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Complete List of Authors:	Hossain, Zakir; Agriculture and Agri-Food Canada, Wang, Xiaoyu; Ottawa Research and Development Centre, Agriculture and Agri-Food Canada, 960 Carling Avenue, Ottawa, Ontario, K1A 0C6 Hamel, Chantal; Quebec Research and Development Centre, Agriculture and Agri-Food Canada, 2560 Boul. Hochelaga, Québec QC G1V 2J3 Knight, J. Diane; University of Saskatchewan, Soil Science Morrison, Malcolm; Agriculture Canada, Research Branch Gan, Y.; Agriculture and Agri-Food Canada,
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Biological nitrogen fixation by pulse crops on the semiarid Canadian Prairie

Zakir Hossain¹, Xiaoyu Wang², Chantal Hamel³, J. Diane Knight⁴, M.J. Morrison², Yantai Gan^{1,*}

¹ *Swift Current Research and Development Centre, Agriculture and Agri-Food Canada, Gate#3, Airport Road East, Swift Current, Saskatchewan, S9H 3X2;*

² *Ottawa Research and Development Centre, Agriculture and Agri-Food Canada, 960 Carling Avenue, Ottawa, Ontario, K1A 0C6;*

³ *Quebec Research and Development Centre, Agriculture and Agri-Food Canada, 2560 Boul. Hochelaga, Québec QC G1V 2J3;*

⁴ *Department of Soil Science, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK, S7N 5A8.*

**Corresponding author (Y. Gan): Email: yantai.gan@agr.gc.ca*

Tel: +1-306-770-4464

fax: +1-306-770-441

Only

Abstract

Hossain, Z., Wang, X., Hamel, C., Knight, J.D., Morrison, M.J. and Gan, Y. 2016. **Biological nitrogen fixation by pulse crops on the semiarid Canadian Prairie. Can. J. Plant Sci. 106:xxx-xxx.** Pulses play a significant role in nitrogen cycling as they fix atmospheric N₂ through symbiosis. However, it is unknown whether there are differences in the ability of biological nitrogen fixation (BNF) among pulse species and individual cultivars. Here we quantified the BNF ability of selected pulse cultivars and determined the effect on crop yield. A total of 25 species-cultivar combinations of chickpea (*Cicer arietinum* L.), dry bean (*Phaseolus vulgaris* L.), faba bean (*Vicia faba* L.), field pea (*Pisum sativum* L.), and lentil (*Lens culinaris* Medik.) were tested in 2008-2010. Pulses had a higher BNF in the wetter 2010 and lower in the drier 2009. In 2010, faba bean and chickpea had highest BNF at 106 kg N ha⁻¹, followed by lentil, field pea and dry bean at 87, 69 and 12 kg N ha⁻¹, respectively. Across years, field pea had most stable BNF ability, fixing 55 kg N ha⁻¹ with an average seed yield of 2418 kg ha⁻¹. There are large differences in BNF and yield among cultivars within a species and the magnitude of the difference varied with years. Large genetic variability in BNF and yield suggest the possibility that pulse cultivars with a higher N₂-fixing ability and seed yield can be developed through selection of N₂-fixing trait.

Keywords: Biological N₂ fixation, chickpea, dry bean, faba bean, field pea, ¹⁵N isotope dilution, lentil

The global human population is predicted to increase to nine billion by 2050, requiring food production to be doubled in order to meet the demand (Godfray 2011). Nitrogen (N) is an essential nutrient element for plants, and acquisition and assimilation of N is of importance to photosynthesis for plant growth and development (Sulieman 2011). Current cropping systems heavily rely on high input of inorganic N-fertilizer (Malhi et al. 2014; Wile et al. 2014), which requires large amounts of non-renewable energy (Wile et al. 2014). Application of N-fertilizer can be detrimental to the environment, potentially leaching nitrate into water sources and contributing to elevated nitrous oxide concentrations in the atmosphere. Therefore, achieving high crop yields with reduced inputs of inorganic fertilizer is one of the main goals for sustainable farming systems. One method to increase crop yield with minimized use of N fertilizer is to include pulse crops in cropping systems to capitalize on their biological nitrogen fixation (BNF) ability (Peoples et al. 2009). Symbiotic N₂ fixation is the major route of fixing atmospheric N₂ and thus contributes to agriculture sustainability (Geurts et al. 2012).

Inclusion of pulse crops in cropping systems can increase the grain yield of subsequent crops (Gan et al. 2003), mitigate nitrogenous greenhouse gas emissions (Lupwayi and Kennedy 2007) and reduce the carbon footprint of grain products (Gan et al. 2014). In addition, the use of annual pulses in a crop sequence can improve soil properties with increased availability of nutrients and soil moisture (Peoples et al. 2009; Williams et al. 2014). Furthermore, the biologically fixed-N₂ can be used directly by the crops and is less susceptible to volatilization, denitrification or leaching (Jensen and Hauggaard-Nielsen 2003) than inorganic fertilizer N applied to crops. It is estimated that BNF contributes 50 to 70 Tg of N annually in agricultural systems (Herridge et al. 2008), accounting for about 16% of global N supply (Liu et al. 2010). In comparison, mineral N fertilizers contribute about half of the global N input while manures,

recycled crop residues, and atmospheric deposition each may contribute 8 to 13% of the total N input (Liu et al. 2010).

Nitrogen naturally occurs in two isotopic forms, ^{15}N and ^{14}N , and ^{14}N is much more abundant relative to ^{15}N . In nitrogen-fixing plant tissue, ^{15}N content is the sum of native N acquired from the soil, N_2 fixed from the atmosphere and N from the applied ^{15}N fertilizer (Danson et al. 1993). The proportion of nitrogen derived from the atmosphere (%Ndfa) is determined by comparing the ^{15}N content of non-fixing reference plants to that of the N_2 fixing plants. Isotope-based techniques are popular in quantifying N_2 -fixation (Carlsson and Huss-Danell 2003), and ^{15}N isotope dilution technique is used widely for measuring time-integrated biological nitrogen fixation in field settings (Unkovich and Pate 2000).

On the semiarid Canadian prairies, the area seeded to chickpea, field pea, and lentil has increased substantially in the recent two decades (FAOSTAT 2014). Many new market classes and cultivars of these pulse crops have been released with the goal of diversifying genetic basis with improved resistance to pests. Several groups have studied the genotypic effect of different pulses such as dry bean (Farid and Navabi 2015), lentil (Sarker and Singh 2015), field pea and lentil (Abi-Ghanem et al. 2011), and field pea (Voisin et al. 2003) on BNF. However, the effect of genotypic variability of pulses on BNF as well as relationship between BNF and crop yield has not been addressed extensively under semiarid growth conditions. Therefore, the objectives of this study were (i) quantify the BNF ability of various pulse crops and genotypes in the semiarid Canadian prairies, and (ii) determine the effect of BNF on plant growth and productivity.

Materials and Methods

Field experiments were conducted at the Agriculture Agri-Food Canada (AAFC) Research Farm near Swift Current, Saskatchewan (50°25'N, 107°44'W) in 2008, 2009 and 2010. The soil was a Brown Chernozem with an organic C content of 20 g kg⁻¹, pH (CaCl₂) of 6.5 in the 0- to 15-cm depth, and a silt loam texture with 28% sand, 49% silt and 23% clay. In each of the three study years, the experiment was established on a different field location on the Research Farm. No pulse crops had been grown on the land for five years before the experiment was initiated.

Treatments and experiment design

Treatments included eight market classes of the pulse crops, each with two or three cultivars, resulting in 23 species-cultivar combinations: (1) field pea with green cotyledons (cvs. CDC Cooper, CDC Striker and Venture), (2) field pea with yellow cotyledons (cvs. CDC Golden, CDC Handel and CDC Meadow), (3) desi chickpea (cvs. CDC Anna, CDC Nika and CDC Vanguard), (4) kabuli chickpea (cvs. Amit, CDC Frontier and CDC Luna), (5) large green lentil (cvs. CDC Glamis, Laird and CDC Sedley), (6) small green lentil (cvs. CDC Meteor, CDC Richlea and CDC Viceroy), (7) extra small red lentil (cvs. CDC Imperial and CDC Robin), and (8) small red lentil (CDC Blaze, CDC Impact and CDC Rouleau). In addition, one cultivar of each of dry bean (cv. Pintium) and faba bean (cv. Blitz) was included in the study. To quantify biological nitrogen fixation by pulses, barley (AC Metcalfe) was used as reference crop. The experiment was arranged in a randomized, complete block design with four replicates.

Plot management

Pulse crops were seeded into standing wheat (*Triticum aestivum* L.) stubble, using a four-row plot seeder, equipped with side-band openers and fertilizer and inoculant boxes. Plot size was 8 ×

2 m. Pulse seeds were inoculated with commercial *Rhizobium* inoculants according to the manufacturer's recommendation. Seeding occurred on 06 May in 2008, 12 May in 2009 and 14 May in 2010 when noon soil temperature at the 10-cm depth was greater than 9 °C (Table 1). The reference barley crop was seeded at a seeding rate of 113 kg ha⁻¹.

Soil of the experimental plots was analyzed each year for pH and major nutrients (Table 2). All plots received a blanket application of 11-51-0 (N-P₂O₅-K₂O) fertilizer applied with the seed to supply a total of 9.5 kg N (start-N) and 20 kg P ha⁻¹. Annual herbicide applications included Trifluralin 10G (0.85 kg a.i. of ethalfluralin ha⁻¹) applied in the previous fall, a pre-seeding application of glyphosate (1.2 L a.e. ha⁻¹) and a post-emergent application of metribuzin (160 g a.i. ha⁻¹) and sethoxydim (30 g a.i. ha⁻¹). Appropriate plant protection measures were taken depending on weather conditions and disease infestation.

Data Collection

At maturity, aboveground plant biomass was sampled from a 1 m² area in each plot, oven-dried at 70 °C for 7 to 10 days to constant weight and weighed. Seeds were threshed and weighed, and straw yield was recorded. Nitrogen accumulation in seed and straw was calculated as the product of biomass and N concentration of the plant parts. N harvest index (NHI) was calculated as the amount of N accumulated in the seed divided by the amount of N accumulated in the aboveground plant biomass. A sub-sample of seed and straw from each plot was analyzed for N concentration by an automated CN analyzer (Carlo-Erba NA-1500 series 2, Milan, Italy).

The %Ndfa was estimated using the ¹⁵N isotope dilution method. Two micro-plots, 0.5 m × 4 rows, were established in accordance with each pulse plot; one within the pulse plot and the other in the adjacent barley pathway. The barley micro-plot was the reference crop for the

BNF measurements. A solution of 10 atom % ^{15}N -ammonium nitrate ($\text{NH}_4^{15}\text{NO}_3$) was applied at a rate of $4 \text{ kg } ^{15}\text{N ha}^{-1}$ to each micro-plot at the 3-4 leaf stage.

At full maturation, the plants in the two inner rows of each micro-plot were hand harvested. The plant samples were separated into seed and straw portions, oven-dried at 70°C to constant weight, ground into fine powder, and stored into glass bottle until analysis. Nitrogen concentration and atom % ^{15}N in the samples of pulse plants and barley reference plants were determined using an automated CN analyzer (Carlo-Erba NA-1500 series 2, Milan, Italy) interfaced to a continuous flow isotope ratio mass spectrometer (Micromass Optima, Manchester, UK). Total N was determined by Kjeldahl digestion method (Takahashi et al. 2001):

Percentage of N derived from atmosphere (%Ndfa) was calculated as:

$$\% \text{Ndfa} = 1 - (\text{atom } \% ^{15}\text{N excess}_{\text{pulse}} / \text{atom } \% ^{15}\text{N excess}_{\text{barley}}) \times 100 \quad (1)$$

Where, atom % ^{15}N excess is the atom % ^{15}N in the pulse or barley shoot in excess of natural abundance level (0.3663).

Total amount of N_2 fixed ($N_{\text{fixed total}}$) was calculated as:

$$\text{N}_2 \text{ fixed}_{\text{total}} = \% \text{Ndfa} \times \text{total N}_{\text{pulse}} \quad (2)$$

Where, total N_{pulse} is the amount of N in the shoot tissue calculated as:

$$\text{Total N}_{\text{pulse}} = \% \text{N}_{\text{shoot}} \times \text{biomass}_{\text{shoot}} (\text{straw} + \text{seed}) \quad (3)$$

Estimates of the amounts of N fixed by pulse plants are based on the assumption that N_2 is fixed homogeneously throughout the plant species.

Statistical analysis

Analyses of variance on different traits, including grain and straw yield, quantity of nitrogen fixed, and NHI were performed using the Proc ANOVA in SAS9.3 (SAS Institute Inc, Cary NC).

Species, cultivar, year and the interactions between species $\times$ year and cultivar $\times$ year were considered fixed effects, while block was considered a random effect. ANOVA was conducted for each year and also in combination across three years, in comparing the differences among pulse species, and among cultivars within the chickpea, field pea and lentil.

Results and Discussion

The environmental conditions during the course of the study varied considerably (Table 1). The killing frost occurred on 21 May in 2009 when pulse seedlings just emerged, which caused significant damage to the seedlings. Total rainfall was the highest in 2010 at 560 mm, and it was 356 mm in 2008 and 214 mm in 2009. The 2009 rainfall was only 74% of the long-term (1983-2012) average.

Seed and straw yields

Seed and straw yields varied significantly ($p < 0.0001$; Table 3) among the pulse species evaluated in the study. Pulses showed significant variation for seed yield in each year. In 2008, field pea showed highest yield at 2244 kg ha⁻¹ and chickpea at 2227 kg ha⁻¹ and they did not differ significantly (Table 4). However, this trend did not continue for chickpea in either 2009 or 2010 but field pea had a trend similar to the previous year with a mean yield of 2039 kg ha⁻¹ in 2009 and 2972 kg ha⁻¹ in 2010. Average yield of lentil was in the medium range over the years with 1904, 1272 and 2313 kg ha⁻¹ in 2008, 2009 and 2010, respectively. Statistically, the yield of lentil was higher than dry bean and chickpea in 2008 and 2010, respectively. Faba bean showed a huge variation for seed yield, and had the lowest yield (886 kg ha⁻¹) along with dry bean (870 kg ha⁻¹) in 2008. Faba bean produced the highest quantity of seed in 2009 and 2010

accompanied by field pea, which was significantly higher than those of chickpea, dry bean and lentil (Table 4). On average, field pea produced highest seed yield and dry bean the lowest, although there was a highly significant effect of growing year and year $\times$ species interaction on seed yield (Table 3).

At the cultivar level, chickpea showed a wide variation in seed yield from year to year but the yield difference among the cultivars was significantly only in 2009 (Fig 1a). In combined analysis of the data, significant effect of growing year ($p < 0.0001$) and year $\times$ cultivar interaction ($p = 0.0172$) on yield was observed (Table 3). For field pea, significant yield differences among the cultivars was observed only in 2010 ($p = 0.05$) where cultivar CDC Meadow produced highest seed yield (3393 kg ha^{-1}) and it was statistically similar to CDC Handel (3251 kg ha^{-1}). In combined analysis, yield variation among the cultivars was significant ($p = 0.0055$) with Venture and CDC Handel at the top of the table (2706 and 2640 kg ha^{-1} , respectively) followed by CDC Meadow (2480 kg ha^{-1}) but all three cultivars were statistically similar. Significant effect of growing year ($p < 0.0001$) and year $\times$ cultivar interaction ($p = 0.0481$) (Table 3) on this trait of field pea cultivars were also observed. Lentil cultivars showed a general trend over the years with moderate yield in 2008, low yield in 2009 and high yield in 2010 (Fig 1c). Of the years, the cultivars showed significant yield differences only in 2009 with CDC Meteor as the best performer followed by CDC Rouleau. In the analysis over years, impact of growing year was highly significant ($p < 0.0001$) along with variation among the cultivars ($p = 0.0003$) but year $\times$ cultivar interaction was not significant (Table 3). Among the cultivars, CDC Meteor was highest performer for seed yield (2371 kg ha^{-1}) followed by CDC Rouleau (2017 kg ha^{-1}) whereas CDC Imperial gave lowest yield (1570 kg ha^{-1}).

For straw yield, pulse species showed significant variation in a specific year and in combined analysis over the years (Table 3). In 2008, field pea produced highest quantity of straw (4095 kg ha^{-1}) followed by lentil (3340 kg ha^{-1}) and chickpea (2675 kg ha^{-1}) (Table 4). In 2009, field pea again produced highest quantity of straw (2234 kg ha^{-1}) and it was statistically similar to those of faba bean and lentil. Highest amount of straw was obtained from faba bean (4634 kg ha^{-1}) in 2010 and it was statistically similar to those of chickpea and lentil. Straw yield of field pea (3481 kg ha^{-1}) was much lower than that of faba bean although seed yield was similar to faba bean for the same year (Table 4). It is possible that seed yield of field pea increases at high moisture condition but straw yield does not increase proportionally. McPhee and Muehlbauer (1999) also observed increased seed yield relative to straw yield in field pea cultivars under elevated moisture condition. In general, straw yield of chickpea was inversely related to seed yield in 2008 and 2010 but it was proportionate to seed yield in 2009. Straw yield of dry bean and faba bean was proportionate to seed yield across years. A similar proportional trend was also observed between straw and seed yield of lentil over the years (Table 4). In combined analysis over years, highly significant effect of growing year and year $\times$ species interaction (Table 3) was observed on this trait.

At the cultivar level, chickpea cultivars showed significant differences for straw yield in 2009 ($p = 0.0089$) and 2010 ($p = 0.0154$) where CDC Frontier showed better performance (Fig 2a). In combined analysis over years, cultivars showed significant differences ($p = 0.0009$) with CDC Frontier as the best performer (3435 kg ha^{-1}) while CDC Anna gave lowest yield (2688 kg ha^{-1}). Desi chickpea plants are typically shorter than kabuli chickpea plants and CDC Anna is a desi cultivar, which explains the lowest straw yield. Impact of growing year ($p < 0.0001$) and year $\times$ cultivar interaction on straw yield were also significant ($p = 0.0063$) (Table 3). However,

pattern of straw yield did not match with the pattern of seed yield of chickpea cultivars. For example, CDC Frontier appeared as top performer for straw yield in combined analysis whereas it positioned at the bottom for seed yield (1531 kg ha^{-1}). Straw quantity produced by field pea cultivars varied significantly only in 2008 ($p = 0.0355$) (Table 3) and cultivar CDC Cooper produced highest amount of straw followed by Venture (Fig 2b). In combined analysis, growing year ($p < 0.0001$) and year $\times$ cultivar interaction ($p = 0.0192$) significantly affected the straw yield. Similar to chickpea, straw yield of field pea cultivars did not match with the seed yield of those cultivars. For example, seed yield of Venture and CDC Handel was highest in combined analysis whereas CDC Handel was at the bottom for straw yield (3138 kg ha^{-1}). For straw yield, lentil cultivars showed highly significant variation ($p < 0.0001$) in 2009 with Laird, CDC Sedley and CDC Glamis at the top of the table and those were significantly different from other cultivars (Fig 2c). All of the above three cultivars belong to large green lentil class and plants of this class are generally bushier. So, higher straw yield is expected from these cultivars. In combined analysis, growing year ($p < 0.0001$) and cultivars ($p = 0.0012$) (Table 2) showed significant effect on straw yield. For overall straw yield, cultivar Laird appeared as highest performer (3934 kg ha^{-1}) followed by CDC Meteor (3340 kg ha^{-1}). Unlike chickpea or field pea, overall seed yield was somewhat aligned to straw yield where CDC Meteor showed highest seed yield (2371 kg ha^{-1}) which was significantly higher than those of other cultivars. Significant effect of genotypes on the seed and straw yield of lentil was reported elsewhere too (Sarker and Singh 2015). Seed and aboveground biomass yield were reported to be differed significantly among chickpea genotypes (Radicetti et al. 2012). Krishnamurthy et al. (2011) also observed significant genotype $\times$ environment interaction for yield among a wide range of chickpea genotypes exposed to heat stress. In another study, Tavakkoli et al. (2012) reported differential effect of faba bean

genotypes on grain yield under salt stress. Carranca et al. (1999) observed significant cultivar effects on seed and straw yields of faba bean and pea depending on experimental conditions. Significant effect of growing year on seed/straw yield of pulses or cultivars suggests that pulses or cultivars of a pulse crop differ in their yield performance depending on environmental conditions. Significant year $\times$ pulse/cultivar interaction was also observed in our study which indicates that pulse species or cultivars within a species respond differently to the environment. Effect of locations and growing years on seed yield of field pea and lentil was reported previously (Ito et al. 2016). So, it is important to select the appropriate pulse crop to maximize the yield in a specific growth condition.

Nitrogen fixation

The amount of N fixed by the symbiotic relationship between legumes and the soil rhizobial bacteria is determined by the relative dependence of the crop on biological nitrogen fixation for growth (i.e. the proportion of the crop N derived from atmospheric N₂, %Ndfa) and the amount of N accumulated by the crop over the growing season (Jensen et al. 2010). In our study, there were significant variations among pulse species for %Ndfa and biological nitrogen fixation over the years (Table 3 & 5). The %Ndfa was generally lowest in 2008 and highest in 2010, and it was highly variable in case of dry bean. In 2008, faba bean showed highest values and it was statistically similar to that of field pea and lentil (Table 5). The %Ndfa of Chickpea was moderate but it was not statistically different to that of field pea or lentil. In 2009, faba bean showed highest value and it was statistically similar to field pea. Lentil and chickpea showed moderate %Ndfa and were statistically similar to field pea. In 2010, faba bean had highest

%Ndfa and was statistically similar to that of chickpea but different from those of field pea and lentil. Dry bean gave lowest %Ndfa value in all three years. In combined analysis over years, the effect of growing year and pulse species on %Ndfa was highly significant ($p < 0.0001$) (Table 3). Among the pulses, faba bean appeared as the top performer with a %Ndfa of 67.48. The values obtained from field pea, lentil and chickpea were statistically similar and dry bean showed lowest %Ndfa (26.31) (Table 5).

At the cultivar level, %Ndfa of chickpea cultivars did not differ significantly but the effect of growing year was highly significant (Table 3). For field pea cultivars, significant variation was observed only in 2009 ($p = 0.0134$) (Table 3) where all the cultivars except CDC Golden grouped together and were superior to that of Golden (32.08) (Fig 3b). Growing year also showed significant effect on %Ndfa of field pea cultivars (Table 3). Among the lentil cultivars, significant variation was observed in 2009 ($p = 0.0154$) and 2010 ($p = 0.0097$). In 2009, cultivar CDC Meteor showed highest value (70.71%) while CDC Richlea showed lowest value (20.11%) (Fig 3c). In 2010, cultivar CDC Rouleau showed highest value (83.25%) while CDC Meteor showed a moderate value (67.48%), but the cultivars were statistically similar. In combined analysis, variation among lentil cultivars was observed with cultivar Laird at the top (62.39%) along with CDC Meteor and CDC Rouleau while CDC Sedley showed lowest %Ndfa (36.45%). Significant effect of growing year ($p < 0.0001$) and year $\times$ cultivar interaction ($p = 0.0481$) (Table 3) on %Ndfa of lentil cultivars was also observed. In semiarid Australia, Jensen et al. (2010) found a %Ndfa for pea, chickpea, lentil and fababean of 56, 50, 60 and 88, respectively, and similarly, Peoples et al. (2009) found the %Ndfa value for pea, chickpea and fababean of 59, 54 and 74, respectively. In a previous Saskatchewan study, the %Ndfa of pea, lentil and fababean was 55, 60 and 88, respectively (Walley et al. 2007). The %Ndfa of pulses in

our study is similar to the values obtained in previous studies. Variation in biological nitrogen fixation is common among pulses and within the genotypes of a specific species and it is sensitive to environmental and edaphic factors (Walley et al. 2007).

Amount of N fixed by individual pulse species showed a wide range of variation and the variation was statistically significant in all three years (Table 5). Dry bean fixed lowest amount of N over the years while faba bean fixed highest amount, a trend which matched with the %Ndfa values obtained in our study. In 2008 and 2009, biological nitrogen fixation by field pea and faba bean was statistically similar while the amount fixed by faba bean in 2010 was much higher than that of field pea. In the analysis over years, biological nitrogen fixation by pulses differed significantly ($p < 0.0001$) and faba bean appeared as the best performer, and was statistically different from other pulses (Table 5). The effect of growing year and year $\times$ species interaction were also highly significant ($p < 0.0001$) for this trait indicating the opportunity of utilizing biological nitrogen fixation potentiality of different pulses in different growth environments to include suitable pulse crops in crop rotation.

We also analyzed biological nitrogen fixation ability of different cultivars within a species. At cultivar level, chickpea cultivars showed significant variation ($p = 0.0389$) in the analysis over years. Cultivar CDC Frontier fixed highest amount of N (65.33 kg ha^{-1}), while the amount was lowest for CDC Anna (39.02 kg ha^{-1}). The impact of growing year on biological nitrogen fixation by chickpea cultivars was highly significant (Table 3). In general, biological nitrogen fixation by the cultivars was low in 2009 and much higher in 2010. For field pea cultivars, only growing year had significant impact on biological nitrogen fixation. Significant differences among the cultivars of lentil were only observed when data from all three years were analyzed combined (Table 3). From the analysis, cultivar Laird fixed highest amount of N (70.32

kg ha⁻¹) while CDC Sedley fixed lowest amount (28.49 kg ha⁻¹), and the trend followed the trend of %Ndfa of lentil cultivars. The effect of growing year on biological nitrogen fixation by lentil cultivars was highly significant ($p < 0.0001$). In general, lentil cultivars showed moderate to good performance for biological nitrogen fixation in 2008 and 2010 while the cultivars had poor to moderate performance in 2009 with an exception of cultivar CDC Meteor which did comparatively better (37.3 kg ha⁻¹). However, overall biological nitrogen fixation by pulses, particularly by chickpea, faba bean and lentil was much higher in 2010 while it was lowest in 2009. It is possible that higher rainfall in 2010 had positive impact on dry matter accumulation of pulses (Table 4). Dry matter is the determining factor of biological nitrogen fixation in legumes (Carlsson and Huss-Danell 2003; Yang et al. 2010) and, therefore, more dry matter accumulation in 2010 may have promoted higher biological nitrogen fixation in pulses, particularly in chickpea, faba bean and lentil. Genotypic background is a key which affect biological nitrogen fixation in pulses, such as pea and lentil (Abi-Ghanem et al. 2011) and peanut (Htoon et al. 2013). Pulse biological nitrogen fixation is extremely sensitive to drought stress (Gálvez et al. 2005; Ladrera et al. 2007; Streeter 2003) which supports our results of low biological nitrogen fixation in 2009 due to low rainfall (Table 1).

Variation in the amount of N fixed by various pulse species has been reported by different groups in the past. Jensen et al. (2010) reported 83 kg ha⁻¹ biological nitrogen fixation by pea while the quantity was 61 kg ha⁻¹ according to Soon and Arshad (2004). In the study of Jensen et al. (2010), biological nitrogen fixation by chickpea, lentil and faba bean was 54, 50 and 135 kg ha⁻¹, respectively, while Peoples et al. (2009) showed that biological nitrogen fixation by chickpea, pea and faba bean was 54, 83 and 118 kg ha⁻¹, respectively. Those trends were somewhat similar to our observation although the amount of N fixed was relatively low in our

study. Previously, Yang et al. (2010) found lowest biological nitrogen fixation rate in Saskatchewan among 10 provinces of Canada, and hypothesized that it was due to more arid conditions in Saskatchewan. Our results also indicate a major impact of growing environment on %Ndfa and biological nitrogen fixation by pulses.

The amount of N fixed by legumes is also reflected in its NHI. In our study, NHI varied significantly among the species in 2008 and 2010 but not in 2009 (Table 3). In 2008, dry bean had highest NHI (0.76) followed by chickpea (0.74) while faba bean had lowest value (0.52) (Table 5). In 2010, dry bean appeared at the top (0.76) followed by field pea (0.70) while chickpea positioned at the bottom of the table with a NHI of 0.31. In the analysis over years, pulses varied significantly for this trait where dry bean gave highest NHI (0.76) followed by field pea and it was lowest for chickpea (0.59). Impact of growing year and year $\times$ species interaction was also highly significant for this trait (Table 3). In general, dry bean produced lower amount of biomass relative to other pulses and straw yield, in particular, was the lowest in all three years. The lowest straw yield contributed to lower shoot N content (straw + seed) and was the determining factor of highest NHI of dry bean.

At cultivar level, cultivars of chickpea varied significantly in 2008 and 2009 (Table 3). In 2008, cultivar CDC Vanguard showed highest value (0.80) and CDC Frontier had lowest value (0.68) (Fig 5a). In 2009, all cultivars except CDC Frontier grouped together and CDC Frontier showed significantly lower NHI (0.63). In 2010, chickpea cultivars had much lower NHI (0.23 – 0.39) compared to previous years. In 2010, the straw N content of the cultivars was much higher than seed N content which contributed to lower NHI. In the analysis over years, effect of growing year on chickpea NHI was highly significant (Table 3). Field pea cultivars showed significant variation for NHI in all three years (Table 3). In 2008, the NHI of field pea cultivars

were relatively low (0.5 – 0.64) in compare to 2009 and 2010, which is a reflection of higher straw yield relative to seed yield. In combined analysis over years, variation was significant among cultivars, and Venture, CDC Handel and CDC Meadow clustered at the top (Fig 5b). Values of CDC Striker and CDC Golden were moderate while NHI of CDC Cooper was lowest (0.61), which is in agreement with higher straw yield by CDC Cooper (Fig 2b). Growing year also showed significant effect on NHI of field pea cultivars (Table 3). For lentil cultivars, NHI varied significantly in all three years (Fig 5c). In combined analysis, effect of growing year, variation among cultivars and year $\times$ cultivar interaction were highly significant (Table 3). Among the cultivars, CDC Viceroy and CDC Meteor gave highest NHI (0.70) while Laird had lowest NHI of 0.58. Seed yield of Laird was moderate while straw yield was highest among the cultivars (Fig 1c and 2c) and these factors contributed to lowest NHI of Laird. Among chickpea, field pea and lentil, the later showed a relatively better utilization of N in grain production. The NHI is used to measure N partitioning in crop plants and gives an indication of plants ability to utilize acquired N for grain production. A lower NHI indicates that N utilization was less for grain yield relative to straw yield, which is particularly true for chickpea in 2010 in our study. Gan et al. (2010) reported a NHI value of 0.86, 0.55 and 0.56 for chickpea, lentil and field pea under rain fed condition that are close to NHI of lentil and filed pea from our study. Genetic variability is reported to exist for NHI across crop genotypes, from species to species and within the genotypes of same species (Fageria and Baligar 2003). Yang et al. (2010) also indicated the influence of different factors, including cultivars, soil types and weather conditions on NHI. The NHI variability across pulse species, cultivars and growing year in our study also supports this notion.

Correlation of %Ndfa with soil N uptake and soil mineral N

The %Ndfa of a legume is not only determined by a legume genotype and rhizobia, but also depends on the interaction between plant-available soil N and legume growth (Unkovich and Pate 2000). Soil mineral N and biological nitrogen fixation are complementary in meeting the N requirements of a legume crop. Studies have demonstrated a negative correlation between biological nitrogen fixation in pulses and the available soil N (Kiers et al. 2003; Salvagiotti et al. 2008; Schipanski et al. 2010). On the other hand, very low concentrations of inorganic N have been reported to increase biological nitrogen fixation (Gan et al. 2004). In our study, %Ndfa value among pulse species was negatively correlated to soil N uptake and soil mineral N (NO_3^- -N and NH_4^+ -N in the soil) (Table 6). In 2008 and 2009, the correlation coefficient of chickpea, lentil and pea were significantly negative with soil N uptake and soil mineral N. In 2010, only pea showed significant negative correlation with soil N uptake and soil mineral N, while the negative correlation of chickpea and lentil was not significant. In the present study, faba bean showed negative correlation in 2008 and positive correlation in 2009 and 2010 between biological nitrogen fixation and N uptake; the inconsistent correlation between the two traits for faba bean was probably because this crop demands more soil N than other pulses such as chickpea (Rose et al. 2016). Faba bean can maintain a high rate of BNF in the presence of high amounts of available N in the soil (Köpke and Nemecek 2010). Higher demand for N and lower inhibitory effect of soil N on BNF by faba bean might be the cause of insignificant negative or positive correlation of faba bean %Ndfa with soil N uptake and soil mineral N observed in our study.

Conclusion

The data obtained from the 3-yr field study allowed the detailed analysis of the effect of pulse species and cultivars on biological nitrogen fixation and its relationship to seed yield. In general, field pea had consistent biological nitrogen fixation ability with highest seed yield across years, suggesting that field pea is more suitable for cultivation across the semiarid Prairie than other pulses evaluated. At the cultivar level, considering biological nitrogen fixation ability and seed yield, the chickpea cultivar Amit, pea cultivar CDC Meadow and lentil cultivar CDC Meteor were more preferable to other cultivars in each of the pulse species for the semiarid environment. Only one fababean and one dry bean cultivar were included in this study as there were no other genotypes available for the experimental area by the time the study was initiated. More research is required to evaluate the BNF ability and yield potential for these two latter pulses in the future. The study highlighted the importance of environmental factors affecting BNF, and particularly the negative effect of low rainfall on BNF and grain yield. Our results show large variation among pulse species and cultivars in BNF and emphasize the importance of selecting appropriate species and cultivar to exploit the potential of BNF in semiarid environment and develop sustainable pulse production systems.

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Figure caption

Fig. 1. Seed yield (kg/ha) of chickpea (a), field pea (b) and lentil (c) cultivars in 2008, 2009 and 2010. Values are presented as mean of four replicates $\pm$ standard error (SE). Yield of chickpea cultivars varied significantly only in 2009 ($p = 0.0141$) while yield of field pea cultivars varied significantly in 2010 ($p = 0.05$). Significant variation among lentil cultivars was observed in 2009 ($p = 0.0175$).

Fig. 2. Straw yield (kg/ha) of chickpea (a), field pea (b) and lentil (c) cultivars in 2008, 2009 and 2010. Values are presented as mean of four replicates $\pm$ SE. Significant variation among chickpea cultivars was observed in 2009 ($p = 0.0083$) and 2010 ($p = 0.0154$) while variation was significant among field pea cultivars in 2008 ($p = 0.0083$). Lentil cultivars differed significantly among themselves only in 2009 ($p = <0.0001$).

Fig. 3. %Ndfa of chickpea (a), field pea (b) and lentil (c) cultivars in 2008, 2009 and 2010. Values are presented as mean of three replicates $\pm$ SE. Chickpea cultivars did not vary significantly among themselves in the study years while variation among pea cultivars was significant in 2008 ($p = 0.0134$). Lentil cultivars showed significant variation in 2009 ($p = 0.0154$) and 2010 ($p = 0.0097$).

Fig. 4. Amount of nitrogen fixed (kg/ha) by chickpea (a), field pea (b) and lentil (c) cultivars in 2008, 2009 and 2010. Values are presented as mean of three replicates $\pm$ SE. Variation among the cultivars of respective pulses was not significant in a specific year.

Fig. 5. Nitrogen harvest index (NHI) of chickpea (a), field pea (b) and lentil (c) cultivars in 2008, 2009 and 2010. Values are presented as mean of four replicates $\pm$ SE. Chickpea cultivars varied significantly in 2008 ($p = 0.0028$) and 2009 ($p = 0.0042$). Field pea cultivars showed significant variation in all three study years ($p = 0.001$, 0.0003 and 0.0005 in 2008, 2009 and 2010, respectively). Lentil cultivars also showed significant variation in all three study years ($p = 0.0273$, <0.0001 and 0.0091 , respectively).

Table 1: Environmental conditions, including rainfall during the 3 years of investigation

Year:	2008	2009	2010	30 year average rainfall (1983-2012)
Seeding Date:	06-May	12-May	14-May	-
Seedbed Temperature at 5 cm:	9 °C	15 °C	17 °C	-
Last Spring Killing Frost:	10-May	21-May	08-May	-
First Fall Killing Frost:	09-Oct	28-Sep	18-Sep	-
Rainfall (mm) by Month:				
April	18.7	15.2	51.0	22.58
May	32.2	25.1	111.6	51.01
June	142.7	37.8	126.3	78.34
July	70.5	51.8	75.9	54.36
August	71.8	61.3	95.8	46.3
September	20.1	22.5	99.3	35.65
Growing Season Rainfall (mm) Total:	356.0	213.7	559.9	288.24

Table 2: Soil pH and nitrogen (N), phosphorus (P), potassium (K), and sulphur (S) status during the 3 years of investigation

pH/nutrient	2008		2009		2010	
	Previous fall	Spring	Previous fall	Spring	Previous fall	Spring
pH	6.5		6.5		6.5	
N (kg ha ⁻¹)	26	42	6	9	9	22
P (kg ha ⁻¹)	23	31	20	28	36	34
K (kg ha ⁻¹)	388	NA	481	NA	326	NA
S (kg ha ⁻¹)	34	NA	21	NA	53	NA

NA – Not analyzed

Table 3: Effect of various pulses on biological nitrogen fixation and yield

Effect	%Ndfa (isotope dilution)	N ₂ fixed (isotope dilution)	NHI	Seed yield (kg ha ⁻¹)	Straw yield (kg ha ⁻¹)
Species comparison*					
2008	0.0002	<.0001	0.0034	<.0001	<.0001
2009	0.0022	0.0253	0.0836	0.0013	0.0022
2010	0.0006	0.0004	<.0001	0.0001	<.0001
Species combined	<.0001	<.0001	<.0001	<.0001	<.0001
Year	<.0001	<.0001	<.0001	<.0001	<.0001
Year × species interaction	0.0561	<.0001	<.0001	<.0001	<.0001
Cultivar comparison**					
Chickpea 2008	0.7014	0.3956	0.0028	0.1654	0.1804
Chickpea 2009	0.4315	0.6395	0.0042	0.0141	0.0083
Chickpea 2010	0.1627	0.0701	0.5161	0.1936	0.0154
Chickpea combined	0.268	0.0389	0.0937	0.4343	0.0009
Year	<.0001	<.0001	<.0001	<.0001	<.0001
Year × cultivar interaction	0.7907	0.0769	0.3445	0.0172	0.0063
Field pea 2008	0.7742	0.4273	0.001	0.0567	0.0355
Field pea 2009	0.0134	0.0703	0.0003	0.2079	0.0797
Field pea 2010	0.8455	0.6509	0.0005	0.0504	0.4707
Field pea combined	0.6578	0.5147	<.0001	0.0055	0.1624
Year	0.0015	0.0001	<.0001	<.0001	<.0001
Year × cultivar interaction	0.2165	0.2181	0.2525	0.0481	0.0192
Lentil 2008	0.7940	0.5509	0.0273	0.3566	0.1018
Lentil 2009	0.0154	0.0676	<.0001	0.0175	<.0001
Lentil 2010	0.0097	0.1438	0.0091	0.1075	0.0702
Lentil combined	0.0474	0.0372	<.0001	0.0003	0.0012
Year	<.0001	<.0001	<.0001	<.0001	<.0001
Year × cultivar interaction	0.0481	0.1831	<.0001	0.8918	0.0580

* Comparison of the differences among species when averaged across cultivars within each species.

** Comparison of cultivars within the chickpea, field pea, and lentil species.

Table 4: Straw and seed yield in various pulse crops

Pulses	Seed yield (kg ha ⁻¹)			Straw yield (kg ha ⁻¹)		
	2008	2009	2010	2008	2009	2010
Chickpea	2227 ± 88 ^{ab}	1520 ± 58 ^b	1263 ± 117 ^c	2675 ± 87 ^c	1667 ± 58 ^{bc}	4612 ± 178 ^a
Dry bean	870 ± 150 ^c	1057 ± 166 ^c	1902 ± 203 ^b	1740 ± 238 ^d	1263 ± 158 ^c	1713 ± 165 ^c
Faba bean	886 ± 132 ^c	1948 ± 264 ^a	3009 ± 343 ^a	2015 ± 217 ^d	2032 ± 225 ^{ab}	4634 ± 328 ^a
Field pea	2244 ± 108 ^a	2039 ± 68 ^a	2972 ± 100 ^a	4095 ± 154 ^a	2234 ± 70 ^a	3481 ± 96 ^b
Lentil	1904 ± 57 ^b	1272 ± 44 ^{bc}	2313 ± 90 ^b	3340 ± 78 ^b	1968 ± 71 ^{ab}	4196 ± 151 ^a
<i>p</i> -value	<.0001	0.0013	0.0001	<.0001	0.0022	<.0001

Data presented as mean ± standard error. Means with the same letters in a column are not significantly different; *p* represents the level of significance.

Table 5: Biological nitrogen fixation and nitrogen harvest index (NHI) in various pulse crops

Pulses	%Ndfa			N fixed (kg ha ⁻¹)			NHI		
	2008	2009	2010	2008	2009	2010	2008	2009	2010
Chickpea	39.22 ± 4.47 ^b	43.31 ± 3.42 ^{bc}	72.15 ± 2.17 ^{ab}	30.59 ± 3.63 ^c	21.02 ± 1.83 ^{bc}	103.59 ± 7.03 ^a	0.74 ± 0.01 ^{ab}	0.73 ± 0.01 ^{ab}	0.31 ± 0.03 ^d
Dry bean	0.72 ± 0.11 ^c	32.45 ± 8.92 ^c	45.76 ± 7.8 ^c	0.76 ± 0.32 ^d	15.37 ± 5.27 ^c	11.87 ± 2.23 ^c	0.76 ± 0.03 ^a	0.77 ± 0.02 ^a	0.76 ± 0.02 ^a
Faba bean	54.34 ± 7.4 ^a	68.39 ± 4.17 ^a	79.72 ± 3.82 ^a	49.52 ± 3.64 ^{ab}	45.35 ± 10.9 ^a	107.69 ± 16.06 ^a	0.52 ± 0.07 ^d	0.72 ± 0.02 ^{ab}	0.61 ± 0.05 ^{bc}
Field pea	43.29 ± 3.4 ^{ab}	56.23 ± 3.31 ^{ab}	62.33 ± 3.86 ^b	58.28 ± 6.15 ^a	36.59 ± 3.78 ^{ab}	68.5 ± 4.77 ^b	0.59 ± 0.01 ^{cd}	0.76 ± 0.01 ^{ab}	0.70 ± 0.01 ^{ab}
Lentil	41.5 ± 3.2 ^{ab}	50.59 ± 2.87 ^b	68.49 ± 3.04 ^b	38.24 ± 3.12 ^{bc}	23.03 ± 2.09 ^{bc}	86.76 ± 5.57 ^{ab}	0.64 ± 0.01 ^{bc}	0.71 ± 0.01 ^b	0.60 ± 0.01 ^c
<i>p</i> -value	0.0002	0.0022	0.0006	<.0001	0.0253	0.0004	0.0034	0.0836	<.0001

Data presented as mean ± standard error. Means with the same letters in a column are not significantly different; *p* represents the level of significance.

Table 6: Correlation coefficient of %Nd_fa with soil N uptake and mineralized N in the soil

Year	Species	Data pairs (n)	Variable	%Nd _f a (isotope dilution)	Soil N uptake
2008	Chickpea	18	Soil N uptake	-0.92***	0.82***
			Mineralized N	-0.81***	
	Faba bean	3	Soil N uptake	-0.99	0.84
			Mineralized N	-0.92	
	Lentil	33	Soil N uptake	-0.75***	0.88***
			Mineralized N	-0.66***	
	Pea	18	Soil N uptake	-0.67**	0.91***
			Mineralized N	-0.77***	
	Dry bean	3	Soil N uptake	-0.99	0.91
			Mineralized N	-0.95	
2009	Chickpea	18	Soil N uptake	-0.92***	0.91***
			Mineralized N	-0.89***	
	Faba bean	3	Soil N uptake	1.00*	0.97
			Mineralized N	0.98	
	Lentil	33	Soil N uptake	-0.58***	0.91***
			Mineralized N	-0.51**	
	Pea	18	Soil N uptake	-0.59**	0.85***
			Mineralized N	-0.69***	
	Dry bean	3	Soil N uptake	-0.98	0.97
			Mineralized N	-1.00*	
2010	Chickpea	18	Soil N uptake	-0.25	0.85***
			Mineralized N	-0.41	
	Faba bean	3	Soil N uptake	0.24	0.99
			Mineralized N	0.08	
	Lentil	33	Soil N uptake	-0.25	0.95***
			Mineralized N	-0.25	
	Pea	18	Soil N uptake	-0.69***	0.85***
			Mineralized N	-0.54*	
	Dry bean	3	Soil N uptake	-0.56	1.00*
			Mineralized N	-0.57	

*, ** and *** significant at 5%, 1% and 0.1% levels, respectively

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.