative is not to rely on fertilizer (Danso et al., 1993). Usually, soil has a slightly higher level of ^{15}N isotope than that found in the atmosphere. The ^{15}N:^{14}N ratio, natural abundance, also known as R can be used to determine how much nitrogen is fixed. Fixation of nitrogen from the atmosphere results in dilution of the ^{15}N in the plant, as nitrogen derived from the atmosphere has a lower ^{15}N:^{14}N ratio than the soil. If the plant were deriving all nitrogen from the soil, the ^{15}N in the plant would be greater than a plant deriving any nitrogen from the atmosphere. This natural abundance method relies on use of a reference crop as does other ^{15}N methods to calibrate nitrogen measurements. Since the natural abundance method deals with such small differences in ^{15}N levels there could be considerable error in measurement, along with the need to have access to equipment sensitive enough to measure these differences (Danso et al., 1993). Working with other nitrogen fixing legumes such as *Trifolium repens* and *Lotus* spp., Steele et al. (1983) discovered that nodules were enhanced in ^{15}N. There was also evidence that different strains of *Rhizobium* had different preferences for ^{15}N, thus the ^{15}N:^{14}N ratio could vary from nodule to nodule based on which *Rhizobium* strain inhabiting the nodule. If there was an expectation that a plant was fixing nitrogen, and the ratio of ^{15}N:^{14}N was higher than expected, researchers may inadvertently underestimate the level of nitrogen fixation occurring.

Danso et al. (1993) discuss the choice of a reference crop and cite one of the major problems with reference crops is the differences in growth habit between the crop being studied and the reference crop. Grasses have commonly been utilized as reference crops (Hardarson and Danso, 1993; Danso et al., 1993). Different plants have different root system architecture and may access different layers of the soil profile resulting in inconsistent acquisition of nitrogen between the reference crop and the nitrogen fixing crop. Park and Buttery (2006) developed two dry bean genotypes, R99 and R69, lacking the ability to fix nitrogen since they do not form nodules, or form poorly developed nodules incapable of nitrogen fixation. The availability of a reference crop in the same species as the crop being studied helps to relieve some problems with differences in growth characteristics. However, genetic differences in growth habit and yield even between genotypes of the same species may contribute to inaccurate estimations of nitrogen fixation.

With each of the methods to measure nitrogen fixation discussed above come challenges to interpreting the data. Traits that have been highly correlated to ARA and ^{15}N methods such as shoot biomass, root mass, nodule rating, and seed yield, would be suitable for the evaluation of the BNF ability of a dry bean genotype. These indirect methods offer efficiency in quickly determining which genotypes are superior nitrogen fixers. These screening methods could easily be integrated into a breeding program to develop superior nitrogen fixing commercial genotypes without considerable increases in cost or time.

Nitrogen fixation research is best conducted in soils with extremely low soil nitrogen levels (Bliss, 1993b). Wolyn et al. (1989) studied progeny from the population developed by Bliss et al. (1989) derived from Puebla 152 and Sanilac. Test plots were considered low in soil nitrogen and fertilizer lacking nitrogen was applied at planting. Several progeny had total biomass nitrogen levels approaching that of Puebla 152, the high fixing parent, and others were more similar to the low fixing parent Sanilac based on total nitrogen, leghemeglobin levels and nodule mass ratings. Wolyn et al. (1989) found a significant correlation (r^2=0.71 $p \leq 0.001$) between nodule mass from the entire root system and seed yield. Nodule mass was determined to be the best indirect measure of BNF.

While developing their non-fixing bean genotypes using the chemical mutagen EMS, Park and Buttery, (1994, 2006), utilized a greenhouse screening method where the purported mutants were grown in a mix of perlite and vermiculite to facilitate the observation of the plants and subsequent replanting of those individuals that exhibited the appropriate non-nodulating phenotype. One-part vermiculite to two-parts perlite mix was used along with the application of a nitrogen free solution. Similarly Souza et al. (2000) and Tsai et al. (1993) utilized an inert growing media for greenhouse screening and provided nutrient solutions with different levels of nitrogen. Tsai et al. (1993) were able to efficiently identify the level of nodulation of the genotypes studied, between Puebla 152 and CIAT-125, a non-nodulating mutant, which had no nodule development in either fertilized or nitrogen free systems with the addition of *Rhizobium* inoculant. Utilizing this method they were able to identify four QTLs contributing to an increase in nodule number and four QTLs contributing to a decrease in nodule number.

Use of an inert material as a growing medium is a suitable choice to support the growth of plants while providing a controlled level of plant nutrients to the system. Growing plants in a greenhouse also offers the benefit of providing a consistent environment for all plants being studied and removes the variation in soil encountered in a research field where pathogens and different root conditions exist. Plants grown in the soil are also difficult to extract with the entire root system intact, often leaving behind finer roots and those roots distant from the main stem. In addition to the ability to recover an entire root system and have greater control on nutrient levels the plants are receiving, another advantage is being able to use a greenhouse to screen at times of the year when field work is at a minimum.

The objective of the current study was to establish an efficient screening system to determine BNF potential of numerous dry bean breeding lines. Requirements for the protocol include that it be simple and efficient as well as economical in terms of cost and time involved in obtaining reliable and reproducible results.

MATERIALS AND METHODS

Advanced breeding lines as well as commercial cultivars were included in the 34 diverse genotypes selected for evaluation of BNF potential. Genotypes selected included representatives of commercially important market classes such as navy, black, cranberry, kidney, pinto and small red and pink seed types. A non nodulating check, R99, was included along with a known high-N fixing check, Puebla 152. R99 is derived from the navy bean Bunsi which was also included. Puebla 152 is a black seeded genotype noted for its improved BNF abilities. (Table 3-2. List of genotypes)

Plastic nursery trade containers were filled with an autoclaved 3:2, perlite: vermiculite volume for volume, mix. Seeds were surface sterilized by soaking in 70% Ethanol for 2 minutes, then soaked in 5% bleach solution for an additional 2 minutes. Seeds were rinsed four times in water for 2 m each rinse. While moist from the rinse, seeds were swirled in *Rhizobium etli* strain UMR 1597 (supplied by P.H. Graham, University of Minnesota) prepared in powdered peat to completely coat the seed. Three seeds were planted into each container and watered thoroughly with tap water. A total of five pots were planted of each genotype. Plants were grown in a greenhouse with supplemental HPS lighting to day length of 12 h. Seedlings were thinned to one seedling per pot when seedlings were large enough to determine that they were healthy but not so late that the removal of a seedling would damage the remaining seedling. Thinning occurred prior to ten days after sowing with seedlings removed by carefully pulling plant from the growing media. At 10 DAP an additional amount, approximately 0.5g of inoculant was spread over the surface of the media and lightly watered in with tap water. Plants were watered as needed with tap water adjusted to a pH of 6.5 throughout the experiment and plants were given 200 ml modified Hoagland's solution twice weekly without nitrogen (Table 3-1). The final nutrient solution was also adjusted for a pH of 6.5. The experiment was repeated three times.

Measurement for plant height and nodule rating were conducted at opening of the first flower plants (See appendix 1). Photographs of the plants were taken with an Olympus camera mounted on a tripod at a distance of approximately 60 cm. Shoots were harvested at the media surface and dried, then weight determined. Roots were removed from the growing media and rinsed with tap water. After gently laying roots out, maximum root length was recorded with a meter stick. The roots were then photographed at an approximate distance of 40 cm such that nodules would be clearly visible in the photograph. After drying, roots and shoots were weighed. Shoot tissue was ground with a Christy-Turner Lab Mill (Ipswitch, Suffolk, UK) to pass through a 1 mm screen. Total N analysis was performed on the shoot biomass using the Kjeldahl Method (A&L Great Lakes Laboratories, Inc., Ft. Wayne, IN, USA).

Root nodule development was visually rated based on nodule number, size and distribution. A scale of 0 to 6 was utilized where the roots of the non-nodulating check R99 was used at the "0" reference. Roots of Puebla 152, the high nitrogen fixing genotype, were used as the reference for a score of "6." Nodule number and size was taken into account as well as placement of those nodules within the root system. Roots with sparse, small, pale nodules were rated lower than roots with large well colored nodules with a higher density on the root system.

The non nodulating check was harvested when its parent line, Bunsi, was harvested since there was risk of the plants not surviving to first bloom due to the lack of nitrogen in the media. In the field, Bunsi exhibited the same agronomic characteristics as R99.

Based on the method of Rennie, (1983), the percent of nitrogen derived from the atmosphere (%Ndfa) was calculated as follows:

%Ndfa=(Nyield(fs)-Nyield(nfs)/Nyield(fs) x 100.

Where Nyield(fs)=the total amount of nitrogen in the biomass of the fixing dry bean genotypes.

Nyield(nfs)=the amount of nitrogen in the biomass of the non-fixing dry bean genotype, R99 (Park and Buttery, 2006)

Relative Nitrogen Growth Rate (R(N)) was calculated according to Park and Buttery, 1989:

R(N)=(logN2-logN1)/t

N2=nitrogen content at harvest time, N1=nitrogen content of seed, t=days from seeding to harvest.

A concurrent study was conducted comparing performance of dry bean genotypes in side by side plots of conventional production and organic production systems. One site for this study was at Kellogg Biological Station where yield trials were conducted in 2007, 2008 and 2009. Seed nitrogen was measured at this site to determine nitrogen fixed by the genotypes in a field setting. Seed nitrogen utilized in the above equation was the average value of three reps obtained from the organic yield trial at Kellogg Biological Station in 2008 since that was the seed source for the current experiment.

Data were analyzed using SAS 9.2 utilizing PROC MIXED.

RESULTS

Plant biomass differed significantly by bean genotype. Puebla 152, had the greatest amount of biomass at an average of 4.36 g plant^{-1}. At the low end of biomass accumulated was the non fixing genotype, R99, at 0.32 g plant^{-1}. All of the market classes accumulated less biomass than the high fixer, and more biomass than the non fixer. Pink, pinto, red, great northern, and black market classes had a significant increase in biomass compared to navy and cranberry market classes. Kidney bean biomass did not differ from navy or cranberry market classes (Table 3-7). Only three kidney bean genotypes yielded greater than the mean of biomass accumulated, 'Montcalm', K05640 and K03204. All other genotypes yielding greater than the mean were of Middle

American origin. Root mass of all Andean genotypes, except the kidney K05604, were lower than the average root mass of all genotypes. Puebla 152 and the black bean 'Zorro', both Middle American genotypes had the highest root mass.

Root mass was positively correlated with nitrogen fixed per plant (r^2=0.83 $p \leq 0.001$). Nodule rating was positively correlated with nitrogen fixed per plant and root mass, although the relationship was moderately strong (r^2=0.52, $p \leq 0.001$ and r^2=0.44 $p \leq 0.001$).

Values for nitrogen fixed per plant ranged from 109mg for Puebla 152 to 6.3mg for R99. Other genotypes that were not significantly different from the non fixing check include 115-11M, 'Michelite', 'Bunsi', 'Seahawk', and 'Sanilac'. Of these five low fixing genotypes, four belong to the navy market class; only 115-11M is a black bean breeding line (Table 3-2)

Puebla 152 had a higher amount of nitrogen fixed per plant than any other genotype studied. While not significantly different than the majority of the genotypes studied, the recently released black bean Zorro and the recently released pinto bean Santa Fe were at the top of the list for nitrogen fixed $plant^{-1}$. The majority of the genotypes studied were not significantly different from one another in nitrogen fixed (Table 3-2). The trend observed with biomass was the same for nitrogen fixed per plant. Those genotypes in the cranberry, kidney, and navy market classes had the lowest amount of nitrogen accumulated in biomass. Red Hawk and the elite breeding line K05604, both kidney beans, were the only genotypes of Andean origin to have nitrogen yield better than the average.

Using the Nitrogen Balance method, or Nitrogen Difference, there is little variability in the genotypes studied for the percent of nitrogen derived from the atmosphere. The range was 88.7% for the pinto bean 'Santa Fe', to 59.4% for the navy bean 'Vista'. Nitrogen balance or the Nitrogen difference analysis yielded little difference between market classes. (Table 3-12)

Root mass accumulated was similar to biomass with the high fixer, Puebla 152, having a greater average root mass than all other genotypes and the non nodulating R99 having significantly less root mass than any other genotype. Again, black bean Zorro was near the top in accumulation of root mass though was not significantly different from many of the other genotypes studied. (See Tables 3-5 and 3-6).

For nitrogen fixed per plant, a similar pattern emerges as in biomass and root mass. Puebla 152 has the greatest amount of nitrogen fixed at over 109 mg N $plant^{-1}$. The non nodulating check, R99, is at the bottom of the list with a total of 6.32 mg N $plant^{-1}$. Pink, small red, pinto, and great northern seed classes fixed a significantly greater amount of nitrogen than navy and cranberry market classes.

DISCUSSION

Despite considerable work in the past to understand and breed for improved nitrogen fixation in dry bean, little progress has been made in using the technologies to actually identify a genotype with superior BNF ability in the field. Only five germplasm lines have been released with improved BNF ability. Work of Bliss et al. (1989) resulted in the registration five genotypes considered high N fixing after testing in both Wisconsin and Brazil. These germplasm lines were derived from an inbred backcross population using Puebla 152 as the donor parent and the type II upright black bean ICA Pijao as the recurrent parent. Bliss et al. (1989) utilized indirect methods in the selection process including total shoot nitrogen, visual nodule score, seed yield and nodule mass of plants grown where nitrogen was limiting along with more direct measures such as acetylene reduction activity and ^{15}N isotope dilution. Total shoot nitrogen was found to be highly correlated with other measures of nitrogen fixed and with seed yield.

Integrating screening methods for improved BNF into a modern breeding program would be advantageous in selecting genotypes with improved agronomic characteristics and also superior BNF. Combining agronomic characteristics with improved nitrogen fixation into a single genotype could offer growers a cultivar with improved productivity while reducing the need for nitrogen inputs. While elite cultivars such as Zorro are an improvement over other commercial cultivars there is still opportunity to increase the level of nitrogen fixation above that of current commercial cultivars.

Using ^{15}N methods Pereira et al. (1989) found that Puebla 152 was the highest fixing line among the 17 bean genotypes studied. Sanilac, a navy bean, was the lowest fixer in the study. They also found that Puebla 152 began fixing nitrogen earlier than most of the bean genotypes studied and that it also nodulated well in the crown area. In the present study, with harvest beginning at bloom, Puebla 152 had likely established a greater number and mass of nodules than the other bean genotypes. Early nitrogen fixation was measured in the present study, since plants were harvested for analysis prior to peak nitrogen fixation at pod filling. However, the early establishment of BNF is an integral component to increasing the seasonal BNF of a dry bean genotype. Increasing the days a dry bean genotype is fixing nitrogen increases the total nitrogen fixed. At maturity fixation stops as seed become the primary sink for photosynthates and not the nodules, favoring early growth nodulation as the most effective way to increase season long fixation.

St. Clair et al. (1988) measured fixation potential of 12 different bean genotypes at different growth stages in the field. The study included four lines derived from an inbred back cross of Puebla 152 and Sanilac and parents which had been identified as superior nitrogen fixers in relation to Sanilac, the low fixing parent with acceptable agronomic qualities. Comparing the R3 stage to the R9 stage, St. Clair et al. (1988) found that the later sampling time was more effective at predicting the season long nitrogen fixation potential. The R3 stage would coincide with the harvest of the genotypes in the present study. Peak nitrogen fixation occurs during development of the seeds, which are a major nitrogen sink, thus sampling prior to that stage may only be effective at measuring those genotypes with improved early N-fixation (St. Clair et al., 1988, Pereira et al., 1989). As mentioned above, earlier fixation may be a desirable trait to improve in order to develop season long superior nitrogen fixing genotypes since fixation ends at maturity since the seeds become a greater sink for resources than nodules. An ideal dry bean genotype with superior nitrogen fixation ability would fix nitrogen earlier in its life cycle and also efficiently mobilize nitrogen from roots and other organs into the seeds.

Aside from the checks, Puebla 152 and R99, the genotypes selected for this study were not chosen based on their BNF ability but for their importance in the breeding program or their importance as commercial cultivars. The 32 genotypes selected likely represent the typical commercial genotypes which have not been selected for improved BNF during their development. In fact selection would have been practiced under condition of applied N that represses N-fixation. This lack of variability for N-fixation observed in this study may indicate a reduction in genetic variability due to the process of selection or lack of variability for the trait among parents. Sources of genetic material with improved BNF, such as Puebla 152, may be necessary to bring in the variation needed to increase BNF in commercial genotypes. Those genotypes more recently developed and selected heavily for yield, such as Zorro or Santa Fe, were near the top in nitrogen fixed and biomass (Table 3-2 and Table 3-8 respectively). There has likely been indirect selection for nitrogen use efficiency and partitioning of assimilates to seed, while directly selecting for yield potential as Zorro and Santa Fe were developed through intense selection for yield, architecture and acceptable maturity for growing conditions in Michigan. However, the majority of the commercial cultivars tested did not exhibit an ability to fix nitrogen. The difference between the best commercial cultivars, such as Zorro, and Puebla 152 demonstrate that there is substantial room for improvement in the BNF ability of modern commercial cultivars. Utilizing methods such as those investigated in the current study, breeding germplasm can be screened prior to inclusion in breeding programs in order to select only those lines that have enhanced BNF ability. Also, early generation screening of resulting breeding lines will help reduce the number of genotypes with improved BNF for further inclusion in advanced yield trials either as parental lines or for potential release as commercial cultivars.

It appears that the navy and cranberry seed classes are low in their nitrogen fixation ability (Table 3-2). The navy beans in this study may have reduced BNF ability do to the inclusion of Sanilac, a known low fixer and Bunsi, the parent line of R99 resulting in skewing the navy seed class towards lower BNF levels. Sanilac has superior seed quality along with determinant growth habit and early maturity which influenced St. Clair and Bliss (1991) to select this genotype to utilize as the recurrent parent with Puebla 152 to develop a commercial genotype with superior BNF ability. Combining the superior agronomic traits from Sanilac with the enhanced BNF and yield of Puebla 152 is a desired outcome in breeding dry bean genotypes with enhanced BNF. There are likely other aspects of the variability of this class that are contributing to reduced biomass accumulation and nitrogen accumulation such as different growth habits or different yield potentials. The cranberry seed class is of Andean origin which, along with the kidney genotypes appear to have reduced ability for nitrogen fixation. Those genotypes of Mesoamerican origin-pinto, pink, red, and great northern seem to be better fixers than those genotypes of Andean origin as well as navy genotypes which are also Mesoamerican. This may be an interaction with the *Rhizobium* strain used in this study as there is evidence that the different gene pools prefer different *Rhizobium* strains. Kipenolt et al. (1992) found that *Rhizobium tropici* strain CIAT 899 preferentially nodulated genotypes of Andean origin. Middle American genotypes were preferentially nodulated by *Rhizobium leguminosarum* bv *phaseoli* strain CIAT 632. Graham (www.rhizobium.umn.edu, accessed December 2009, and personal communication March 2008) indicated that *Rhizobium etli* UMR1597, the strain used in the current study, was isolated from Brazil. It has been the best overall inoculant for dry bean genotypes regardless of dry bean origin in his studies. Graham (personal communication) indicated, though, that efficiency of this strain may be superior when used with Middle American genotypes as compared to Andean genotypes. Vasquez-Arroyo et al. (1998) discovered that even within the indigenous strains (those that were already present in the field soil) in Durango, Mexico, that not all *Rhizobium* strains were as effective at competing for nodulation sites. In addition, Vasques-Arroyo et al. (1998) found that different *Rhizobium* strains had different abilities to fix nitrogen. Differences between the Andean and Middle American gene pools other than BNF ability may contribute to reduced biomass and nitrogen yield. Characteristics such as nitrogen partitioning as well as maturity or growth habit may also reduce yield in Andean genotypes when compared to Middle American genotypes.

Seed yield and biomass are also components of nitrogen fixation as seeds and vegetative organs of the plant are sinks for nitrogen. Plants that yield a greater amount of seed or biomass may have a larger pool of nitrogen available to them stored in the stems and leaves. Kidney and cranberry seed classes typically experience lower yields than smaller seed sizes (Kelly et al., 1987). This difference in productivity may explain the poor performance of the larger seed classes when compared to the smaller, Middle American derived genotypes. Cichy et al. (2009) noticed that a reduction in seed yield was linked to the *fin*, gene for determinacy in Andean genotypes which is located on linkage group B1.

In researching phosphorus uptake Cichy et al. (2009) hypothesize that either *fin* has pleiotropic effects resulting in a reduction of yield or is linked to other gene(s) responsible for the reduction. The difference in growth habit that was responsible for reduced phosphorus uptake in Andean bean genotypes may be responsible for the reduced levels of nitrogen acquired found in the current study.

The lack of variation observed for %Ndfa in the nitrogen balance (difference) method could be explained by different growth characteristics of the genotypes studied. The estimates of %Ndfa below 100% demonstrate that there was some level of nitrogen available to the plants, which would likely be around 6 mg, the average nitrogen in R99 biomass. This nitrogen may come from different sources, possibly the seed, water, and dust settling from the air. Larger seeded types such as kidney beans have a larger amount of stored nutrients for the developing seedling as opposed to genotypes with smaller seeds and thus less capacity to store nitrogen. Those genotypes that are better at scavenging this nitrogen from the soil may have an augmented nitrogen fixed estimate. Accordingly those genotypes could have an advantage throughout the experiment since they would have more nitrogen to devote to developing nodules, increasing nitrogen fixed in the long term. Development of nodules is aided by a small application of nitrogen early in plant development. An application of nitrogen immediately after germination and subsequently eliminating nitrogen from the nutrient solution might provide a more uniform test environment, allowing those genotypes that are good fixers, but poor nitrogen scavengers, to nodulate and reach levels closer to their potential.

Nodule rating has been shown to accurately predict those lines with improved nitrogen fixation potential (Park and Buttery, 1989). In this study, there was a high correlation between nodule rating and nitrogen fixation measurements (r^2=0.52, $p \leq 0.0001$), with smaller correlations between nodule rating and evaluations such as nitrogen balance (r^2=0.36, $p \leq 0.0001$) and relative growth in nitrogen (r^2=0.3, $p \leq 0.0001$) (Table 3-13). Kidney beans average 3.6 on a scale of 0 to 6 for nodule appearance. This is considerably higher than seed classes that accumulated a larger amount of nitrogen, such as pinto or red and pink seed classes. While the nodules were well developed it is possible that the genotypes nodules were not as efficient as those with lower nodule ratings on a nodule for nodule basis. Additionally, there may be differences in partitioning and mobilization of the nitrogen available into parts of the plant other than the roots. Since seed was sterilized and growing media was autoclaved prior to sowing and a known efficient *Rhizobium* strain, identified by Graham (www.rhizobium.umn.edu, accessed December 2009) as one of the best inoculants, was applied, it could be expected that the kidney beans nodulated with the same rhizobia as the other genotypes did. This difference in efficiency could be explained by the strain/host interaction, kidney beans may not fix nitrogen optimally with *Rhizobium etli* strain UMR 1597. Meschini et al. (2008) determined that *Rhizobium* strains preferentially inoculate dry bean genotypes from similar regions of origin. *Rhizobium etli* is likely of Middle American origin which might help to explain the improved performance of Middle American bean genotypes when compared to Andean bean genotypes inoculated with *R. etli* strain UMR1597.

Characteristics that are correlated with nitrogen fixed include root mass, plant height and nodule rating. The volume of roots produced would seem to determine the number of nodules able to be formed, with more potential infection sites there would be in increase in the number of nodules formed. It is likely that plants with upright architecture possess a better anchorage which results in a more expansive root system to provide the needed support with the resulting root area also having an increased amount of fine roots which are involved in nodulation.

Root mass shows some relationship to nitrogen fixed based on high correlation (r^2=0.83, $p \leq 0.0001$). Selection of lines based on above ground architecture is common while selection for root architecture is rarely practiced. It has been noted that indeterminate plants also have indeterminate roots, root mass would be larger in relation to those that are determinate (Wolyn et al., 1991). Plants approaching or exceeding shoot to root ratio of 3.0 seem to be among those that are also better at fixing nitrogen. Those genotypes with a significantly increased shoot to root ratio over R99 and Sanilac (table 3-9), are the same lines that exhibited increased levels of nitrogen in the biomass (table 3-12).

Nodule rating has been considered an acceptable means to select plants for improved nitrogen fixation and the significant correlation (r^2=0.52 $p \leq 0.001$) between nitrogen fixed and nodule rating offers support for selecting plants based on visual appearance of nodules (rating scale from 0 to 6). In addition, root length had a significant positive correlation with nodule rating, which could imply a greater number of infection sites for *Rhizobium*. A root system that spreads a greater distance is able to access a greater range of the soil profile, both depth and horizontally, increasing access not only to different nutrients in the soil but also has an increase opportunity to contact immobile rhizobia. Roots in this study were confined to the volume of the container and the long roots exceeded the dimensions of the container, wrapping around the perimeter of the container one or more times. Those genotypes with shorter root systems also wrapped around the perimeter of the pot thus suggesting that the longer roots could form more associations than the shorter roots since they all accessed the same area in the container. Root mass is strongly correlated with nitrogen fixed (r^2=0.83 $p \leq 0.001$), Relative growth in nitrogen (RN) (r^2=0.65 $p \leq 0.001$), and N Balance (r^2=0.43 $p \leq 0.001$) while root length is correlated with nodule rating (r^2=0.43 $p \leq 0.001$). (See table 3-13).

It would seem that root characteristics are important in the BNF process and attention to root attributes in considering the fixation ability of a genotype would be advantageous.

Plant height is difficult to evaluate in a greenhouse due to the difference in growth habit when compared to field conditions. Plants are generally more etiolated owing to reduced light levels in the greenhouse while those plants that are type II, which do not vine excessively in the field, do so in the greenhouse. Plant height was measured as an indication of vigor; however, biomass is a more encompassing and useful trait. Plants such as Sanilac produced a vine that was very thin and wiry due to overall lack of vigor. Sanilac was tall, yet had a low biomass (tables 3-8 and 3-10). The plants with the lowest amount of biomass tended to be in the navy seed class. A plant that is fixing a greater amount of nitrogen would likely be able to grow a more of foliage, which would photosynthesize at a greater rate thus having a greater amount of carbohydrates to supply the nodules for greater nitrogen fixation. Selecting for increased biomass, or yield, indirectly selects those genotypes with improved BNF.

The ratio of the shoot weight to the root weight offers some perspective on where the nitrogen is being partitioned, and a level of the efficiency of the growth. Investment in roots is necessary to support plant growth, though increase in yield, the top portion of the plant is critical for increased yield. While there were differences between seed classes and several genotypes the rank of those genotypes and seed classes was not the same as in nitrogen fixed, nitrogen balance, or %Ndfa. The cranberry bean 'Capri', a consistently low nitrogen fixer, ranked in the middle of the scale of shoot to root ratio, whereas 'Matterhorn', a great northern, had a low shoot to root ratio. The efficient transport of nitrogen from nodules and roots to the rest of the plant is an important aspect of nitrogen fixation. Those plants retaining nitrogen in the roots are not as likely to have high levels of biomass accumulation. Nitrogen partitioning is a critical trait that cannot be ignored when selecting a genotype for improved BNF. The ultimate goal is to move the nitrogen into the seed which is the harvestable organ. Though, nitrogen remaining in the straw and roots may provide nitrogen for the next season's crop, it does not contribute to the economic yield of a grain legume crop.

Puebla 152 had the highest nitrogen fixed, nodule rating, biomass, root mass, and height. These combined characteristics would be a useful screen for identifying genotypes with superior nitrogen fixation abilities. Both the non nodulating R99 navy and Sanilac were low in these characteristics. Those genotypes fixing increased amounts of nitrogen above the low/non fixers were more similar to Puebla 152 in these traits. There is some evidence that later maturity helps increase BNF. There was no significant difference among the seed classes (table 3-13) and the genotypes for nitrogen fixed per day. This would suggest that an increase in days to maturity could explain the increase in nitrogen fixation in longer maturity genotypes.

CONCLUSION

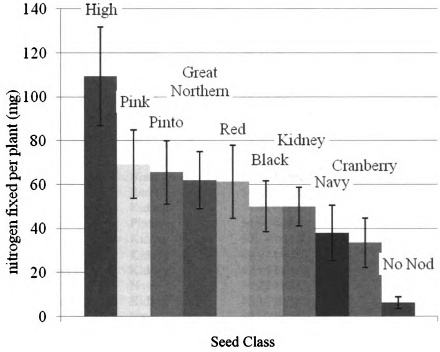
Growing plants in a system that limits nitrogen and using indirect measures to evaluate the BNF ability of different bean genotypes can be a useful tool in selecting bean genotypes for improved nitrogen fixation. The high fixing check, Puebla 152, accumulated greater biomass, larger root mass, had a higher nodule rating, and the greatest accumulation of nitrogen, 109 mg N $plant^{-1}$ compared to the next highest fixers, the pinto bean Santa Fe and the black bean Zorro 72 mg N $plant^{-1}$ and 70 mg N $plant^{-1}$, respectively. The lowest fixers, mostly in the navy seed class, had low biomass, low root mass, and a lower nodule rating than those determined to be greater nitrogen fixers. Traits such as total biomass and root mass may be used along with total biomass nitrogen accumulation and a visual nodule score to screen genotypes quickly and in an efficient manner. This greenhouse assay can be performed during times when field work is at a minimum and also decrease the time necessary to properly evaluate genotypes based on BNF. Genotypes which possess similar root mass, biomass, nodule score, and biomass nitrogen combined with upright, vigorous, architecture could be selected in a breeding program utilizing the above protocols. Reaching a balance between adequate root growth with improved biomass, and resulting yield, is an essential requirement for a successful bean genotype.

In addition to the above mentioned characteristics important for genotypes having improved nitrogen fixation, this study demonstrated that screening genotypes in the greenhouse in inert media and fertilized with nitrogen free solution can be an effective method to identify dry bean genotypes with enhanced nitrogen fixation ability. Convenience of observing the roots in an enclosed system as well as flexibility in timing of the screen in relation to field season are advantages to this system. While considerably different than conditions in the field, the greenhouse screening identified both the poor yielding genotypes in the field as well as the higher yielding genotypes in the field. Zorro performed well under organic and conventional systems as well as being identified as a superior nitrogen fixer in the greenhouse. Similarly, the navy bean genotypes yielded poorly in the field and also performed poorly in amount of nitrogen fixed per plant. While the non nodulating genotype R99 was inconsistent in its performance between organic and conventional sites, it fixed the lowest amount of nitrogen in the greenhouse study.

Table 3-1 Modified Hoagland's nutrient solution utilized in the greenhouse study of nitrogen fixation of 34 dry bean (*Phaseolus vulgaris* L.) genotypes.

	Amount in 1 L stock solution	Amount per L stock solution for final dilution.
K_2HPO_4	116 g	3 ml
$CaCl_2$	366g	1ml
$MgSO_4$	245g	1ml
$KHSO_4$	218g	1ml
The following were prepared as a single stock solution		1ml of the minor nutrient solution
$CuSO_4$	.051g	
$NaMoO_4*2H_2O$	0.12g	
$MnCl_2*4H_2O$	1.81g	
H_3BO_2	2.86g	
$ZnSO_4$	0.22g	

Figure 3-1. Nitrogen fixed per dry bean plant (*Phaseolus vulgaris* L.) from 10 seed classes evaluated for nitrogen fixation under greenhouse conditions in East Lansing, MI in September and December 2008 and March 2009[1].



[1] The high genotype was Puebla 152 and the no-nod was R99.

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Table 3-2. Nitrogen Fixed $plant^{-1}$ of 34 dry bean (*Phaseolus vulgaris* L.) genotypes evaluated for nitrogen fixation ability under greenhouse conditions in East Lansing, MI in September and December 2008 and March 2009. [1]

Genotype	Seed class	Nitrogen fixed $plant^{-1}$(mg)
Puebla 152	Black, High Fixer	109.48 a[2]
Santa Fe	Pinto	72.34 b
Zorro	Black	70.71 bc
T39	Black	69.97 bc
Sedona	Pink	69.30 bc
P06131	Pinto	68.65 bc
Buster	Pinto	67.16 bcd
TARS-SR05	Red	66.25 bcde
Matterhorn	Great Northern	62.06 bcdef
K05604	Kidney	61.94 bcdef
Jaguar	Black	58.40 bcdef
Merlot	Red	56.25 bcdef
Red Hawk	Kidney	54.91 bcdef
Othello	Pinto	54.65 bcdef
Montcalm	Kidney	50.14 bcdefg
K03240	Kidney	49.97 bcdefg
USDK-CBB-15	Kidney	49.85 bcdefg
N05324	Navy	48.22 bcdefg
Chinook Select	Kidney	46.13 bcdefg
N05311	Navy	46.12 bcdefg
B05039	Black	46.02 bcdefg
B04431	Black	44.80 bcdefg
Beluga	Kidney	44.10 bcdefg
B05055	Black	42.79 bcdefg
CELRK	Kidney	41.84 bcdefg
Condor	Black	41.05 bcdefg
115-11M	Black	38.56 bcdefgh
Michelite	Navy	38.56 cdefgh
Capri	Cranberry	33.56 defgh
Vista	Navy	33.34 efgh
Bunsi	Navy	29.32 fgh
Seahawk	Navy	28.95 fgh
Sanilac	Navy	19.98 gh
R99	Navy, No Nod	6.32 h
Mean		50.64
Tukey's LSD ($p \leq 0.05$)		17.3

[1]See appendix A for days to first bloom [2]Genotypes followed by the same letter are not significantly different.

Table 3-3. Nodule rating for 34 dry bean (*Phaseolus vulgaris* L.) genotypes evaluated for nitrogen fixation under greenhouse conditions in East Lansing, MI September and December 2008 and March 2009. [1]

Genotype	Seed class	Nodule rating[2]
Puebla-152	High Fixer	5.2
Othello	Pinto	4.0
Chinook Select	Kidney	3.9
Buster	Pinto	3.8
USDK-CBB-15	Kidney	3.7
Red Hawk	Kidney	3.7
K03240	Kidney	3.7
CELRK	Kidney	3.6
Beluga	Kidney	3.6
TARS-SR05	Red	3.5
Sedona	Pink	3.5
Montcalm	Kidney	3.5
K05604	Kidney	3.4
Merlot	Red	3.4
Capri	Cranberry	3.3
Santa Fe	Pinto	3.2
Zorro	Black	3.1
T39	Black	3.0
N05311	Navy	2.8
B05055	Black	2.8
Jaguar	Black	2.8
Seahawk	Navy	2.7
P06131	Pinto	2.6
Michelite	Navy	2.6
B05039	Black	2.6
Bunsi	Navy	2.6
Matterhorn	Great Northern	2.5
N05324	Navy	2.5
115-11M	Black	2.5
Vista	Navy	2.4
Sanilac	Navy	2.3
B04431	Black	2.3
Condor	Black	2.2
R99	No Nod	0
Mean		3.0

[1] See Appendix A for days to first bloom. [2]Nodule rating scale 0= no nodules 6= extensive nodulation

Table 3-4. Nodule rating for 10 dry bean (*Phaseolus vulgaris* L.) averaged by seed class evaluated under greenhouse conditions for nitrogen fixation ability in East Lansing, MI in September and December 2008 and March 2009.[1]

Seed class	Nodule Rating[2]
High,Puebla 152	5.2
Kidney	3.6
Pink	3.5
Red	3.4
Pinto	3.4
Navy	3.4
Cranberry	3.3
Black	2.7
Great Northern	2.5
Non Nodulating, R99	0
Mean	3.1

[1] See Appendix A for days to first bloom [2]Nodule rating scale 0= no nodules 6= heavy nodulation

Table 3-5. Average root mass of 10 dry bean (*Phaseolus vulgaris* L.) market classes evaluated at the first flower stage for nitrogen fixation ability under greenhouse conditions in East Lansing, MI in September and December 2008 and March 2009.[1]

Seed Class[2]	Root Mass (g)
High, Puebla 152	1.62 a[2,3]
Great Northern	1.01 b
Pink	0.96 b
Pinto	0.89 bc
Black	0.87 bcd
Small Red	0.86 bcd
Kidney	0.73 bcd
Cranberry	0.61 cd
Navy	0.58 cd
Non Nodulating, R99	0.28 e
Mean	0.84
Tukey's LSD($p \leq 0.05$)	0.29

[1]See Appendix A for days to first bloom [2]The high genotype was Puebla 152 and the no-nod was R99 [3]Seed classes followed by the same letter are not significantly different.

Table 3-6. Root mass of 34 dry bean (*Phaseolus vulgaris* L.) genotypes evaluated for nitrogen fixation ability under greenhouse conditions in East Lansing, MI in September and December 2008 and March 2009.[1]

Genotype	Seed Class	Root Mass (g)
Puebla 152	High Fixer	1.62 a[2]
P06131	Pinto	1.17 ab
Zorro	Black	1.16 ab
Matterhorn	Great Northern	1.01 bc
Jaguar	Black	0.98 bcd
Sedona	Pink	0.96 bcde
B04431	Black	0.95 bcde
K05604	Kidney	0.95 bcde
T39	Black	0.93 bcde
B05039	Black	0.91 bcde
TARS-SR05	Red	0.90 bcde
Santa Fe	Pinto	0.86 bcdef
Merlot	Red	0.83 bcdef
Buster	Pinto	0.79 bcdef
N05311	Navy	0.78 bcdef
Red Hawk	Kidney	0.77 bcdef
USDK-CBB-15	Kidney	0.76 bcdef
B05055	Black	0.73 bcdefg
Othello	Pinto	0.72 bcdefg
K03240	Kidney	0.72 bcdefg
N05324	Navy	0.72 bcdefg
CELRK	Kidney	0.70 cdefg
Condor	Black	0.66 cdefg
Beluga	Kidney	0.65 cdefg
115-11M	Black	0.64 cdefg
Chinook Select	Kidney	0.64 cdefg
Michelite	Navy	0.63 cdefg
Montcalm	Kidney	0.62 cdefg
Capri	Cranberry	0.61 cdefg
Vista	Navy	0.54 defg
Seahawk	Navy	0.52 efg
Bunsi	Navy	0.43 fg
Sanilac	Navy	0.28 g
R99	No Nod	0.28 g
Mean		0.78
Tukey's LSD ($p \leq 0.05$)		0.46

[1]See Appendix A, days to first bloom [2]Genotypes followed by the same letter are not significant.

Table 3-7. Average biomass of 10 dry bean (*Phaseolus vulgaris* L.) seed classes evaluated for nitrogen fixation ability under greenhouse conditions in East Lansing, MI in September and December 2008 and March 2009.[1]

Seed Class[2]	Biomass (g)
High, Puebla 152	4.36 a[2]
Pink	2.23 b
Pinto	2.22 b
Small Red	2.13 b
Great Northern	1.92 b
Black	1.89 b
Kidney	1.63 bc
Navy	1.22 c
Cranberry	1.17 c
Non Nodulating, R99	0.32 d
Mean	1.91
Tukey's LSD ($p \leq 0.05$)	0.65

[1]See Appendix A for days to first bloom [2]Averages followed by the same letter are not significantly different.

Table 3-8. Biomass of 34 dry bean (*Phaseolus vulgaris* L.) genotypes evaluated for nitrogen fixation ability under greenhouse conditions in East Lansing, MI in September and December 2008 and March 2009[1].

Genotype	Seed Class	Biomass (g)
Puebla 152	Black	4.36 a[2]
Zorro	Black	2.83 b
P06131	Pinto	2.47 bc
TARS-SR05	Red	2.47 bcd
Santa Fe	Pinto	2.37 bcde
Jaguar	Black	2.35 bcdef
Sedona	Pink	2.24 bcdefg
T39	Black	2.21 bcdefg
Buster	Pinto	2.21 bcdefg
K03240	Kidney	1.96 bcdefgh
Matterhorn	Great Northern	1.92 bcdefghi
K05604	Kidney	1.86 bcdefghi
Othello	Pinto	1.83 bcdefghi
Merlot	Red	1.80 cdefghi
B05039	Black	1.79 cdefghi
Red Hawk	Kidney	1.78 cdefghi
B04431	Black	1.77 cdefghi
USDK-CBB-15	Kidney	1.65 cdefghi
B05055	Black	1.58 cdefghij
Condor	Black	1.55 cdefghij
N05324	Navy	1.54 cdefghij
Montcalm	Kidney	1.51 cdefghij
Beluga	Kidney	1.46 defghij
N05311	Navy	1.45 efghij
CELRK	Kidney	1.44 efghij
115-11M	Black	1.39 efghij
Chinook Select	Kidney	1.34 fghij
Michelite	Navy	1.27 ghijk
Capri	Cranberry	1.17 hijk
Vista	Navy	1.17 hijk
Seahawk	Navy	0.95 hijk
Bunsi	Navy	0.87 ijk
Sanilac	Navy	0.58 jk
R99	Navy No Nod	0.32 k
Mean		1.75
LSD		1.01

[1]See Appendix A for days to first bloom [2]Genotypes followed by the same letter are not significantly different.

Table 3-9. Shoot to root ratio of 34 dry bean (*Phaseolus vulgaris* L.) genotypes evaluated for nitrogen fixation ability under greenhouse conditions in East Lansing, MI in September and December 2008 and March 2009[1].

Genotype	Seed Class	Shoot to Root Ratio
TARS-SR05	Red	3.3 a[2]
Buster	Pinto	3.3 ab
Puebla 152	High Fixer	2.9 abc
K03240	Kidney	2.9 abc
Santa Fe	Pinto	2.8 abc
Othello	Pinto	2.6 abc
Montcalm	Kidney	2.6 abc
Beluga	Kidney	2.5 abc
Zorro	Black	2.5 abc
Jaguar	Black	2.5 abc
Condor	Black	2.4 abc
T39	Black	2.4 abcd
Red Hawk	Kidney	2.4 abcd
Capri	Cranberry	2.3 abcd
B05055	Black	2.3 abcd
N05324	Navy	2.3 abcd
115-11M	Black	2.3 abcd
B04431	Black	2.2 abcd
Merlot	Red	2.2 abcd
CELRK	Kidney	2.2 abcd
USDK-CBB-15	Kidney	2.2 abcd
Vista	Navy	2.2 abcd
Sedona	Pink	2.2 abcd
Sanilac	Navy	2.2 abcd
P06131	Pinto	2.1 bcd
I81010	Bunsi	2.0 dc
B05039	Black	2.0 dc
K05604	Kidney	2.0 dc
Chinook Select	Kidney	1.9 dc
Matterhorn	Great Northern	1.9 dc
Seahawk	Navy	1.8 dc
Michelite	Navy	1.8 dc
N05311	Navy	1.7 dc
R99	No Nod	1.2 d
Mean		2.29
LSD		1.23

[1]See Appendix B for days to first bloom.
[2]Genotypes followed by the same letter are not significantly different.

Table 3-10. Shoot to root ratio of 10 dry bean (*Phaseolus vulgaris* L.)seed classes evaluated under greenhouse conditions for nitrogen fixation ability in East Lansing, MI in September and December 2008 and March 2009[1].

Seed Class	Shoot to root ratio
High, Puebla 152	2.91 a[2]
Red	2.76 ab
Pinto	2.68 abc
Cranberry	2.34 abc
Kidney	2.32 abc
Black	2.28 abc
Pink	2.17 abc
Navy	2.07 bc
Great Northern	1.92 d
Non Nodulating, R99	1.18 d
Mean	2.26
Tukey's LSD ($p \leq 0.05$)	0.78

[1]See Appendix B for days to first bloom.
[2]Seed classes followed by the same letter are not significantly different.

Table 3-11. Nitrogen fixed per day of 10 dry bean (*Phaseolus vulgaris* L.) seed classes evaluated for nitrogen fixation under greenhouse conditions in East Lansing, MI in September and December 2008 and March 2009[1].

Seed class	N fixed per day (mg)
High	1.23 a[2]
Pink	1.23 a
Pinto	1.09 a
Red	1.06 a
Great Northern	1.02 a
Kidney	0.90 a
Black	0.83 a
Cranberry	0.73 ab
Navy	0.70 ab
Non Nodulating	0.09 b
Mean	0.89
Tukey's LSD ($p \leq 0.05$)	0.7

[1]See Appendix B for days to first bloom.
[2]Seed classes followed by the same letter are not significantly different.

Table 3-12. Percent nitrogen derived from the atmosphere (%Ndfa) of 34 dry bean (*Phaseolus vulgaris* L.) genotypes evaluated for nitrogen fixation ability under greenhouse conditions in East Lansing, MI in September and December 2008 and March 2009[1].

Genotype	Seed Class	%Ndfa
Puebla 152	High Fixer	94.2 a[2]
Santa Fe	Pinto	91.3 a
T39	Black	91.1 a
Sedona	Pink	90.9 a
Buster	Pinto	90.6 a
TARS-SR05	Red	90.5 a
P06131	Pinto	90.1 a
K05604	Kidney	89.8 a
Matterhorn	Great Northern	89.8 a
Jaguar	Black	89.2 a
Zorro	Black	89.0 a
Merlot	Red	88.8 a
Red Hawk	Kidney	88.5 a
B05039	Black	86.3 a
Montcalm	Kidney	87.4 a
K03240	Kidney	87.4 a
Othello	Pinto	87.4 a
USDK-CBB-15	Kidney	87.3 a
N05324	Navy	86.9 a
N05311	Navy	86.3 a
Chinook Select	Kidney	86.3 a
B04431	Black	85.9 a
Beluga	Kidney	85.7 a
B05055	Black	85.2 a
CELRK	Kidney	84.9 a
Condor	Black	84.6 a
115-11M	Black	83.6 a
Michelite	Navy	83.6 a
Capri	Cranberry	81.2 a
Vista	Navy	81.0 a
Bunsi	Navy	78.4 a
Seahawk	Navy	78.2 a
Sanilac	Navy	68.4 a
R99	Non Nodulating	0 b
Mean		87.5
Tukey's LSD (p<0.05)		63.5

[1]See Appendix B for days to first bloom. [2]Genotypes followed by the same letter are not significantly different.

Table 3-13. Correlations among growth characteristics of 34 bean (*Phaseolus vulgaris* L.) genotypes evaluated for nitrogen fixation under greenhouse conditions in East Lansing, MI in September and December 2008 and March 2009[1, 2].

	Days to flower	Height	Root Length	Biomass	Root Mass	Percent Nitrogen	Nitrogen Fixed	Seed Nitrogen	Rn[3]	Shoot to Root Ratio	Nitrogen Balance
Days to flower	1.00										
Height	-0.033	1.00									
Root Length	0.17***	0.06	1.00								
Biomass	0.12*	0.59***	0.11*	1.00							
Root Mass	0.004	0.48***	0.11*	0.83***	1.00						
Percent Nitrogen	-0.42***	0.04	-0.04	0.0	0.09	1.00					
Nitrogen Fixed	-0.04	0.55***	0.8	0.94***	0.83***	0.29***	1.00				
Seed Nitrogen	-0.43***	0.17***	-0.15**	0.08	0.04	0.22***	0.13**	1.00			
Rn[3]	-0.29***	0.42***	0.08	0.71***	0.65***	0.39***	0.79***	-0.23***	1.00		
Shoot to Root Ratio	0.12*	0.16***	0.16**	0.32***	-0.13**	-0.08	0.26***	0.12*	0.11*	1.00	
Nitrogen Balance	0.03	0.24***	0.24***	0.45***	0.43***	0.18***	0.46***	0.17***	0.32***	0.19***	1.00
Nodule Rating	-0.2***	0.42***	0.43***	0.51***	0.44***	0.23***	0.52***	0.44***	0.30**	0.17**	0.36***

[1] Data from three reps averaged. [2]*=significant at $p \leq 0.05$; **=significant at $p \leq 0.01$, ***=significant at $p \leq 0.001$, [3]Rn=relative growth in nitrogen.

SUMMARY

A comparison of results from the nitrogen fixation screening in the greenhouse to yield trials conducted in the different production systems in the field reveals a similar trend: In the field Middle American genotypes produced higher yields and acquired greater amounts of nitrogen than Andean genotypes. Middle American genotypes produced greater biomass and fixed greater amounts of nitrogen than those genotypes of Andean origin in the greenhouse screening. Black, pink and red seed classes as a group performed well under low nitrogen conditions. Kidney and cranberry market classes yielded poorly under low nitrogen conditions.

The black bean genotype 'Zorro' performed consistently well under organic and conventional production systems as well as fixing a high amount of nitrogen per plant in greenhouse trials. Zorro is a modern commercial cultivar which was recently released (Kelly et al., 2009). The black bean genotype 'Jaguar' also performed well under both organic and conventional production systems.

The germplasm small red line TARS-SR05 performed well under both organic and conventional production systems. TARS-SR05 was ranked 8th for nitrogen fixed per plant (Table 3-2). This genotype will likely provide a source for improving nitrogen fixation but also development of genotypes better suited to organic production systems and having better root rot resistance, stress tolerance and overall root health.

The navy genotype 'Michelite' released prior to the common use of pesticides and fertilizers did not perform well under organic or conventional production systems.

Similar to the other navy beans evaluated, Michelite was inferior for nitrogen fixation (Table 3-2). Those modern genotypes selected for disease resistance and superior yield appear better suited to organic production as they have undergone rigorous selection for yield.

Under the organic production system at all sites and years, a moderate correlation (r^2=0.15, p≤0.0001) was observed between the amounts of nitrogen fixed in the greenhouse with yield as obtained in field trials. Biomass in the greenhouse had a slightly stronger correlation (r^2=0.18, p≤0.0001) with yield from field trials. The amount of nitrogen fixed per acre in field trials correlated with nitrogen fixed in the greenhouse, (r^2=0.12, p≤0.05) as well as greenhouse biomass (r^2=0.17, p≤0.001). The genotypes selected for evaluation were selected for their importance in breeding programs and commercial production. TARS SR05 was included based on its development under stress conditions which may be better suited for organic production. It is likely that stronger relationships would be observed when evaluating populations which have been created by crossing genotypes of divergent nitrogen fixation ability. Such a population could be generated by crossing a genotype identified as strong nitrogen fixers such as Puebla 152 and a commercial cultivar that fixes less nitrogen.

Nitrogen fixed in the greenhouse along with biomass in the greenhouse may be used to select genotypes with superior early nitrogen fixation ability as well as greater yield under field evaluation in early generation selection programs.

Summary Table 1. Pearson Correlations of 32 dry bean (*Phaseolus vulgaris* L.) genotypes grown under organic production at KBS, Gratiot County, and Tuscola County from 2007 to 2009.

	Greenhouse N	Greenhouse Biomass	Greenhouse N Balance	Field-Yield
Greenhouse N	1.00			
Greenhouse Biomass	0.94***[1]	1.00		
Greenhouse N Balance	0.77***	0.72***	1.00	
Field-Yield	0.15***	0.18***	0.05	1.00
Field-N Yield	0.12*	0.17**	0.11	0.75***

[1]*, $p \leq 0.05$, **, $p \leq 0.01$, ***$p \leq 0.001$

Summary Table 2. Ranking of the five highest yielding over all sites and years, and highest nitrogen fixed, of 32 dry bean (*Phaseolus vulgaris* L.) genotypes.

Rank	**Organic Yield**	**Conventional Yield**	**Greenhouse Nitrogen Fixed plant^{-1}**
1	TARS-SR05	**Zorro**	Puebla 152[1]
2	**Zorro**	TARS-SR05	Santa Fe
3	N05311	R99	**Zorro**
4	B05055	Jaguar	T39
5	Jaguar	Condor	Sedona

[1]Puebla 152 was not included in field studies do to its lack of adaptation to production in Michigan.

Appendix

Table A1. Small seeded dry bean (*Phaseolus vulgaris* L.) genotypes grown organically at Kellogg Biological Station in 2007. Planted 30 May 2007.

Entry	Name	Yield CWT/ACRE	100 Seed Wt	Days to Flower	Days to Maturity	Lodging	Height	Des score
N05311	N03611/B01749	22.0	19.9	47.5	92.0	2.5	31.9	3.8
N97774	Seahawk	21.7	22.7	40.8	92.0	3.0	27.5	4.5
B04431	I01892/Jaguar	21.2	23.2	47.0	89.8	2.5	33.8	3.5
B04554	Zorro	20.6	22.4	47.3	90.0	2.5	31.2	4.3
I92002	Vista	20.5	19.4	45.0	92.0	2.5	35.1	4.3
I07112	R99	20.2	18.4	42.5	91.8	2.8	28.6	4.0
N05324	N00838/N00809//N00792	19.2	21.5	44.8	92.0	2.8	35.5	4.0
I81066	T39	18.8	19.8	45.5	92.0	3.0	36.9	4.5
I01892	115-11M	17.4	20.5	46.5	92.3	2.8	30.8	5.0
B05055	34-27/Jag*2/SEL1308//HR45/Kaboon	17.1	20.1	46.3	90.8	2.5	27.3	4.5
N61001	Michelite	16.9	19.7	46.0	92.3	2.5	40.1	4.3
I81010	Bunsi	16.7	20.0	41.8	90.5	2.8	26.4	4.8
B00101	Condor	16.3	20.8	47.0	92.0	3.0	29.3	4.5
B95556	Jaguar	16.3	19.0	46.8	90.8	2.3	27.2	4.3
B05039	35-5/Jag*2/SEL138//HR45/Kaboon	16.1	22.5	45.0	90.8	2.5	27.6	5.0
I07111	R69	5.3	15.5	43.8	92.0	2.8	23.3	4.0
	Average	17.9	20.3	45.2	91.4	2.7	30.8	4.3
	LSD (p=0.05)	4.7	1.7	2.5	4.0	0.7	6.3	1.4
	LSD (p=0.01)	6.1	2.2	3.2	5.2	0.9	8.2	1.8
	Coefficient of Variation	18.6	6.0	3.9	3.1	19.0	14.4	23.2

Table A2. Medium and large seeded dry bean (*Phaseolus vulgaris* L.) genotypes grown organically at Kellogg Biological Station in 2007. Planted 30 May 2007.

Entry	Name	Yield CWT/ACRE	100 Seed Wt	Days to Flower	Days to Maturity	Lodging	Height	Des score
P06131	P02646/P02630	17.0	35.8	43.2	92.0	2.8	28.2	4.0
I99117	Buster	16.8	26.8	41.7	91.8	2.8	28.2	4.0
G93414	Matterhorn	16.0	37.1	42.2	92.3	2.5	26.8	4.3
R98026	Merlot	15.9	35.3	45.4	92.0	3.0	31.5	4.5
I05101	USDK-CBB15	15.5	49.4	42.0	92.0	3.0	25.9	4.5
S00809	Sedona	14.4	33.1	43.8	92.0	2.5	33.3	4.3
K90101	Red Hawk	14.3	48.3	39.1	90.8	2.5	32.1	5.0
K03601	Chinook Select	14.1	50.4	40.0	89.8	2.5	30.5	3.5
P04205	Santa Fe	13.6	35.9	43.5	90.5	2.8	29.1	4.8
K90902	Beluga	13.1	49.7	41.7	90.0	2.5	29.2	4.3
C99833	Capri	12.9	52.4	36.8	92.0	2.5	30.8	3.8
K74002	Montcalm	12.2	50.9	40.5	90.8	2.5	30.4	4.5
I90013	CELRK	11.5	48.6	35.5	92.3	2.8	32.6	5.0
K03240	Red Hawk*2/Negro San Luis-140	11.4	48.4	38.7	90.8	2.3	34.2	4.3
K05604	K00604/X02151	10.6	49.5	41.1	92.0	3.0	34.2	4.5
	Average	14.0	42.8	40.8	91.4	2.7	30.4	4.3
	LSD (p=0.05)	4.9	6.0	1.8	4.0	0.7	3.4	1.4
	LSD (p=0.01)	6.4	7.8	2.3	5.2	0.9	4.4	1.8
	Coefficient of Variation	24.8	10.0	3.1	3.1	19.0	7.8	23.2

Table A3. Small seeded dry bean (*Phaseolus vulgaris* L.) genotypes grown conventionally at Kellogg Biological Station in 2007. Planted 30 May 2007.

Entry	Name	Yield CWT/ACRE	100 Seed Wt	Days to Flower	Days to Maturity	Lodging	Heigh t	Des score
N05324	N00838/N00809//N00792	25.3	20.2	45.0	92.0	2.8	25.7	4.0
B05039	35-5/Jag*2/SEL1308//HR45/Kaboon	24.8	23.3	45.3	90.8	2.5	25.4	5.0
B04431	I01892/Jaguar	23.7	23.0	48.5	89.8	2.5	26.1	3.5
B04554	Zorro	22.6	21.8	47.0	90.0	2.5	27.4	4.3
N97774	Seahawk	22.6	22.0	41.0	92.0	3.0	24.8	4.5
I92002	Vista	22.6	20.9	44.8	92.0	2.5	24.5	4.3
N05311	N03611/B01749	22.2	18.7	47.3	92.0	2.5	25.2	3.8
B05055	34-27/Jag*2/SEL1308//HR45/Kaboon	21.8	21.7	45.8	90.8	2.5	24.9	4.5
N61001	Michelite	21.2	20.6	46.5	92.3	2.5	34.4	4.3
I81066	T39	19.2	21.4	46.5	92.0	3.0	29.6	4.5
I01892	115-11M	18.1	21.0	47.5	92.3	2.8	25.7	5.0
B00101	Condor	17.9	22.2	48.3	92.0	3.0	27.4	4.5
B95556	Jaguar	16.7	20.1	48.8	90.8	2.3	25.4	4.3
I07112	R99	16.4	16.2	43.8	91.8	2.8	27.3	4.0
I81010	Bunsi	16.3	19.3	40.5	90.5	2.8	26.6	4.8
I07111	R69	14.1	14.6	43.8	92.0	2.8	24.9	4.0
	Average	20.4	20.4	45.6	91.4	2.7	26.6	4.3
	LSD (p=0.05)	5.2	0.8	2.6	4.0	0.7	5.0	1.4
	LSD (p=0.01)	6.8	1.0	3.3	5.2	0.9	6.5	1.8
	Coefficient of Variation	18.0	2.7	4.0	3.1	19.0	13.2	23.2

Table A4. Medium and large seeded dry bean (*Phaseolus vulgaris* L.) genotypes grown conventionally at Kellogg Biological Station in 2007. Planted 30 May 2007.

Entry	Name	Yield CWT/ACRE	100 Seed Wt	Days to Flower	Days to Maturity	Lodging	Height	Des score
I99117	Buster	23.4	37.2	40.8	91.8	2.8	28.5	4.0
R98026	Merlot	19.1	39.4	44.2	92.0	3.0	31.3	4.5
S00809	Sedona	16.2	39.1	43.2	92.0	2.5	33.7	4.3
K90902	Beluga	15.5	46.4	41.5	90.0	2.5	29.1	4.3
K03240	Red Hawk*2/Negro San Luis-140	13.3	44.5	39.1	90.8	2.3	37.0	4.3
K03601	Chinook Select	13.1	45.6	40.0	89.8	2.5	36.0	3.5
K74002	Montcalm	13.0	48.3	40.6	90.8	2.5	35.0	4.5
K05604	K00604/X02151	12.8	44.1	39.9	92.0	3.0	38.9	4.5
I05101	USDK-CBB15	12.8	48.9	40.9	92.0	3.0	30.0	4.5
P04205	Santa Fe	12.7	37.6	41.4	90.5	2.8	30.5	4.8
K90101	Red Hawk	12.7	46.0	40.1	90.8	2.5	37.7	5.0
I90013	CELRK	11.2	44.2	36.5	92.3	2.8	36.1	5.0
C99833	Capri	10.4	48.4	36.7	92.0	2.5	37.0	3.8
I84002	Othello	9.9	36.9	39.1	92.0	2.8	32.0	4.0
P06131	P02646/P02630	9.4	39.4	42.1	92.0	2.8	31.2	4.0
G93414	Matterhorn	9.2	35.3	43.0	92.3	2.5	28.9	4.3
	Average	13.4	42.6	40.6	91.4	2.7	33.3	4.3
	LSD (p=0.05)	4.4	1.1	1.5	4.0	0.7	3.8	1.4
	LSD (p=0.01)	5.8	1.4	1.9	5.2	0.9	4.9	1.8
	Coefficient of Variation	23.4	1.8	2.6	3.1	19.0	8.0	23.2

Table A5. Small seeded dry bean (*Phaseolus vulgaris* L.) genotypes grown conventionally in Gratiot County in 2007. Planted 12 June 2007.

Entry	Name	Yield CWT/ACRE	100 Seed Wt	Days to Flower	Height
B05039	35-5/Jag*2/SEL1803//HR45/Kaboon	17.5	23.1	45.3	35.2
I01892	115-11M	17.4	22.7	47.5	42.0
N05311	N03611/B01749	17.3	19.9	47.3	38.2
B00101	Condor	17.2	19.9	48.3	39.0
I92002	Vista	14.1	20.5	44.8	33.4
B95556	Jaguar	12.2	20.0	48.8	31.5
B04554	Zorro	18.5	22.6	47.0	40.8
B05055	34-27/Jag*2/SEL1308//HR45/Kaboon	17.3	20.1	45.8	32.3
N05324	N008838/N00809//N00792	15.6	19.6	45.0	35.5
B04431	I01892/Jaguar	17.0	23.3	48.5	41.3
I81066	T39	16.9	20.2	46.5	38.0
I81010	Bunsi	13.5	17.0	40.5	31.7
N97774	Seahawk	14.7	20.5	41.0	30.7
N61001	Michelite	14.3	21.1	46.5	41.5
I07112	R99	14.2	17.4	43.8	34.5
I07111	R69	10.3	17.8	43.8	37.0
	Average	15.5	20.3	45.6	36.4
	LSD (p=0.05)	3.5	1.6	2.6	5.4
	LSD (p=0.01)	4.6	2.0	3.3	7.0
	Coefficient of Variation	16.0	5.4	4.0	10.5

A6. Medium and large seeded dry bean (*Phaseolus vulgaris* L.) genotypes grown organically in Gratiot County in 2007. Planted 12 June 2007.

Entry	Name	Yield CWT/ACRE	100 Seed Wt	Days to Flower	Height
S00809	Sedona	21.7	37.3	43.8	44.9
R98026	Merlot	20.6	37.3	45.4	48.1
P06131	P02646/P02630	20.4	37.4	43.2	42.5
I99117	Buster	19.4	35.1	41.7	40.7
I84002	Othello	18.7	33.7	38.2	39.4
P04205	Santa Fe	17.4	36.5	43.5	39.4
I05101	USDK-CBB15	16.3	44.3	42.0	36.2
G93414	Matterhorn	15.7	30.9	42.2	36.0
K03601	Chinook Select	14.8	43.9	40.0	45.2
C99833	Capri	12.0	43.5	36.8	36.4
K05604	K00604/X02151	11.5	40.3	41.1	42.7
K74002	Montcalm	10.8	43.4	40.5	42.7
K90902	Beluga	10.5	42.6	41.7	41.7
K90101	Red Hawk	10.0	37.9	39.1	39.7
K03240	Red Hawk*2/Negro San Luis-140	9.6	38.3	38.7	42.6
I90013	CELRK	7.1	37.4	35.5	36.7
	Average	14.8	38.7	40.8	40.9
	LSD (p=0.05)	4.3	2.7	1.8	6.7
	LSD (p=0.01)	5.5	3.5	2.3	8.8
	Coefficient of Variation	20.3	4.9	3.1	11.7

Table A7. Medium and large seeded dry bean (*Phaseolus vulgaris* L.) genotypes grown conventionally in Gratiot County in 2007. Planted 12 June 2007.

Entry	Name	Yield CWT/ACRE	100 Seed Wt	Days to Flower	Height
S00809	Sedona	20.2	33.7	43.2	42.3
R98026	Merlot	22.2	36.6	44.2	45.0
P06131	P02646/P02630	24.5	37.2	42.1	41.0
I99117	Buster	22.3	32.4	40.8	39.5
I84002	Othello	23.5	36.5	39.1	40.3
P04205	Santa Fe	20.7	38.3	41.4	39.3
I05101	USDK-CBB15	14.8	43.0	40.9	36.5
G93414	Matterhorn	21.6	31.8	43.0	36.5
K03601	Chinook Select	12.7	44.1	40.0	40.3
C99833	Capri	19.2	47.3	36.7	39.0
K05604	K00604/X02151	13.7	42.5	39.9	46.3
K74002	Montcalm	12.1	43.2	40.6	44.8
K90902	Beluga	8.1	45.4	41.5	39.3
K90101	Red Hawk	11.3	39.8	40.1	45.3
K03240	Red Hawk*2/Negro San Luis-140	8.9	38.5	39.1	43.8
I90013	CELRK	6.9	36.3	36.5	38.0
	Average	16.4	39.2	40.6	41.0
	LSD (p=0.05)	4.7	3.0	1.5	4.8
	LSD (p=0.01)	6.1	3.9	1.9	6.2
	Coefficient of Variation	20.3	5.5	2.6	8.2

Table A8. Small seeded dry bean (*Phaseolus vulgaris* L.) genotypes grown organically at Kellogg Biological Station in 2008. Planted 1 June 2008.

Entry	Names	Yield CWT/ACRE	100 Seed Wt	Days to Flower	Days to Maturity	Lodging	Height	Des score
I07148	TARS SR05	16.1	24.1	42.2	94.8	1.0	47.9	4.0
B95556	Jaguar	15.7	19.2	41.7	84.7	1.0	27.3	4.0
N05324	N00838/N00809//N00792	13.7	17.5	38.2	87.9	1.0	30.2	4.0
I81066	T39	13.5	18.7	38.6	89.7	1.0	36.1	3.5
B04554	Zorro	13.2	20.4	42.3	90.1	1.0	38.9	4.5
I81010	Bunsi	12.9	18.8	39.0	95.7	2.5	36.4	2.5
I01892	115-11M	12.6	20.2	41.5	90.0	2.0	47.3	4.5
N61001	Michelite	12.5	16.2	37.2	87.6	3.0	44.3	3.5
N05311	N03611/B01749	12.4	16.7	41.9	89.7	1.0	36.4	5.0
B00101	Condor	12.2	18.6	39.1	85.0	1.0	33.6	3.5
B05055	34-27/Jag*2/SEL1308//HR45/Kaboon	12.1	17.2	42.9	87.1	1.0	33.2	3.0
N97774	Seahawk	12.0	19.6	40.4	85.3	1.5	40.5	3.0
I07112	R99	11.8	17.3	37.3	105.6	2.0	46.8	3.0
I92002	Vista	11.5	17.6	40.2	90.2	1.0	38.6	3.5
B04431	I01892/Jaguar	11.4	19.9	43.6	95.5	1.0	37.1	4.0
B05039	35-5/Jag*2/SEL1308//HR45/Kaboon	9.1	20.9	42.5	97.1	1.0	30.5	2.5
	Average	12.7	18.9	40.5	91.0	1.4	37.8	3.6
	LSD (p=0.05)	3.8	1.4	1.5	4.3	0.2	4.6	0.6
	LSD (p=0.01)	4.9	1.8	1.9	5.5	0.3	6.0	0.8
	Coefficient of Variation	21.2	5.1	2.6	3.3	10.8	8.7	11.5

Table A9. Medium and large seeded dry bean (*Phaseolus vulgaris* L.) gentoypes grown organically at Kellogg Biological Station in 2008. Planted 1 June 2008.

Entry	Name	Yield CWT/ACRE	100 Seed Wt	Days to Flower	Days to Maturity	Lodging	Height	Des score
I99117	Buster	15.4	35.7	35.2	80.0	2.0	54.5	3.0
P04205	Santa Fe	14.9	38.0	35.9	80.0	1.5	49.5	5.0
S00809	Sedona	14.3	33.0	35.1	80.0	2.0	59.8	4.5
K90101	Red Hawk	14.2	44.9	32.7	80.0	1.5	29.6	3.5
P06131	P02646/P02630	13.9	39.3	33.3	80.0	1.0	45.0	5.0
I84002	Othello	13.7	38.0	32.1	80.0	2.5	55.9	2.5
I90013	CELRK	13.1	52.9	32.0	80.0	1.0	35.0	2.5
G93414	Matterhorn	12.9	34.7	35.4	80.0	2.0	55.0	5.0
R98026	Merlot	12.5	36.3	37.4	80.0	2.5	50.4	3.5
C99833	Capri	11.9	51.7	31.6	80.0	1.5	35.0	4.0
K03601	Chinook Select	11.6	45.8	34.1	80.0	1.5	35.4	4.0
K74002	Montcalm	10.8	47.3	34.0	80.0	1.0	49.3	4.0
I05101	USDK CBB15	10.7	42.5	34.3	80.0	2.0	34.3	4.0
K90902	Beluga	10.4	43.6	37.0	82.5	1.5	35.9	3.0
K03204	Redhawk/Negro San Luis-140	9.9	43.0	35	80.0	1.5	45.2	3.5
K05604	K00604/X02151	8.3	49.7	34.2	85.0	2.0	35.4	4.0
	Average	12.4	42.3	34.3	80.5	1.7	44.1	3.8
	LSD (p=0.05)	3.0	3.9	1.2	1.6	0.4	5.6	0.6
	LSD (p=0.01)	3.9	5.0	1.6	2.0	0.5	7.3	0.8
	Coefficient of Variation	17.1	6.5	2.5	1.4	17.4	9.1	11.7

Table A10. Small seeded dry bean (*Phaseolus vulgaris* L.) genotypes grown conventionally at Kellogg Biological Station in 2008. Planted 1 June 2008.

Entry	Name	Yield CWT/ACRE	100 Seed Wt	Days to Flower	Days to Maturity	Lodging	Height	Des score
N05324	N00838/N00809//N00792	21.6	20.0	38.4	100.2	1.1	43.1	4.4
B00101	Condor	20.9	20.8	40.5	92.8	1.6	37.3	4.7
I01892	115-11M	20.8	23.9	37.7	97.7	2.1	42.5	4.6
B04554	Zorro	20.3	22.7	40.5	90.2	1.6	36.6	5.1
I81010	Bunsi	20.2	21.0	37.8	97.5	3.1	30.0	2.5
I92002	Vista	20.1	21.3	38.5	96.7	2.4	46.1	4.0
B04431	I01892/Jaguar	19.6	22.4	40.8	99.9	1.3	47.2	4.2
B95556	Jaguar	19.5	19.8	40.6	95.2	1.1	37.4	4.9
B05039	35-5/Jag*2/SEL1308//HR45/Kaboon	18.3	23.0	38.4	103.4	2.3	39.9	4.5
N61001	Michelite	17.8	20.5	39.2	91.4	3.5	25.5	3.4
N05311	N03611/B01749	17.7	22.0	39.5	92.8	0.9	37.4	5.6
N97774	Seahawk	17.6	27.3	41.2	90.0	2.6	37.6	3.5
I81066	SEL-BTS, T39	16.4	22.4	40.1	92.0	1.7	41.6	4.7
B05055	34-27/Jag*2/SEL1308//HR45/Kaboon	14.6	20.6	41.3	97.7	1.1	37.8	3.4
I07148	TARS SR05	10.9	24.5	44.8	95.3	2.5	40.0	3.6
I07112	R99	10.2	17.0	38.6	106.1	3.6	44.0	2.2
	Average	17.9	21.8	39.9	96.2	2.0	39.0	4.1
	LSD (p=0.05)	4.8	2.2	1.4	2.5	0.7	3.3	0.7
	LSD (p=0.01)	6.2	2.9	1.9	3.3	0.9	4.3	1.0
	Coefficient of Variation	18.8	7.2	2.6	1.9	23.3	6.0	12.8

Table A11. Medium and large seeded dry bean (*Phaseolus vulgaris* L.) genotypes grown conventionally at Kellogg Biological Station in 2008. Planted 1 June 2008.

Entry	Name	Yield CWT/ACRE	100 Seed Wt	Days to Flower	Days to Maturity	Lodging	Height	Des score
G93414	Matterhorn	20.2	37.0	33.0	82.5	1.0	47.5	4.0
C99833	Capri	19.9	57.7	32.4	80.0	1.0	32.5	3.5
I90013	CELRK	19.8	60.7	30.7	80.0	1.0	32.5	3.0
I84002	Othello	18.9	42.0	34.2	80.0	3.0	40.0	2.9
S00809	Sedona	18.8	40.7	36.5	85.0	2.5	60.0	3.0
I99117	Buster	18.6	41.8	36.5	82.5	3.0	55.0	3.0
P06131	P02646/P02630	17.4	45.2	36.7	80.0	1.0	40.0	5.0
P04205	Santa Fe	16.3	45.2	34.0	82.5	1.5	35.0	4.0
K03601	Chinook Select	16.2	52.6	35.7	82.5	1.0	37.5	4.1
K05604	K00604/X02151	15.5	56.2	35.4	82.5	1.5	45.0	4.0
K90101	Redhawk	14.9	53.7	35.1	82.5	1.5	35.0	3.5
K90902	Beluga	14.8	50.4	34.5	82.5	1.5	32.5	3.5
R98026	Merlot	14.3	43.6	35.3	80.0	1.0	50.0	5.0
K03240	Redhawk*2/Negro San Luis-140	14.0	52.8	33.8	80.0	2.0	35.0	3.0
K74002	Montcalm	13.5	53.7	32.6	82.5	1.0	47.5	3.0
I05101	USDK-CBB15	12.5	53.9	37.1	82.5	1.0	46.5	3.0
	Average	16.6	49.2	34.6	81.7	1.5	42.0	3.6
	LSD (p=0.05)	3.4	2.8	1.1	1.5	0.3	4.1	0.5
	LSD (p=0.01)	4.4	3.6	1.4	1.9	0.4	5.3	0.6
	Coefficient of Variation	14.5	4.0	2.3	1.3	15.3	6.9	8.9

Table A12. Small seeded dry bean (*Phaseolus vulgaris* L.) genotypes grown organically in Gratiot County in 2008. Planted 12 June 2008.

Entry	Name	Yield CWT/ACRE	100 Seed Wt	Days to Flower	Days to Maturity	Lodging	Height	Des score
B95556	Jaguar	10.4	21.4	41.7	84.7	1.0	27.3	4.0
I01892	115-11M	10.0	23.7	41.5	90.0	2.0	47.3	4.5
I07148	TARS SR05	9.5	26.2	42.2	94.8	1.0	47.9	4.0
N97774	Seahawk	9.4	24.6	40.4	85.3	1.5	40.5	3.0
B04554	Zorro	8.7	23.7	42.3	90.1	1.0	38.9	4.5
N05311	N03601/B01749	8.4	19.3	41.9	89.7	1.0	36.4	5.0
B00101	Condor	8.2	23.0	39.1	85.0	1.0	33.6	3.5
I81066	T39	7.8	23.1	38.6	89.7	1.0	36.1	3.5
I92002	Vista	7.6	25.4	40.2	90.2	1.0	38.6	3.5
B05055	34-27/Jag*2/SEL1308//HR45/Kaboon	7.1	21.0	42.9	87.1	1.0	33.2	3.0
N61001	Michelite	6.8	20.4	37.2	87.6	3.0	44.3	3.5
N05324	N00838/N00809//N00792	6.7	23.2	38.2	87.9	1.0	30.2	4.0
B04431	I01892/Jaguar	6.1	24.4	43.6	95.5	1.0	37.1	4.0
B05039	35-5/Jag*2/SEL1308//HR45/Kaboon	5.9	22.3	42.5	97.1	1.0	30.5	2.5
I81010	Bunsi	3.8	20.8	39.0	95.7	2.5	36.4	2.5
I07112	R99	1.6	18.6	37.3	105.6	2.0	46.8	3.0
	Average	7.4	22.6	40.5	91.0	1.4	37.8	3.6
	LSD (p=0.05)	2.6	0.9	1.5	4.3	0.2	4.6	0.6
	LSD (p=0.01)	3.4	1.2	1.9	5.5	0.3	6.0	0.8
	Coefficient of Variation	25.1	2.9	2.6	3.3	10.8	8.7	11.5

Table A13. Medium and large seeded dry bean (*Phaseolus vulgaris* L.) genotypes grown organically in Gratiot County in 2008. Planted 12 June 2008.

Entry	Name	Yield CWT/ACRE	100 Seed Wt	Days to Flower	Days to Maturity	Lodging	Height	Des score
R98026	Merlot	19.0	41.6	37.4	80.0	2.5	50.4	3.5
S00809	Sedona	16.0	41.6	35.1	80.0	2.0	59.8	4.5
P06131	P02646/P02630	14.7	48.0	33.3	80.0	1.0	45.0	5.0
I84002	Othello	13.6	41.6	32.1	80.0	2.5	55.9	2.5
P04205	Santa Fe	13.4	46.9	35.9	80.0	1.5	49.5	5.0
G93414	Matterhorn	12.0	39.3	35.4	80.0	2.0	55.0	5.0
C99833	Capri	11.3	51.4	31.6	80.0	1.5	35.0	4.0
I99117	Buster	10.5	41.3	35.2	80.0	2.0	54.5	3.0
I05101	USDK-CBB15	8.4	48.4	34.3	80.0	2.0	34.3	4.0
K74002	Montcalm	8.0	47.5	34.0	80.0	1.0	49.3	4.0
K03601	Chinook Select	7.9	48.6	34.1	80.0	1.5	35.4	4.0
I90013	CELRK	7.9	52.0	32.0	80.0	1.0	35.0	2.5
K03240	Redhawk*2/Negro San Luis-140	7.0	44.9	35.0	80.0	1.5	45.2	3.5
K90902	Beluga	6.9	51.3	37.0	82.5	1.5	35.9	3.0
K05604	K00604/X02151	6.9	49.2	34.2	85.0	2.0	35.4	4.0
K90101	Redhawk	6.1	44.3	32.7	80.0	1.5	29.6	3.5
	Average	10.6	46.1	34.3	80.5	1.7	44.1	3.8
	LSD (p=0.05)	3.2	2.8	1.2	1.6	0.4	5.6	0.6
	LSD (p=0.01)	4.1	3.6	1.6	2.0	0.5	7.3	0.8
	Coefficient of Variation	21.3	4.2	2.5	1.4	17.4	9.1	11.7

Table 14. Small seeded dry bean (*Phaseolus vulgaris* L.) genotypes grown conventionally in Gratiot County in 2008. Planted 12 June 2008.

Entry	Name	Yield CWT/ACRE	100 Seed Wt	Days to Flower	Days to Maturity	Lodging	Height	Des score
B04554	Zorro	19.4	24.3	40.5	90.2	1.6	36.6	5.1
I01892	115-11M	18.8	24.9	37.7	97.7	2.1	42.5	4.6
I92002	Vista	18.6	24.5	38.5	96.7	2.4	46.1	4.0
B04431	I01892/Jaguar	18.3	26.8	40.8	99.9	1.3	47.2	4.2
B95556	Jaguar	18.3	22.6	40.6	95.2	1.1	37.4	4.9
I81066	T39	16.9	23.8	40.1	92.0	1.7	41.6	4.7
N97774	Seahawk	16.5	26.2	41.2	90.0	2.6	37.6	3.5
I07148	TARS SR05	16.4	27.4	44.8	95.3	2.5	40.0	3.6
B00101	Condor	16.4	24.2	40.5	92.8	1.6	37.3	4.7
N05324	N00838/N00809//N00792	16.3	23.7	38.4	100.2	1.1	43.1	4.4
N05311	N03611/B01749	16.3	21.8	39.5	92.8	0.9	37.4	5.6
B05055	34-27/Jag*2/SEL1308//HR45/Kaboon	15.2	22.0	41.3	97.7	1.1	37.8	3.4
B05039	35-5/Jag*2?SEL1308//HR45/Kaboon	13.8	25.7	38.4	103.4	2.3	39.9	4.5
N61001	Michelite	11.9	21.0	39.2	91.4	3.5	25.5	3.4
I81010	Bunsi	9.1	21.4	37.8	97.5	3.1	30.0	2.5
I07112	R99	7.8	18.9	38.6	106.1	3.6	44.0	2.2
	Average	15.6	23.7	39.9	96.2	2.0	39.0	4.1
	LSD (p=0.05)	3.7	1.2	1.4	2.5	0.7	3.3	0.7
	LSD (p=0.01)	4.8	1.5	1.9	3.3	0.9	4.3	1.0
	Coefficient of Variation	16.7	3.5	2.6	1.9	23.3	6.0	12.8

Table A15. Medium and large seeded dry bean (*Phaseolus vulgaris* L.) genotypes grown conventionally in Gratiot County in 2008. Planted 12 June 2008.

Entry	Name	Yield CWT/ACRE	100 Seed Wt	Days to Flower	Days to Maturity	Lodging	Height	Des score
S00809	Sedona	22.4	42.2	36.5	85.0	2.5	60.0	3.0
P06131	P02646/P02630	20.0	49.6	36.7	80.0	1.0	40.0	5.0
R98026	Merlot	19.1	42.7	35.3	80.0	1.0	50.0	5.0
I99117	Buster	17.9	43.7	36.5	82.5	3.0	55.0	3.0
P04205	Santa Fe	15.2	48.5	34.0	82.5	1.5	35.0	4.0
G93414	Matterhorn	14.1	40.0	33.0	82.5	1.0	47.5	4.0
I84002	Othello	12.6	40.9	34.2	80.0	3.0	40.0	2.9
K03601	Chinook Select	11.3	50.1	35.7	82.5	1.0	37.5	4.1
K74002	Montcalm	10.5	49.2	32.6	82.5	1.0	47.5	3.0
C99833	Capri	10.0	52.7	32.4	80.0	1.0	32.5	3.5
I05101	USDK-CBB15	9.9	50.0	37.1	82.5	1.0	46.5	3.0
I90013	CELRK	9.6	51.0	30.7	80.0	1.0	32.5	3.0
K90101	Redhawk	9.2	47.2	35.1	82.5	1.5	35.0	3.5
K03240	Redhawk*2/Negro San Luis-140	8.8	46.9	33.8	80.0	2.0	35.0	3.0
K05604	K00604/X02151	8.5	51.3	35.4	82.5	1.5	45.0	4.0
K90902	Beluga	7.9	49.6	34.5	82.5	1.5	32.5	3.5
	Average	12.9	47.2	34.6	81.7	1.5	42.0	3.6
	LSD (p=0.05)	2.6	2.8	1.1	1.5	0.3	4.1	0.5
	LSD (p=0.01)	3.4	3.6	1.4	1.9	0.4	5.3	0.6
	Coefficient of Variation	14.1	4.1	2.3	1.3	15.3	6.9	8.9

Table A16. Small seeded dry bean (*Phaseolus vulgaris* L.) genotypes grown organically at Kellogg Biological Station in 2009. Planted 16 June 2009.

Entry	Name	Yield CWT/ACRE	100 Seed Wt	Days to Flower	Stand Count	Days to Maturity	Lodging	Height	Des score
B04431	I01892/Jaguar	35.1	24.9	49.5	24.0	99.8	2.0	55.3	4.0
N05324	N00838/N00809//N00792	33.5	22.0	44.0	24.5	97.9	1.5	54.0	5.0
N05311	N03601/B01749	33.2	23.4	46.0	25.3	98.9	2.1	52.0	5.0
B04554	Zorro	33.0	23.3	48.5	25.5	95.3	1.0	55.3	6.0
I01892	115-11M	30.8	23.2	48.5	23.0	98.9	2.5	54.3	4.0
I07148	TARS SR05	30.0	26.3	48.0	19.0	98.8	3.0	48.0	2.5
B00101	Condor	29.8	23.0	47.5	29.8	97.7	3.0	43.0	3.0
B05055	34-27/Jag*2/SEL1308//HR45/Kaboon	26.6	21.9	49.5	29.0	99.7	1.5	55.9	4.0
I07112	R99	26.1	19.4	45.0	17.5	95.2	3.4	47.9	3.0
B95556	Jaguar	25.4	20.9	47.0	30.0	95.1	1.4	60.4	5.5
N97774	Seahawk	25.0	24.8	44.5	23.0	97.6	3.4	58.7	1.5
B05039	35-5/Jag*2/SEL1308//HR45/Kaboon	24.3	24.8	48.0	26.0	99.6	2.0	46.8	4.0
I81010	Bunsi	23.1	22.0	44.0	22.0	99.7	3.5	50.7	2.5
I81066	T39	22.9	23.5	46.0	25.5	99.8	3.1	47.9	2.0
N61001	Michelite	18.1	20.0	42.0	22.0	89.4	3.1	47.6	2.0
I92002	Vista	16.8	20.6	43.5	25.8	89.6	4.0	38.1	1.5
	Average	27.1	22.8	46.3	24.5	97.1	2.5	51.0	3.5
	LSD (p=0.05)	7.2	1.6	2.5	4.8	1.9	0.6	5.0	0.8
	LSD (p=0.01)	9.4	2.1	3.2	6.2	2.4	0.8	6.5	1.0
	Coefficient of Variation	18.8	5.0	3.8	13.8	1.4	17.1	7.0	15.5

Table A17. Medium and large seeded dry bean (*Phaseolus vulgaris* L.) genotypes grown organically at Kellogg Biological Station in 2009. Planted 16 June 2009.

Entry	Name	Yield CWT/ACRE	100 Seed Wt	Days to Flower	Stand Count	Days to Maturity	Lodging	Height	Des score
I99117	Buster	32.6	41.7	41.9	16.0	87.5	3.3	37.5	3.1
R9802	Merlot	28.5	39.0	43.6	18.0	90.0	1.4	61.0	4.6
P06131	P02646/P02630	28.0	44.4	42.0	16.8	90.0	1.5	49.5	4.5
K03601	Chinook Select	26.8	48.6	39.1	12.0	94.5	2.6	40.0	3.0
S00809	Sedona	26.5	40.8	43.5	16.3	87.5	2.4	57.0	3.6
G93414	Matterhorn	25.9	34.7	40.5	25.0	82.5	1.5	41.0	4.5
P04205	Santa Fe	25.9	44.8	40.0	14.8	87.5	2.4	49.5	4.0
C99833	Capri	22.7	54.5	39.0	14.3	88.5	2.6	31.5	2.4
I84002	Othello	22.5	39.0	39.2	19.3	80.0	2.6	36.5	3.0
I05101	USDK-CBB15	21.5	51.1	40.3	14.8	97.0	1.5	35.0	3.0
K03240	Redhawk*2/Negro San Luis-140	21.4	48.6	35.0	16.0	90.0	2.6	39.0	3.0
K74002	Montcalm	20.7	50.8	38.8	14.0	96.5	2.1	33.5	3.9
K05604	K00604/X02151	20.5	46.7	41.0	14.8	89.0	2.1	36.5	3.0
K90101	Redhawk	19.3	46.7	39.4	18.0	91.5	2.6	37.0	3.0
K90902	Beluga	19.0	54.5	39.2	15.5	92.5	2.1	33.0	4.5
I90013	CELRK	12.1	58.7	39.1	15.8	87.5	1.6	29.5	2.5
	Average	23.4	46.5	40.1	16.3	89.5	2.2	40.4	3.5
	LSD (p=0.05)	4.5	3.1	1.5	4.0	2.4	0.4	3.9	0.6
	LSD (p=0.01)	5.9	4.0	1.9	5.2	3.1	0.6	5.1	0.8
	Coefficient of Variation	13.7	4.7	2.6	17.2	1.9	14.2	6.9	12.7

Table A18. Small Seeded dry bean (*Phaseolus vulgaris* L.) genotypes grown conventionally at Kellogg Biological Station in 2009. Planted 16 June 2009.

Entry	Name	Yield CWT/ACRE	100 Seed Wt	Days to Flower	Stand Count	Days to Maturity	Lodging	Height	Des score
N0532	N00838/N00809//N00792	42.0	24.0	47.5	25.8	100.0	1.4	54.0	4.5
B04554	Zorro	40.8	25.5	49.5	23.5	100.0	1.5	55.0	4.5
B00101	Condor	38.8	23.8	48.5	27.5	102.5	2.0	48.5	4.5
B04431	I01892/Jaguar	37.8	25.7	50.5	17.0	102.5	0.9	52.0	4.5
I07112	R99	36.2	23.5	45.0	19.5	102.5	3.9	47.5	3.0
B95556	Jaguar	33.7	22.9	49.0	31.8	100.0	1.6	44.0	4.5
I01892	115-1M	32.9	25.6	49.0	19.3	105.0	3.1	48.5	3.5
B05039	35-5/Jag*2/SEL1308//HR45/Kaboon	30.9	26.1	52.0	26.8	105.0	1.4	44.5	4.5
I81010	Bunsi	29.6	23.7	42.0	18.5	105.0	4.0	41.5	2.0
I07148	TARS SR05	29.2	26.2	48.5	20.0	102.5	3.1	54.5	2.5
N0531	N03611/B01749	27.9	26.3	44.5	22.8	105.0	2.7	57.5	4.0
N9777	Seahawk	27.8	26.0	43.5	23.0	102.5	3.5	47.0	2.5
B05055	34-	26.8	23.4	52.0	28.8	105.0	1.6	44.0	3.5
I81066	T39	26.3	24.2	50.0	21.3	107.7	3.5	50.5	3.0
I92002	Vista	19.8	24.5	48.5	21.5	108.0	2.3	54.5	2.5
N6100	Michelite	17.1	22.3	46.5	15.3	110.0	4.5	40.0	2.0
	Average	31.1	24.6	47.9	22.6	103.9	2.6	49.0	3.5
	LSD (p=0.05)	10.5	2.0	7.4	7.4	4.2	0.5	3.8	0.6
	LSD (p=0.01)	13.6	2.6	9.6	9.6	5.5	0.6	4.9	0.8
	Coefficient of Variation	23.9	5.8	2.2	23.0	2.9	13.0	5.5	13.2

Table A19. Medium and large seeded dry bean (*Phaseolus vulgaris* L.) genotypes grown conventionally at Kellogg Biological Station in 2009. Planted 6 June 2009.

Entry	Name	Yield CWT/ACRE	100 Seed Wt	Days to Flower	Stand Count	Days to Maturity	Lodging	Height	Des score
I99117	Buster	43.4	44.9	43.0	14.8	97.5	3.0	54.0	2.1
R98026	Merlot	41.0	42.1	44.5	20.3	95.0	3.0	55.4	3.1
S00809	Sedona	40.8	47.4	43.5	16.1	97.5	2.5	52.6	3.5
K03601	Chinook Select	35.9	61.2	39.0	11.3	95.0	2.0	41.1	4.5
P04205	Santa Fe	35.4	50.3	43.5	14.2	92.5	3.5	49.8	3.5
G93414	Matterhorn	35.1	39.4	40.5	23.5	95.0	1.5	54.1	5.0
K03240	Redhawk*2/Negro San Luis-140	33.5	56.8	40.5	15.0	95.0	2.5	38.9	2.6
K90902	Beluga	33.4	61.8	39.5	19.0	97.5	2.5	41.9	4.0
P06131	P02646/P02630	33.3	51.4	40.5	16.8	95.0	2.0	63.6	4.5
K74002	Montcalm	31.3	59.5	40.5	11.4	95.0	3.0	44.9	3.5
I84002	Othello	31.3	44.2	40.5	22.2	92.5	3.0	40.0	2.0
I05101	USDK-CBB-115	29.1	58.9	39.5	16.5	92.5	1.5	38.1	3.1
K90101	Redhawk	29.0	60.8	49.0	16.8	95.0	2.0	38.1	4.1
C99833	Capri	26.0	65.3	40.0	17.6	90.0	2.0	35.9	2.8
K05604	K00604/X02151	21.8	61.2	40.5	13.3	92.5	2.0	36.1	3.4
I90013	CELRK	20.1	72.3	39.0	14.4	95.0	1.5	39.9	2.9
	Average	32.5	54.8	40.8	16.5	94.5	2.3	45.3	3.4
	LSD (p=0.05)	8.8	4.1	0.7	4.9	3.1	0.5	3.5	0.6
	LSD (p=0.01)	11.5	5.4	0.9	6.3	4.0	0.6	4.5	0.8
	Coefficient of Variation	19.2	5.3	1.1	20.9	2.3	14.1	5.4	13.1

Table A20. Small seeded dry bean (*Phaseolus vulgaris* L.) genotypes grown organically in Tuscola County in 2009. Planted 16 June 2009.

Entry	Name	Yield CWT/ACRE	100 Seed Wt	Days to Flower	Stand Count
B04554	Zorro	31.9	22.0	42.0	28.3
I07148	TARS SR05	30.6	23.4	42.5	17.8
I92002	Vista	30.1	20.2	45.0	26.3
N05311	N03601/B01749	30.0	19.1	47.0	27.3
B05055	34-27/Jag*2/SEL1308//HR45/Kaboon	28.6	20.5	42.5	29.3
I01892	115-11M	27.4	23.1	44.5	22.8
B00101	Condor	27.1	21.9	45.0	28.3
B05039	35-5/Jag*2/SEL1308//HR45/Kaboon	26.5	22.7	44.0	29.8
I81066	T39	26.4	21.9	44.5	22.3
N97774	Seahawk	25.8	22.4	43.0	18.3
N05324	N00838/N00809//N00792	24.0	19.3	43.5	19.3
N61001	Michelite	23.6	18.4	43.0	23.8
B95556	Jaguar	23.5	20.0	43.0	31.8
B04431	I01892/Jaguar	23.3	23.6	46.5	26.0
I81010	Bunsi	22.2	20.5	44.5	20.3
I07112	R99	21.7	19.8	46.0	11.8
	Average	26.4	21.2	44.2	23.9
	LSD (p=0.05)	5.0	1.6	1.8	6.7
	LSD (p=0.01)	6.5	2.1	2.9	8.7
	Coefficient of Variation	13.3	5.5	2.9	19.7

Table A21. Medium and large seeded dry bean (*Phaseolus vulgaris* L.) genotypes grown organically in Tuscola County in 2009. Planted 16 June 2009.

Entry	Name	Yield CWT/ACRE	100 Seed Wt	Days to Flower	Stand count
R98026	Merlot	29.8	37.9	42.0	21.1
P06131	P02646/P02630	29.6	41.5	42.3	13.8
P04205	Santa Fe	25.2	35.8	42.6	18.4
S00809	Sedona	24.5	38.5	42.0	19.5
G93414	Matterhorn	24.1	31.8	40.4	21.2
K90101	Red Hawk	22.8	44.7	40.0	14.3
C99833	Capri	22.1	56.5	37.6	15.5
I99117	Buster	20.9	35.1	41.3	13.9
I84002	Othello	20.4	33.7	41.6	17.7
K74002	Montcalm	17.4	47.5	39.0	11.0
I05101	USDK CBB-115	17.1	50.0	39.2	15.7
K03240	Redhawk 2*/Negro San Luis 140	17.0	44.1	41.0	22.0
K03601	Chonook Select	15.9	45.7	39.6	13.4
K05604	K00604/X02151	15.8	48.0	39.1	15.0
K90902	Beluga	13.4	46.2	39.1	17.1
I90013	CELRK	12.3	53.7	38.1	17.4
	Average	20.5	43.2	40.3	16.7
	LSD (p=0.05)	7.7	3.0	0.8	4.1
	LSD (p=0.01)	10.0	3.9	1.0	5.4
	Coefficient of Variation	26.4	4.9	1.4	17.6

Appendix B

Appendix B1. Average day to harvest of 34 dry bean (*Phaseolus vulgaris* L.) genotypes evaluated for nitrogen fixation ability under greenhouse conditions in East Lansing, MI in 2008 and 2009.

Genotype	Average days to harvest
Red Hawk	37.8
Sanilac	39.0
Chinook Select	39.9
USDK-CBB-15	40.3
K03240	40.3
CELRK	40.7
Montcalm	40.8
Beluga	40.8
Capri	40.9
K05604	42.3
Bunsi	42.7
R99	46.4
Seahawk	47.8
Othello	48.1
T-39	48.3
Sedona	48.9
Merlot	50.5
Matterhorn	50.7
Santa Fe	50.7
Buster	51.0
N05324	52.6
115-M	52.8
TARS-SR05	53.0
P06131	53.1
Jaguar	53.3
Zorro	53.4
Michelite	53.7
N05311	54.4
Condor	54.9
B04431	56.3
Puebla 152	56.4
Vista	56.6
B05055	58.6
B05039	58.7
Mean	48.7

Appendix C1. Average Percent seed N of 32 dry bean (*Phaseolus vulgaris* L.) genotypes each year grown under organic and conventional production systems at Kellogg Biological Station in 2007 to 2009.

	Organic			Conventional		
	2007	2008	2009	2007	2008	2009
Merlot	2.9[1]	2.9	2.8	3.5	3.2	3.6
Sedona	3.4	2.3	2.9	3.7	3.1	3.7
T39	3.6	3.8	3.8	3.8	3.9	3.9
N05311	3.3	3.9	3.8	4.2	3.6	4.2
Zorro	3.6	3.5	3.6	4.2	3.5	3.8
P06131	3.2	2.4	3.3	3.9	3.4	4.1
115-11M	3.4	3.4	3.6	3.9	3.9	3.4
Condor	2.6	2.9	3.7	3.9	3.9	3.9
Santa Fe	2.9	2.8	3.3	4.2	3.3	3.9
TARS-SR05		3.8	3.9		3.9	3.9
Jaguar	3.2	3.5	3.6	4.2	3.8	3.9
B04431	3.4	3.2	3.4	3.5	3.8	3.7
Buster	2.8	2.7	2.8	3.7	3.6	3.6
B05055	3.7	2.9	4.1	4.5	4.2	4.4
Matterhorn	3.3	3.5	3.1	3.8	3.8	3.9
N05324	2.2	3.2	3.8	3.9	4.1	3.9
USDK-CBB-15	3.6	2.9	3.3	4.6	4.4	3.9
Othello	3.1	3.2	2.7	3.6	3.5	3.4
Seahawk	3.5	3.4	3.6	3.5	3.6	3.8
B05039	3.7	3.3	3.8	3.7	4.3	4.4
Vista	3.5	3.7	3.6	3.9	4.1	4.4
Capri	3.4	3.1	3.2	3.6	3.9	3.8
Michelite	3.6	3.6	3.5	3.9	3.9	3.9
Bunsi	3.7	3.3	3.7	4.3	4.2	3.9
Red Hawk	4.1	3.9	3.2	4.6	4.4	3.8
Montcalm	3.9	3.7	3.4	4.4	4.4	3.9
Chinook Select	3.6	3.5	3.1	3.9	3.2	3.8
K03240	3.9	3.4	3.6	4.5	3.3	3.7
Beluga	3.8	4.2	3.4	4.4	4.3	3.9
K05604	3.9	3.3	3.3	4.4	4.2	3.9
CELRK	3.8	3.4	3.2	4.2	3.6	3.8
R99	2.2	3.4	2.8	2.9	3.7	3.8
Mean	3.3	3.3	3.4	4.0	3.8	3.9

[1]Nitrogen values as determined by the Kjeldahl method.

Appendix D1. Average Percent seed protein of 32 dry bean (*Phaseolus vulgaris* L.) genotypes each year grown under organic and conventional production systems at Kellogg Biological Station in 2007 to 2009.

					Organic			
	2007	2008	2009	Mean	2007	2008	2009	Mean
Merlot	18.8[1]	18.1	17.5	18.1	21.9	20.0	22.5	21.5
Sedona	21.3	14.4	18.1	17.9	23.1	19.4	23.1	21.9
T39	22.5	23.8	23.8	23.3	23.8	25.0	25.0	24.6
N05311	20.6	25.0	23.8	23.1	26.3	22.5	26.3	25.0
Zorro	22.5	21.9	22.5	22.3	26.3	21.9	23.8	24.0
P06131	20.0	15.0	20.6	18.5	25.0	21.3	25.6	24.0
115-11M	21.3	21.3	22.5	21.7	25.0	24.4	21.3	23.5
Condor	16.3	18.1	23.1	19.2	24.4	25.0	24.4	24.6
Santa Fe	18.8	17.5	20.6	19.0	26.3	20.6	24.4	23.8
TARS-SR05		23.8	24.4	24.1		25.0	24.4	24.7
Jaguar	20.0	21.9	22.5	21.5	26.3	23.8	24.4	24.8
B04431	21.3	20.0	21.3	20.8	21.9	23.8	23.1	22.9
Buster	17.5	16.9	17.5	17.3	23.1	22.5	22.5	22.7
B05055	23.1	18.8	25.6	22.5	28.1	26.3	27.5	27.3
Matterhorn	20.6	21.9	19.4	20.6	23.8	23.8	25.0	24.2
N05324	13.8	20.0	23.8	19.2	24.4	25.6	24.4	24.8
USDK-CBB-15	22.5	18.1	20.6	20.4	28.8	27.5	24.4	26.9
Othello	19.4	20.0	16.9	18.8	22.5	21.9	21.3	21.9
Seahawk	21.9	21.3	22.5	21.9	21.9	22.5	23.8	22.7
B05039	23.1	20.6	23.8	22.5	23.1	26.9	27.5	25.8
Vista	21.9	23.1	22.5	22.5	24.4	25.6	27.5	25.8
Capri	21.3	19.4	20.0	20.2	22.5	24.4	23.8	23.5
Michelite	22.5	22.5	21.9	22.3	24.4	24.4	25.0	24.6
Bunsi	23.1	20.6	23.1	22.3	26.9	26.3	24.4	25.8
Red Hawk	25.6	24.4	20.0	23.3	28.8	27.5	23.8	26.7
Montcalm	24.4	23.1	21.3	22.9	27.5	27.5	24.4	26.5
Chinook Select	22.5	21.9	19.4	21.3	25.0	20.0	23.8	22.9
K03240	24.4	21.3	22.5	22.7	28.1	20.6	23.1	24.0
Beluga	23.8	26.3	21.3	23.8	27.5	26.9	25.0	26.5
K05604	24.4	20.6	20.6	21.9	27.5	26.3	24.4	26.0
CELRK	23.8	21.3	20.0	21.7	26.3	22.5	23.8	24.2
R99	13.8	21.3	17.5	17.5	18.8	23.1	23.8	21.9
Mean	20.5	20.7	21.3	21.1	24.2	23.9	24.3	24.4

[1]Percent Protein=%N x 6.25

Literature Cited

Alvaro, A., T. Vargas, and P.H. Graham. 1989. Cultivar and pH effects on competition for nodule sites between isolates of *Rhizobium* in bean. Plant and Soil 117:195-200.

Badgley, C, J. Moghtader, E. Quintero, E. Zakem, M.J. Chapell, K. Aviles-Vazquez, A. Samulon, and I. Perfecto. (2007) Organic agriculture and the global food supply. Renewable Agriculture and Food Systems 22:86-108.

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

Beebe, S., P.W. Skroch, J. Tohme, M.C. Dugue, F. Pedraza, and J. Nienheus. 2000. Structure of genetic diversity among common bean landraces of Middle American origin based on correspondence analysis of RAPD. Crop Science 40:264-273.

Beckie, H.J. and S.A. Brandt. 1997. Nitrogen contribution of field pea in annual cropping systems. 1. Nitrogen residual effect. Canadian Journal of Plant Science 77:311-322.

Bliss, F.A. 1993a. Breeding Common Bean for improved biological nitrogen fixation. Plant and Soil 152:71-79.

Bliss, F.A. 1993b. Utilizing the potential for increased nitrogen fixation in common bean. Plant and Soil 152:157-160.

Bliss, F.A. P.A.A. Pereira, R.S. Araujo, R.A. Henson, K.A. Kmiecik, J.R. McFerson, M.G. Teixeira and C.C. Da Silva. 1989. Registration of five high nitrogen fixing common bean germplasm lines. Crop Science 29:240-241

Buttery, B.R., S.J. Park, and D.J. Hume. 1992. Potential for increasing nitrogen fixation in grain legumes. Canadian Journal of Plant Science 72:323-349.

Carr, P.M., H.J. Kandel, P.M. Porter, R.D. Horsley, and S.F. Zwinger. 2006. Wheat cultivar performance on certified organic fields in Minnesota and North Dakota. Crop Science 46:1963-1971.

Cichy, K.A., M.W. Blair, C.H. Galeano Mendoza, S.S. Snapp, and J.D. Kelly. 2009. QTL Analysis of root architecture traits in low phosphorus tolerance in and Andean bean population. Crop Science 49:59-68.

Danso, S.K.A., G. Hardarson, and F. Zapata. 1993. Misconceptions and practical problems in the use o f15N soil enrichment techniques for estimating N2 fixation. Plant and Soil 152:25-52.

Da Silva, P.M., S.M. Tsai, and R. Bonetti. 1993. Response to inoculation and N fertilization for increased yield and biological nitrogen fixation of common bean (*Phaseolus vulgaris*. L). Plant and Soil 152:123-130.

De Jenson, C.E., J. Kurl, and J. Percich. 2004. Integration of *Bacillus-Rhizobium* inoculants and tillage to manage dry bean and soybean root rot. Phytophathology 94:s144-s144.

Deoliveira, L.A., and P.H. Graham. 1990. Speed of nodulation and competitive ability among strains of *Rhizobium leguminosarum* bv *phasioli*. Archives of Microbiology 153:311-315.

Dimitri, C. and L. Oberholtzer. 2009. Meeting market demand in the organic sector: Handler-supplier relationships in the face of tight supply. Renewable Agriculture and Food Systems 24:137-145.

Dubois, J.D., and R.H. Burris. 1986. Comparative study of N uptake and distribution in 3 lines of common bean (*Phaseolus vulgaris* L.) at early pod filling stage. Plant and Soil 93:79-86.

Elizondo-Barron, J., R.J. Pasini, D.W. Davis, D.D. Stuthman, and P.H. Graham. 1999. Response to selection for seed yield and nitrogen (N2) fixation in common bean (*Phaseolus vulgaris*. L). Field Crops Research 62:119-128.

George, T. and P.W. Singleton. 1992. Nitrogen assimilation traits and dinitrogen fixation in soybean and common bean. Agronomy Journal 84:1020-1028.

Gepts, P. 1988. A Middle American and an Andean common bean gene pool. In Genetic Resources of Phaseolus Beans. Kluwer Academic Publishers, Dordrecht, Netherlands. Pp. 375-390.

Gepts, P. 1998. Origin and evolution of common bean: past events and recent trends. Hortscience 33:1119-1135.

Graham, P.H. 1981. Some problems of nodulation and symbiotic nitrogen fixation in *Phaseolus vulgaris* L.-A Review. Field Crops Research 4:289-290.

Graham, P.H. and P. Ranalli. 1997. Common Bean (*Phaseolus vulgaris* L.). Field Crops Research 53:131-146.

Graham, P.H. and S.R. Temple. 1984. Selection for improved nitrogen fixation in Glycine max L Merr. and *Phaseolus vulgaris*. L. Plant and Soil 82:315-327.

Graham, P.H. and C.P. Vance. 2000. Nitrogen fixation in perspective: an overview of research and extension needs. Field Crops Research 65: 93-106.

Hardarson, G. and S.K.A. Danso. 1993. Methods for measuring biological nitrogen fixation in grain legumes. Plant and Soil 152:19-23.

Henson, R.A. 1993. Measurement of N2 fixation by common bean in Central Brazil as affected by different reference crops. Plant and Soil 152:53-58.

Herridge, D.F. and S.K.A. Danso. 1995. Enhancing crop legume N2 fixation through selection and breeding. Plant and Soil 174:51-82.

Ibarra-Perez, F.J., B Ehdaie, and J.G. Waines. 1997. Estimation of outcrossing rate in common bean. Crop Science 37:60-65.

Kami, J., V.B. Valesquez, D.G. Debouck, and P. Gepts. 1995. Identification of presumed ancestral DNA sequences of phaseolin in *Phaseolus vulgaris*. Proceedings of the National Academy of Science 92:1101-1104.

Kelly, J.D., Adams, M.W. and Varner, G.V. 1987. Yield stability of determinate and indeterminate dry bean cultivars. Theoretical and Applied Genetics. 74:516-521.

Kelly, J.D., G.V. Varner, and B. Long. 2010. Registration of 'Santa Fe' Pinto Bean. Journal of Plant Registrations 4:12-16.

Kelly, J.D., G.V. Varner, O'Boyle, P., and B. Long. 2009. Registration of 'Zorro' Black Bean. Journal of Plant Registrations 3:226-230.

Kelly, J.D., K.A. Schneider, and J.M. Kolkman. 1999. Breeding to improve yield. in Developments in Plant Breeding Vol. 7. Common bean improvement in the Twenty First Century. ed. S.P. Singh. Kluwer Acad. Pub. Dordrecht, The Netherlands.

Kipe-Nolt, J.A., C.M. Montealegre, and J. Tohme. 1992. Restriction of nodulation by the broad host range *Rhizobium tropici* strain CIAT899 in while accessions of *Phaseolus vulgaris* L. New Phytologist 120:489-494.

Kipe-Nolt, J.A. and K.E. Giller. 1993. A field evaluation using the 15N isotope dilution method of lines of *Phaseolus vulgaris* L. bred for increased nitrogen fixation. Plant and Soil 152:107-114.

Lindemann, W.C., and C.R. Glover. 2003. Nitrogen fixation by legumes. Guide A-129. Cooperative Extension Service, New Mexico State University, Las Cruces, NM USA.

Maiti, R.K. 1997. Chapter 6, BNF. Phaseolus Spp. Bean Science. Pp 249-280. Science Publishers, Inc. Enfield, NH, USA.

McFerson, J.R., F.A. Bliss and J.C. Rosas. 1982. Selection for enhanced nitrogen fixation in common beans *Phaseolus vulgaris* L. in: Graham and Harris BNF Technology for tropical agriculture, 39-44. Cali Colombia CIAT.

Meschini, E.P., F.A. Blanco, M.E. Zanetti, M.P. Beker, H. Kuster, A. Pueher, and O.M. Aguilar. 2008. Host genes involved in nodulation preference in common bean (*Phaseolus vulgaris* L.)-Rhizobium etli symbiosis revealed-by suppressor subtractive hybridization. Molecular Plant-Microbe Interactions 21:459-468.

Muller, S., P.A.A. Pereira, and P. Martin. 1993. Effect of different levels of mineral nitrogen on nodulation and N2 fixation of two cultivars of common bean (*Phaseolus vulgaris*. L). Plant and Soil 152:139-143.

Pacovsky, R.S. and G. Fuller. 1991. Nitrogen assimilation and partitioning in two nitrogen fixing cultivars of *Phaseolus vulgaris* L. Plant and Soil 132:139-148.

Park, S.J. and B.R. Buttery. 1989. Identification and characterization of common bean (*Phaseolus vulgaris* L.) lines well nodulated in the presence of high nitrate. Plant and Soil 119: 237-244.

Park, S.J and B.R. Buttery. 1994. Inheritance of non nodulation and ineffective nodulation mutants in common bean (*Phaseolus vulgaris*. L) Agriculture Canada 85:1-3.

Park, S.J. and B.R. Buttery 2006. Registration of ineffective nodulation mutant R69 and nonnodulation mutant R99 common bean genetic stocks. Crop Science 46:1415-1417.

Patterson, T.G. and T.A. La Rue. 1983. Nitrogen-fixation by soybeans –seasonal and cultivar effects, and comparison of estimates. Crop Science 23:488-492.

Pena-Cabriales, J.J. and J.Z. Castellanos. 1993. Effects of water stress on N2 fixation and grain yield of *Phaseolus vulgaris* L. Plant and Soil 152:151-155.

Pena-Cabriales, J.J., O.A. Grageda-Cabrera, V. Kola, and G. Hardarson. 1993. Time course of N2 fixation in common bean (*Phaseolus vulgaris* L). Plant and Soil 152:115-121.

Peoples, M.B., A.M. Bowman, R.R. Gault, D.F. Herridge, M.H. McCallum, K.M. McCormick, R.M. Norton, I.J. Rochester, G.J. Scammell and G.D. Schwenke. 2001. Factors regulating the contributions of fixed nitrogen by pasture and crop legumes to different farming systems of eastern Australia. Plant and Soil 228:29-41.

Peoples, M.B., H. Hauggaard-Nielsen, and E.S. Jensen. The potential environmental benefits and risks derived from Legumes in Rotations. 2009. In Nitrogen Fixation in Crop Production. D.W. Emerich and H.B. Krishnan, editors. American Society of Agronomy.

Peoples, M.B, D. F. Herridge and J. K. Ladha.1995. Biological Nitrogen Fixation:An efficient source of Nitrogen for sustainable agriculture production? Plant and Soil 174:3-28.

Pereira, P.A.A. and F.A. Bliss. 1987. Nitrogen fixation and plant growth of common bean (*Phaseolus vulgaris*. L) at different levels of phosphorus availability. Plant and Soil 104:79-84.

Pereira, P.A.A., R.H. Burris and F.A. Bliss. 1989. 15N-determined dinitrogen fixation potential of genetically diverse bean lines (*Phaseolus vulgaris* L). Plant and Soil 120:171-179

Perret, X. and W.J. Broughton. 1998. Rapid identification of *Rhizobium* strains by targeted PCR fingerprinting. Plant and Soil 204:21-34.

Piha, M.I. and D.N. Munns. 1987. Nitrogen fixation of common bean compared to other legumes. Agronomy Journal 79:690-696.

Ray, J.D., L.G. Heatherly, and F.B. Fritschi. 2006. Influence of large amounts of nitrogen on nonirrigated and irrigated soybean. Crop Science 46:52-60.

Rennie, R.J. 1983. Comparison of N balance and 15N isotope dilution to quantify N2 fixation in field grown legumes. Agronomy Journal 76:785-790.

Rosas, J.C., J.A. Castro, E.A. Robleto, and J. Handelsmann. 1998. A method for screening *Phaseolus vulgaris* L. germplasm for preferential nodulation with a selected R. etli strain. Plant and Soil 203:71-78.

Ross, P.J. and A.E. Martin. 1970. A rapid procedure for preparing gas samples for N-15 determinations. Analyst (London) 95:817-822.

St. Clair, D.A. and F.A. Bliss. 1991. Intrapopulation recombination for 15 N-determined dinitrogen fixation in common bean. Plant Breeding 106: 215-225.

St. Clair, D.A., D.J. Wolyn, J. Dubois, R.H. Burris, and F.A. Bliss. 1988. Field comparison of dinitrogen fixation determined with nitrogen -15-depleted and nitrogen-15-enriched ammonium sulfate in selected inbred backcross lines of common bean. Crop Science 28:773-778.

Serraj, R. and T.R. Sinclair. 1998. N2 fixation response to drought in common bean (*Phaseolus vulgaris* L.). Annals of Botany 82:229-234.

Shirtliffe, S.J., J.K. Vessey, B.R. Buttery, and S.J. Park. 1995. Comparison of growth and N accumulation of common bean (*Phaseolus vulgaris* L) cv. OAC Rico and its two nodulation mutants, R69 and R99. Canadian Journal of Plant Science 76:73-83.

Singh, S.P. 1982. A key for identification of different growth habits of *Phaseolus vulgaris* L. Annual Report of the Bean Improvement Cooperative. 25:92-94.

Singh, S.P. 2007. Drought resistance in the race Durango dry bean landraces and cultivars. Agronomy Journal 99:1219-1225.

Singh, S.P. and D.T. Westermann. 2002. A single dominant gene controlling resistance to soil zinc deficiency in common bean. Crop Science 42:1071-1074.

Singh, S.P. P. Gepts, and D.G. Debouck. 1991. Races of common bean (*Phaseolus vulgaris*, Fabaceae). Economic Botany 43:379-396.

Singh, S.P., H. Teran, C. G. Munoz, J.M. Osorno, J.C. Takegami and M.D.T. Thun. 2000. Low soil fertility tolerance in landraces and improved common bean genotypes. Crop Science 43:110-119.

Singh, S.P., H. Teran, M. Lema, D.M. Webster, C.A. Strausbaugh, P.N. Miklas, H.F. Schwartz and M.A. Brick. 2007. Seventy-five years of breeding dry bean of the Western USA. Crop Science 47:981-989.

Singh, S.P. H. Teran, C.G. Munoz-Perea, M. Lema, M. Dennis, R. Hays, R. Parrott, K. Mulberry, D. Fulmer, and J. Smith. 2009. Dry bean landrace and cultivar performance in stressed and nonstressed organic and conventional production systems. Crop Science 49:1859-1866.

Smith, J.R., S.J. Park, J.S. Beaver, P.N. Miklas, C.H. Canaday and M. Zapata. 2007. Registration of TARS-SR05 multiple disease resistant dry bean germplasm. Crop Science 47:457-458

Soon, Y.K. and M.A. Arshad. 2002. Comparison of the decomposition and N and P mineralization of canola, pea and wheat residues. Biology and Fertility of Soils 36:10-17.

Souza, A.A., R.L. Boscariol, D.H. Moon, L.E.A. Camargo and S.M. Tsai. 2000. Effects of *Phaseolus vulgaris* QTL in controlling host-bacteria interaction under two levels of nitrogen fertilization. Genetics and Molecular Biology 23:155-161.

Steele, K.W., P.M. Bonish, R.M. Daniels and G.W. O'Hara. 1983 Effect of rhizobial strain and host plant on nitrogen isotopic fractionation in legumes. Plant Physiology 72:1001-1004.

Talbott, H.J., W.J. Kenworthy, and J.O. Legg. 1982. Field comparison of the N-15 and difference-methods of measuring nitrogen-fixation. Agronomy Journal 74:799-804.

Thomas, R.J., J.R. McFerson, L.E. Schrader, and F.A. Bliss. 1984. Composition of bleeding sap nitrogen from lines of field grown *Phaseolus vulgaris* L. Plant and Soil 79:77-88

Tsai, S.M., R. Bonetti, S.M. Agbala, and R. Rossetto. 1993. Minimizing the effect of mineral nitrogen on biological nitrogen fixation in common bean by increasing nutrient levels. Plant and Soil 152:131-138.

Vasquez-Arroyo, J., A. Sessitsch, E. Martinez, and J.J. Pena-Cabriales. 1998. Nitrogen fixation and nodule occupancy by native strains of *Rhizobium* on different cultivars of common bean (*Phaseolus vulgaris* L.). Plant and Soil 204:147-154.

Walley, F.L., G.W. Clayton, P.R. Miller, P.M. Carr and G.P. Lafond. 2007. Nitrogen economy of pulse crop production in the northern Great Plains. Agronomy Journal 99:1710-1718.

Weiser, G.C., K.F. Grafton and D.L. Berryhill. 1985. Nodulation of dry beans by commercial and indigenous strains of Rhizobium phaseoli. Agronomy Journal 77:856-859.

Witty, J.F. 1983. Estimating N2-fixation in the field using N-15-labelled fertilizers-some problems and solutions. Soil Biology and Biochemistry 15:631-639.

Wolyn, D.J., J. Atewell, P.W. Ludden, and F.A. Bliss. 1989. Indirect measures of N2 fixation in common bean (*Phaseolus vulgaris* L.) under field conditions-the role of lateral root nodules. Plant and Soil 113:181-187.

Wolyn, D.J., D.A. St. Clair, J. DuBois, J.C. Rosas, R.H. Burris and F.A. Bliss. 1991. Distribution of nitrogen in common bean (*Phaseolus vulgaris* L.) genotypes selected for differences in nitrogen fixation ability. Plant and Soil 138:303-311.