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Nitrogen use efficiency of irrigated dry bean (*Phaseolus vulgaris* L.) genotypes in southern Alberta

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Abstract: Although dry bean (*Phaseolus vulgaris* L.) is a legume capable of fixing nitrogen, fertilizer N is usually recommended for its production in Canada because it is believed to be an inefficient N fixer. Using genotypes with high N use efficiency (NUE) would reduce the amount of applied N, which usually has deleterious environmental effects. We evaluated 22 bean genotypes for N uptake efficiency (NUpE) in a greenhouse trial, and 16 genotypes for N use efficiency (NUE) and its components: NUpE and N utilization efficiency (NUE) in two seasons (2012 and 2013) of a field trial at 30 kg N ha⁻¹ (30N) and 100 kg N ha⁻¹ (100N) soil levels. Root biomass and surface area were highly correlated with NUpE in the greenhouse, where NUpE at 100N was 48% lower than that at 30N. In the field trial, grain yield was highly correlated with NUpE and NUE at both 30N and 100N. The NUpE at 100N was 65% lower than that at 30N, and NUE at 100N was 68% lower than that at 30N. The low NUE at 100N in the field trial was mainly due to the difference in NUpE. This study also identified nine genotypes including five germplasm lines (PI 136692, GH-196, UNS-117, UI-239 and LEF2RB) and four cultivars (Othello, Viva, AC Redbond and Island) that were both N-efficient (at 30N) and N-responsive (to 100N).

Key words: Nitrogen uptake efficiency, nitrogen utilization efficiency, nitrogen use efficiency, root surface area

Abbreviations: 30N = 30 kg N ha⁻¹; 100N = 100 kg N ha⁻¹; DM = dry matter; HI = Harvest index; NUE = Nitrogen use efficiency; NUpE = Nitrogen uptake efficiency; NUtE = Nitrogen utilization efficiency.

Introduction

Limitation in food supply for a growing world human population and harmful effects of intensive agriculture on the environment are major challenges in agricultural production. Nitrogen is an essential element for plant growth and is the nutrient taken up in the largest amount. In the last 40 years, the amount of synthetic N applied to crops has risen dramatically, from 12 to 104 Tg per year (Mulvaney et al. 2009), resulting in significant increases in yield but with considerable adverse impacts on the environment throughout the world. In most intensive agricultural production systems, about 50-70% of the fertilizer N applied to crops is not taken up by the crops and is lost by ammonia volatilisation, denitrification and leaching into the soil (Belanger et al. 2003; Asghari and Cavagnaro, 2011).

Fertilizer N is usually applied to dry bean (*Phaseolus vulgaris* L.) in Canada, presumably due to the perception that dry bean is a poor N₂ fixer (Hardarson et al. 1993). In southern Alberta, where dry beans are grown under irrigation, it is only in soils (0-30 cm depth) testing more than 100 kg N ha⁻¹ (for row-cropped beans) or 120 kg N ha⁻¹ (for solid seeded beans) that no fertilizer N is recommended (Alberta Agriculture, Food and Rural Development, 2013). Otherwise, recommended rates range from 10 to 110 kg N ha⁻¹, depending on soil test N level. The increasing cost of fertilizer N and the negative environmental impact of excessive fertilizer N application make it desirable to reduce its applications. These levels of fertilizer N application can be reduced by using bean varieties that are efficient at fixing

atmospheric N₂ (Akter et al. 2014). If such varieties cannot be identified and fertilizer N has to be used, then use of genotypes with high NUE would reduce fertilizer application rates.

NUE has been studied and quantified extensively (Good et al. 2004; Ladha et al. 2005; Dawson et al. 2008). The most-used definition of NUE is the ratio between an output (biological or economic yield) and N input (supply) (Ladha et al. 2005). Nitrogen supply can be difficult to quantify, especially if N mineralization during plant growth is included. However, when emphasis is on differences between crop genotypes rather than crop/fertilizer management practices, a commonly used NUE definition is that of Moll et al. (1982): grain yield per unit of available N in the soil, i.e., soil N and fertilizer N. With that definition, NUE is a product of NUpE, i.e., crop N uptake per unit of available N from soil and fertilizer, and NUtE, i.e., grain yield per unit crop N uptake (Moll et al. 1982; Good et al. 2004; Dawson et al. 2008). Therefore, NUE can be increased by increasing plant N uptake from the soil (NUpE), increasing the conversion of accumulated plant N into grain yield (NUtE), or both. Many factors affect NUpE. They include management factors that improve synchrony between fertilizer N supply and crop demand to minimise N losses from the plant root zone, e.g., seeding rate (Dai et al. 2013), crop rotation/sequence (Lupwayi and Soon 2016), and the 4Rs of nutrient management: applying the right fertilizer type at the right rate, right time and right place (McKenzie et al. 2001; Venterea et al. 2016). However, genetic differences in plant root architecture may also affect NUpE. Efficient N uptake depends on increased root morphological features like size and distribution in the soil profile, which not only maximize interception and uptake of N fertilizers but also reduce N losses to deeper soil layers and groundwater, thereby increasing NUE (Lynch 2013; Ning et al. 2014). For example, increases in root size (root dry weight, root length, and root density) have been reported to improve N uptake and yield in maize (Mu et al. 2015). NUtE is a plant physiological trait whose differences could be due to genetic differences in internal N requirements for metabolism, ability to mobilize and distribute absorbed N to and from different plant organs, photosynthetic efficiency or environmental factors (Ladha et al. 2005). Whether

improvements in NUE result from NUpE, NUtE, or both depends on many factors that include how these traits are measured or derived, the amount of fertilizer N applied, environmental conditions and the germplasm under evaluation. Worku et al. (2007) found that NUE of tropical maize hybrids was influenced similarly by both NUpE and NUtE. However, Moll et al. (1982), working on maize, Dhugga and Waines (1989), working on hexaploid and tetraploid spring wheat, Gouis et al. (2000), working on winter wheat, and Rotundo et al. (2014), working on soybean, showed that NUpE was more important than NUtE in determining NUE of these crops.

Most NUE studies have been conducted in cereals crops. Very few such studies have been conducted in legumes (Vadez et al. 1999; Dorcinvil et al. 2010; Rotundo et al. 2014), presumably because legumes obtain some of their N from the atmosphere through biological N₂ fixation. Vadez et al. (1999) reported that dry bean lines with high NUE that had been inoculated with *Rhizobium leguminosarum* biovar *phaseoli* produced more root and shoot biomass than inefficient genotypes. After evaluating the NUE and grain yields of 36 dry bean lines under optimum and limiting soil N and P supply, Dorcinvil et al. (2010) recommended that phenotypic traits like root architecture (including specific root volume, number of root crowns, root mass and root/shoot ratios) be examined to explain the observed NUE and yield differences between the genotypes. In Canada, barley (Anbessa et al. 2009) and potato (Zebarth et al. 2004) genotypes have been screened for NUE, but similar characterization of dry bean genotypes has not been done. The objectives of this study were (a) to relate the root characteristics of dry bean genotypes to their N acquisition and biomass (DM) production in a greenhouse study, and (b) to evaluate them for NUE and grain yield in the field to identify genotypes capable of producing high yields in limiting and optimum soil N environments. Such genotypes could be used directly in dry bean production or in bean breeding programs to improve overall NUE of dry bean cultivars.

Materials and Methods

Greenhouse trial

A greenhouse evaluation of NUpE was conducted with 22 dry bean genotypes that consisted of the major market classes grown in commercial fields, germplasm lines, and R99, a non-nodulating mutant (Table 1). The experiment had two N levels: 30 kg N ha⁻¹ (i.e., 15 mg N kg⁻¹ soil) and 100 kg N ha⁻¹ (i.e., 50 mg N kg⁻¹ soil). The soil for the experiments was obtained from Purple Springs, Alberta and was mixed with gravel and vermiculite in a 1:1:1 mixture by weight. Gravel and vermiculite were added in order to facilitate the root washing. Calcium nitrate [Ca(NO₃)₂] was added to the mixture to make 30 and 100 kg N ha⁻¹ (30N and 100N, respectively), taking into account the initial nitrate content of the soil (Table 2), calculated using the relationship: 1 kg ha⁻¹ = 2 x mg kg⁻¹ soil. Tree pots (TP915R: 22.7 cm x 39.4 cm, Stuewe and Sons, Tangent, OR, USA) were filled with either the 30N soil or 100N soil. The pots were arranged in a randomized complete block design, with three replications separated in time under controlled environment conditions: 23/17°C day/night temperatures, 16/8 h light/dark photoperiod, 60% relative humidity and approximately 280 µmol m⁻² s⁻¹ photosynthetically active radiation (PAR) at the top of the pots during the experiment. Three uniformly-sized dry bean seeds of each genotype were planted in each pot, and seedlings were thinned to one per pot after emergence.

Plants were fertilized weekly with a modified N-free Hoagland's solution (Hershey 1994) and watered as needed. Each genotype was harvested at the beginning of flowering: the earliest genotype was harvested 35 days after sowing and the latest 46 days after sowing. Plants were harvested by cutting them at soil level and shoot DM was determined after drying at 55°C for one week. Because of the dense root system of crops in the pots, the total root system was separated into two parts by soil depth: 0-20 cm and 20-40 cm. After washing the roots with water, root surface area of each of the two parts was estimated by analysis of scanned images. Cleaned roots were placed in clear Plexiglas tray

with submerged water and scanned with an Epson Perfection 4990 Photo scanner (Epson Canada, Toronto, Ontario). Root DM was determined after drying the roots at 55°C for one week. Total root DM and root surface area were determined by summing the root surface area and root DM of the two soil depths. To determine N content of the shoot from each experiment, 5-6 mg of ground shoot sample was weighed into a tin capsule (8 × 5 mm) and the capsule was then closed, compressed and placed in 96-well micro plates. Samples were analyzed for N using the Carlo Erba NA2100 automated nitrogen analyzer (Carlo Erba Strumentazione, Milan, Italy), and NUpE was calculated as follows (Moll et al. 1982):-

$$\text{NUpE (kg crop N kg}^{-1} \text{ soil N)} = \text{Crop N uptake} / \text{Available soil N} \dots\dots\dots (1)$$

Where NUpE = N uptake efficiency; Available soil N = Soil + fertilizer N; Crop N uptake = aboveground crop N, i.e., shoot N.

Field trial

The field trial was conducted in 2012 and 2013 on a Black Chernozem soil at Lethbridge Research and Development Centre, Lethbridge (49°7N and 112.833°W), Alberta. Both the 2012 and 2013 fields of the experiment had been depleted of N by uniformly growing unfertilized wheat for 10 and 5 years, respectively. Before seeding, soil samples were taken randomly from 5 spots from 15 and 30 cm depths and analyzed for N and other essential elements (P, K, Zn, S and B) at a commercial soil testing laboratory (Down to Earth Labs Inc., Lethbridge, AB). The results are presented in Table 2.

From the 22 bean genotypes that had been screened in the greenhouse study, 16 were selected on the basis of NUpE to cover a wide range of NUE for the field experiment. The plants were grown at 30N and 100N soil N levels. The experimental design was split-plot, with N level as main plots and

genotypes as sub-plots, replicated four times. Each sub-plot consisted of four 3-m long rows spaced at 63 cm, and the seeding rate was 28 seeds m⁻². All fertilizers were broadcast and incorporated prior to seeding, which was done on May 30 in 2012 and May 21 in 2013. The extra N required to add to soil test levels to make 30N or 100N was applied as urea. Phosphorus and Zn fertilizers were applied according to soil test recommendations. Irrigation was supplied as required throughout the season. The plots were kept free of weeds by application of herbicides Imazamox (at 0.03 kg a.i. ha⁻¹) and Bentazon (at 1.23 L ha⁻¹), and by hand weeding at later stages of crop growth. Copper hydroxide (at 2.47 kg a.i. ha⁻¹) was applied at flowering to prevent common bacterial blight. The growing-season weather conditions at the field sites were recorded.

At physiological maturity (on August 18, 2012 and August 23, 2013), a 30 cm length of two rows of each plot was harvested to determine seed and straw yields and N contents. The same protocol described above (greenhouse trial) was used to determine N content of the straw and grain, and NUE and its components were calculated as follows (Moll et al. 1982):-

NUE (kg grain DM kg⁻¹ soil N) = Crop grain yield / Available soil N.....(2)

NUpE (kg crop N kg⁻¹ soil N) = See Equation (1) above

NUE (kg grain DM kg⁻¹ crop N) = Crop grain yield / crop N uptake.....(3)

Where NUE = N use efficiency; NUpE = N uptake efficiency; Available soil N = soil + fertilizer N; Crop N uptake = aboveground crop N, i.e., grain N + straw N.

The yield HI was calculated as grain DM / (grain DM + straw DM).

At maturity (on September 7, 2012 and September 10, 2013), seed yield was determined from two centre rows of each plot by undercutting the plants and, 10 days later, harvesting with a small-plot combine.

Statistical analysis

Data from the greenhouse trial were subjected to analysis of variance (ANOVA) using the PROC GLM procedure of SAS (SAS institute Inc. 2008). Soil N level, genotype and the interaction of the two factors were considered as fixed effects. Data from both years of the field trial were analyzed by using the PROC Mixed procedure of SAS (SAS institute Inc. 2008). Year, replication nested within year, and interactions involving these factors were considered as random effects, whereas soil N level, genotypes and their interaction were considered as fixed effects. In both the greenhouse and field trials, differences between treatments were considered to be significant at 5% level of significance. Means were separated by the least significant difference (LSD) method. Main-effect results are presented in tables where no soil N x genotype interaction was observed, and interaction results are presented graphically where interaction was observed. Relationships between the measured genotype traits were evaluated by correlation analysis.

Results

Greenhouse trial

The greenhouse study was conducted to pre-screen the 22 bean genotypes for shoot and root traits prior to inclusion in the field study. There were significant soil N and bean genotype effects, with no factor interactions, on shoot DM, root DM and root surface area (Table 1). Shoot DM, root DM and root surface area increased by 36, 59 and 49%, respectively, at 100N soil level relative to the 30N soil level (Table 1). Averaged over both soil N levels, shoot DM ranged from 14.85 to 6.19 g plant⁻¹, with a mean of 9.38 g plant⁻¹. The top three genotypes in shoot DM were TLP-19, Othello and ChinookRRR, and the bottom three were Viva, Arikara Yellow and SEA 5. Root DM ranged from 4.05 to 0.98 g plant⁻¹ and the mean was 1.73 g plant⁻¹; the top three genotypes were Othello, LEF 2RB and Topaz, while the

bottom three were AC Black Diamond, AC Redbond and Winchester. The range in root surface area was 88.04 to 29.54 cm² per plant⁻¹ with a mean of 47.91 cm² plant⁻¹; the top three genotypes were Othello, UNS-117 and Topaz, and the bottom three were AC Redbond, AC Black Diamond and Arikara Yellow.

The interaction between soil N and genotype in NUpE was significant (Fig. 1). At 30N, NUpE ranged from 1.33 to 0.28 kg crop N kg⁻¹ soil N and the mean was 0.71 kg crop N kg⁻¹ soil N. The top three genotypes were Othello, TLP-19 and ChinookRRR, and the bottom three were R99, Arikara Yellow and Viva. The average NUpE at 100N was 0.33 kg crop N kg⁻¹ soil N, with a range of 0.41 to 0.25 kg crop N kg⁻¹ soil N. The top three genotypes were Othello, TLP-19 and R99, and the bottom three were Arikara Yellow, Viva and B98311. Therefore, NUpE at 100N was less than half (about 48%) of that at 30N.

There were significant correlations between NUpE and the other traits, both at 30N and 100N (Table 3). The correlation coefficients at 30N ranged from $r = 0.938$ ($P < 0.001$) between NUpE and shoot DM, to $r = 0.361$ ($P = 0.003$) between NUpE and root surface area. At 100N, the range was from $r = 0.723$ ($P < 0.001$) between NUpE and shoot DM, to $r = 0.290$ ($P = 0.018$) between NUpE and root surface area.

Field trial weather conditions

The growing-season weather conditions at the field sites are presented in Table 4. The average air temperature did not deviate much from normal (30-year average) temperatures in any month of the growing season in either year. The rainfall patterns were different between the two years. In 2012, May and June were 37% and 48% wetter than normal, respectively. The rest of the season was drier than normal, but the plants were irrigated. In 2013, June and July were 84% and 22% wetter than normal, respectively. September rainfall was 66% above average. Soil moisture deficits (lower-than-normal rainfall) were corrected by supplemental irrigation, but there was no corrective action for excessive rainfall.

Field trial

The combined analysis of soil N and bean genotype effects in both years of the field trial (Table 5) revealed interactions between soil N and bean genotype in NUpE, NUE and grain yield (Table 5). Genotype differences, regardless of soil N level, were observed for NUpE and HI. NUpE at 30N ranged from 5.25 to 2.95 kg crop N kg⁻¹ soil N, with a mean of 4.17 kg crop N kg⁻¹ soil N (Fig. 2a). The top three genotypes in NUpE were PI 136692, Hyden and LEF2RB while the bottom three were R99, Resolute and ChinookRRR. At 100N, the range was 1.86 to 1.20 kg crop N kg⁻¹ soil N; mean 1.47 kg crop N kg⁻¹ soil N (Fig. 2a). The top three performers at 100N were PI 136692, UNS-117 and GH-196, and the bottom three were ChinookRRR, AC Redbond and Resolute. Therefore, NUpE at 100N (1.47 kg crop N kg⁻¹ soil N) was 65% lower than that at 30N (4.17 kg crop N kg⁻¹ soil N). There was no soil N effect on NUtE, which ranged from 20.63 to 9.87 kg crop yield kg⁻¹ crop N with a mean of 17.70 kg crop N kg⁻¹ crop N (Table 6). The top three performers in NUtE were Resolute, Othello and Island, and the bottom three were FR266, ChinookRRR and R99.

The NUE of the genotypes also varied with soil N level, i.e., there was a soil N x genotype interaction. At 30N soil level, NUE ranged from 96.43 to 39.01 kg grain yield kg⁻¹ soil N with a mean of 76.40 kg grain yield kg⁻¹ soil N, and from 30.81 to 12.46 kg grain yield kg⁻¹ soil N with a mean of 24.48 kg grain yield kg⁻¹ soil N at 100N (Fig. 2b). At 30N, the top three genotypes in NUE were LEF2RB, GH-196 and PI 136692, and the three genotypes with the lowest NUE were FR266, R99 and ChinookRRR. At 100N, the three genotypes with the highest NUE were Othello, PI 136692 and UNS-117, and the bottom three performers were FR266, ChinookRRR and R99 (Fig. 2b). Therefore, NUE at 100N (24.48 kg grain yield kg⁻¹ soil N) was 68% lower than that at 30N (76.60 kg grain yield kg⁻¹ soil N).

Genotype grain yields also depended on soil N level. At 30N soil level, grain yield ranged from 2893 to 1170 kg ha⁻¹ with a mean of 2292 kg ha⁻¹, and from 3081 to 1246 kg ha⁻¹ with a mean of 2448 kg

ha⁻¹ soil N at 100N (Fig. 3). At 30N, the top three genotypes in grain yield were LEF2RB, GH-196 and PI 136692, and the three genotypes with the lowest grain yield were FR266, R99 and ChinookRRR. At 100N, the three genotypes with the highest grain yield were Othello, PI 136692 and UNS-117, and the bottom three yielders were FR266, ChinookRRR and R99 (Fig. 3). There was no soil N effect on HI, which ranged from 0.51 to 0.28 with a mean of 0.46 (Table 6). The top three performers in HI were Island, GH-196 and Othello, and the bottom three were FR266, R99 and ChinookRRR.

The interaction between soil N and bean genotypes in grain yield can be depicted by plotting genotype yields at 30N against yields at 100N (Fig. 4). The lines showing mean yields at each N level divide the figure into four quadrants, categorizing the genotypes into N-efficient (or inefficient) and N-responsive (or non-responsive) (Nyikako et al 2014). This categorization showed that nine of the sixteen genotypes were efficient N users at low soil N and responsive to high soil N (in the “efficient, responsive” quadrant of Fig. 4). These genotypes were PI 136692, GH-196, LEF2RB, Othello, UI-239, Viva, Island, AC Redbond and UNS-117. At the other end of the scale were the genotypes FR266, ChinookRRR, R99 and TLP-19 that were inefficient N users at low soil N and not responsive to high soil N. The genotypes Topaz and Hyden were efficient N users at low soil N but non-responsive to high soil N, while the genotype Resolute was inefficient at low soil N but responsive to high soil N.

Correlations between grain yield and NUpE, NUtE, NUE and HI were all highly significant ($P < 0.001$) both at 30N and 100N (Table 7). Correlations between NUE and grain yield were 1.000 because NUE was calculated as grain yield divided by a constant (either 30 at 30N or 100 at 100N). At 30N, the correlation between NUpE and NUE or grain yield was stronger ($r = 0.736$, $P < 0.001$) than that between NUtE and NUE or grain yield ($r = 0.0442$, $P < 0.001$). These correlations were almost the same at 100N ($r = 0.610$ vs 0.635). One noteworthy relationship is the negative correlation between NUpE and NUtE at both soil N levels (Table 7). One reason for this relationship is that genotype Resolute had one of the lowest NUpE (Fig. 2a) but the highest NUtE (Table 6).

Discussion

Defining NUE as a ratio of grain yield to N supply really measures plant response to available N. The mean NUE of bean genotypes in the field trial were 76.40 and 24.48 kg grain kg⁻¹ soil N at 30N and 100N soil levels, respectively. In other studies in which NUE was calculated similarly (in which case it is sometimes called partial factor productivity of N, PFPN), it ranged from 30.2 to 62.3 kg grain kg⁻¹ N for barley genotypes in which soil available N ranged from 45 to 269 kg ha⁻¹ in Alberta (Anbessa et al. 2009). In rice genotypes in China, NUE ranged from 72.4 to 83.4 kg grain kg⁻¹ N at 100 kg N ha⁻¹, from 42.6 to 47.6 kg grain kg⁻¹ N at 200 kg N ha⁻¹, and from 32.3 to 33.1 kg grain kg⁻¹ N at 100 kg N ha⁻¹ (Ju et al. 2015). One component of NUE is NUpE, which was 4.17 and 1.47 kg crop N kg⁻¹ soil N at 30N and 100N, respectively, in the field study. In maize genotypes, NUpE ranged from 1.75 to 2.28 kg crop N kg⁻¹ soil N at 56N and from 0.43 to 0.77 kg crop N kg⁻¹ soil N at 224N (Moll et al. 1982). One difference between N uptake (accumulation) in legume crops like dry bean and cereals is that legume crops obtain some of their N through biological N₂ fixation (although dry bean is believed to be an inefficient N₂ fixer), especially at low soil N level. This difference may explain the different magnitudes of NUpE (and NUE) between our study and other studies.

In the greenhouse trial, genotype Othello was consistently among the top three genotypes in root surface area, root DM, NUpE and shoot DM. Root DM and root surface area were both highly correlated with NUpE, especially at 30N. In two different seasons with regard to rainfall (Table 4), Othello was one of the nine genotypes that were efficient N users at low soil N and responsive to high soil N (Fig. 4). This result suggests that plant root characteristics were important for NUpE. Roots are the plant organs that acquire nutrients (and water) from the soil. In a field study in China, rice varieties with high NUE also had greater root DM, greater root density and deeper roots (among other traits)

than the varieties with low NUE at 0, 100 and 200 kg N ha⁻¹ but not at 300 kg N ha⁻¹ (Ju et al. 2015). Similar results have been reported in winter wheat (Rasmussen et al. 2015) and other nonlegume crops like oilseed rape (*Brassica napus* L.) (Kamh et al. 2005). Although root traits are important in NUpE, they are rarely studied in field experiments because they are difficult to quantify. Other factors that affect NUpE are management factors that minimise N losses from the root zone, e.g., seeding rate (Dai et al. 2013), crop rotation/sequence (Lupwayi and Soon 2016), use of nitrification inhibitors (Alonso-Ayuso et al. 2016), and the 4Rs of nutrient management: applying the right fertilizer type at the right rate, right time and right place (McKenzie et al. 2001; Venterea et al. 2016).

In the greenhouse trial, NUpE at 100N was less than half (46%) of that at 30N. In the field trial, NUpE was 65% lower at 100N than at 30N, and NUE was 68% lower at 100N than at 30N. The low NUE at 100N in the field trial was mainly due to the difference in NUpE because NUtE did not differ much between the two N levels. Therefore, most of the fertilizer N applied at high rates was not taken up by the bean plants. Similar results of decreasing NUpE (and NUE) with increasing soil N have been observed in cereals (Moll et al. 1982; Anbessa et al. 2009; Ju et al. 2015), potatoes (Zerbath et al. 2004), and oilseed rape (Nyikako et al. 2014).

The other component of NUE is NUtE. Therefore, the ideal bean genotypes would be those that acquire more N from the soil (high NUpE) and/or use the acquired N efficiently (high NUtE). NUtE is a plant physiological trait, which can be targeted in dry bean breeding programs. Differences between bean genotypes in NUtE were observed in the field trial, but soil N had no effect. The mean NUtE was 17.70 kg grain yield kg⁻¹ crop N, which is lower than the 18.3–24.5 g seed DM g⁻¹ crop N range reported for chickpea genotypes (Koutroubas et al. 2009), presumably because no N was applied to the chickpea. In eastern Canada, Zebarth et al. (2004) also reported differences between potato genotypes in NUtE. Such differences in NUtE could be due to genetic differences in internal N requirements for metabolism, ability to mobilize and distribute absorbed N to and from different plant organs, photosynthetic

efficiency or environmental factors (Ladha et al. 2005). Although there was no soil N effect on NUtE in our study, some studies have reported increased NUtE at high fertilizer N rates (Chen et al. 2016; Koutroubas et al. 2016). Correlations between NUtE and NUE or grain yield were highly significant, but it is noteworthy that correlations between NUtE and NUpE were negative at both soil N levels even though they are both components of NUE. This negative correlation was partly explained by the inconsistent ranking of one genotype (Resolute) in these two components of NUE. Our observation of stronger correlation of NUE with NUpE than with NUtE at low soil N level has also been reported in field studies with oilseed rape (Berry et al. 2010; Nyikako et al. 2014).

Conclusion

This study identified nine dry bean genotypes that can be grown in either low-N or high-N soil environments: PI 136692, GH-196, LEF2RB, Othello, UNS-117, UI-239, Viva, AC Redbond and Island. The genotypes Topaz and Hyden performed better in low-N soil than high-N soil, and vice-versa for Resolute. Genotypes FR266, ChinookRRR and R99, and to some extent, TLP-19 were poor in either environment. Of the nine N-efficient and responsive genotypes, AC Redbond (red bean), Island (pinto bean) and Viva (pink bean) are early maturing cultivars currently grown in Alberta. Root biomass and surface area were highly correlated with NUpE in the greenhouse although assessment of root traits may be cumbersome when evaluating several hundred lines in a breeding program. However, selection for high grain yield, a trait routinely measured in breeding programs, in low soil-N or high soil-N environments may result in dry bean lines with high NUE and NUpE. The N-efficient and responsive cultivars may enable dry bean growers to reduce nitrogen input without compromising seed yield and quality.

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Figure captions

Fig. 1. Interaction between soil N and genotype in N uptake efficiency (NUpE) in the greenhouse trial.

Fig. 2. Interaction between soil N and genotype in N uptake efficiency (NUpE) (a) and N use efficiency (NUE) (b) in both years of the field trial.

Fig. 3. Interaction between soil N and genotype in grain yield in both years of the field trial.

Fig. 4. Mean bean genotype yields at 30 kg N ha⁻¹ (30N) and 100 kg N ha⁻¹ (100N) in the both years of the field trial. The lines showing mean yields at each N level divide the figure into four quadrants, categorizing the genotypes into N-efficient (or inefficient) and N-responsive (or non-responsive).

Table 1. Shoot dry matter (DM), root DM and root surface area of the dry bean genotypes, and the effect of soil N, in the greenhouse trial.

Treatment	Genotype growth	Shoot DM	Root DM	Root surface area
	habit ^b , and market class	(g plant ⁻¹)	(g plant ⁻¹)	(cm ² plant ⁻¹)
Soil N (kg ha⁻¹)				
30		7.96	1.34	38.55
100		10.81	2.13	57.28
LSD (0.05)		0.50	0.20	3.54
Genotype				
TLP-19	II, Black	14.85 ¹	1.93 ⁷	50.32 ⁸
Othello	III, Pinto	14.31 ²	4.05 ¹	88.04 ¹
ChinookRRR	I, Red kidney	11.65 ³	1.52 ¹²	36.55 ¹⁷
Resolute	II, Great Northern	10.68 ⁴	1.19 ¹⁹	34.50 ¹⁸
FR266	I, White kidney	10.63 ⁵	1.50 ¹³	44.23 ¹⁴
Topaz	II, Pinto	10.53 ⁶	2.26 ³	67.27 ³
AC Black Diamond	II, Black	10.28 ⁷	0.98 ²²	30.43 ²¹
LEF2RB	III, Carioca	10.21 ⁸	2.70 ²	58.63 ⁴
Winchester	II, Pinto	10.12 ⁹	1.10 ²⁰	34.26 ¹⁹
Island	II, Pinto	9.21 ¹⁰	1.80 ¹⁰	51.72 ⁷
UI-239	II, Red	8.96 ¹¹	1.35 ¹⁷	37.60 ¹⁶
AC Redbond	II, Red	8.63 ¹²	1.03 ²¹	29.54 ²²
GH-196	II, Pinto	8.53 ¹³	1.93 ⁶	55.03 ⁵

PI 136692	III, Red	8.47 ¹⁴	1.84 ⁸	48.20 ¹⁰
AC Polaris	II, Great Northern	8.06 ¹⁵	1.55 ¹¹	53.12 ⁶
R99	II, Navy	7.88 ¹⁶	1.96 ⁵	49.26 ⁹
UNS-117	III, Pink	7.75 ¹⁷	1.99 ⁴	69.48 ²
B98311	II, Black	7.54 ¹⁸	1.81 ⁹	48.05 ¹²
Hyden	II, Navy	7.46 ¹⁹	1.48 ¹⁴	39.38 ¹⁵
SEA 5	III, Cream	7.43 ²⁰	1.48 ¹⁵	47.12 ¹³
Arikara Yellow	I, Yellow	7.00 ²¹	1.25 ¹⁸	33.27 ²⁰
Viva	III, Pink	6.19 ²²	1.38 ¹⁶	48.08 ¹¹
Mean		9.38	1.73	47.91
LSD _{0.05} ^a		1.65	0.67	11.74

Notes: Superscript numbers on each mean are the rankings of the 22 genotypes in each column.

^aLSD_{0.05} = Least significant difference at 0.05 significance level.

^bType I = determinate bush growth habit; Type II = indeterminate bush growth habit;

Type III = Indeterminate prostrate with weak stem.

Table 2. Soil properties in the greenhouse and field experiments.

Experiment	Depth	Organic matter	pH	NO ₃ -N	P-Kelowna ^a	K	Zn	S	B
	(cm)	(g kg ⁻¹)		(kg ha ⁻¹)	(kg ha ⁻¹)	(kg ha ⁻¹)	(kg ha ⁻¹)	(kg ha ⁻¹)	(kg ha ⁻¹)
Greenhouse	0-30	13.0	7.5	22.4	52.7	799	2.13	112	0.45
Field, 2012	0-15	41.0	7.9	13.2	87.9	1489	0.85	43	0.56
	15-30	33.0	8.0	8.5	43.9	1102	0.38	34	0.45
Field, 2013	0-15	33.0	7.6	21.1	9.0	1949	0.56	67	0.78
	15-30	24.0	7.9	19.1	4.5	1553	0.16	61	0.78

^aKelowna-extractable P (van Lierop 1988).

Table 3. Correlations between the crop traits at 30 and 100 kg N ha⁻¹ soil levels (30N and 100N, respectively) using data in individual replicates (n= 66) of the greenhouse trial.

	Shoot DM	Root DM	Root surface area
	Correlation coefficient		
30N			
Root DM	0.453 (P <0.001)		
Root surface area	0.327 (P = 0.008)	0.844 (P <0.001)	
NUpE	0.938 (P <0.001)	0.500 (P < 0.001)	0.361 (P = 0.003)
100N			
Root DM	0.430 (P <0.001)		
Root surface area	0.282 (P = 0.022)	0.868 (P <0.001)	
NUpE	0.723 (P <0.001)	0.319 (P = 0.009)	0.290 (P = 0.018)

Table 4. Growing season (May to September) precipitation and temperature at the study sites.

Month	2012	2013	30-yr mean ^a	2012	2013	30-yr mean
	Total rainfall (mm)			Mean air temperature (°C)		
May	68	56	50	10.7	13.0	11.5
June	125	155	84	15.5	15.5	15.4
July	16	50	41	20.0	18.6	18.4
August	20	26	40	19.0	19.6	17.9
September	7	67	40	15.5	15.9	13.0
Total/Mean	236	354	255	16.1	16.5	15.2

^a1981–2010.

Table 5. Summary of analysis of variance results (ProcMixed for fixed effects only) in both years of the field trial.

Effect	DF ^a	NUpE ^b	NUtE ^c	NUE ^d	Grain yield	HI ^e
Soil N (N)	1	NS	NS	NS	NS	NS
Genotype (G)	15	**	**	**	**	**
N x G	15	**	NS	**	*	NS

Notes: ** = significant at 0.01 significance level, * = significant at 0.05 significance level, NS = not significant at 0.05 significance level.

^aDF = Degrees of freedom.

^bNUpE = N uptake efficiency.

^cNUtE = N utilization efficiency.

^dNUE = N use efficiency.

^eHI = Harvest index.

Table 6. Nitrogen utilization efficiency (NUE) and harvest index (HI) of dry bean genotypes, and the effect of soil N, averaged over both years of the field trial.

Treatment	NUE ^c (kg grain yield kg ⁻¹ crop N)	HI ^d
Soil N (kg ha⁻¹)		
30	18.59	0.47
100	16.81	0.43
SE ^a	1.82	0.07
LSD _{0.05} ^b	NS	NS
Genotype		
Resolute	20.63 ¹	0.47 ⁹
Othello	20.57 ²	0.50 ³
Island	19.96 ³	0.51 ¹
AC Redbond	19.81 ⁴	0.48 ⁶
Topaz	19.55 ⁵	0.48 ⁷
UI-239	19.11 ⁶	0.49 ⁵
Viva	18.88 ⁷	0.50 ⁴
GH-196	18.72 ⁸	0.51 ²
UNS-117	18.36 ⁹	0.47 ¹⁰
LEF2RB	18.05 ¹⁰	0.44 ¹²
PI 136692	17.24 ¹¹	0.48 ⁸
TLP-19	16.48 ¹²	0.41 ¹³
Hyden	15.84 ¹³	0.47 ¹¹

R99	15.75 ¹⁴	0.31 ¹⁵
ChinookRRR	14.32 ¹⁵	0.36 ¹⁴
FR 266	9.87 ¹⁶	0.28 ¹⁶
SE	1.20	0.03
LSD _{0.05}	2.56	0.06

Notes: Superscript numbers on each mean, where there were genotype differences, are the rankings of the 16 genotypes in the column.

^aSE = Standard error of the difference of two means.

^bLSD_{0.05} = Least significant difference at 0.05 significance level.

^cNUtE = N utilization efficiency.

^dHI = Harvest index.

Table 7. Correlations between the crop traits at 30 and 100 kg N ha⁻¹ soil levels (30N and 100N, respectively) using data in individual replicates (n = 128) of both years of the field trial.

	Grain yield	HI	NUpE	NUtE
	Correlation coefficient (probability)			
30N				
HI ^a	0.723 (P < 0.001)			
NUpE ^b	0.736 (P < 0.001)	-0.473 (P < 0.001)		
NUtE ^c	0.442 (P < 0.001)	0.390 (P < 0.001)	-0.254 (P = 0.004)	
NUE ^d	1.000 (P < 0.001)	0.723 (P < 0.001)	0.736 (P < 0.001)	0.442 (P < 0.001)
100N				
HI	0.473 (P < 0.001)			
NUpE	0.610 (P < 0.001)	-0.001 (P = 0.993)		
NUtE	0.635 (P < 0.001)	0.601 (P < 0.001)	-0.198 (P = 0.025)	
NUE	1.000 (P < 0.001)	0.473 (P < 0.001)	0.610 (P < 0.001)	0.635 (P < 0.001)

^aHI = Harvest index.

^bNUpE = Nitrogen uptake efficiency.

^cNUtE = Nitrogen utilization efficiency.

^dNUE = Nitrogen use efficiency. Correlations between NUE and grain yield are perfect because NUE was calculated as grain yield divided by a constant (either 30 at 30N or 100 at 100N).

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.