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Nodulation and nitrogen accumulation in pulses vary with species, cultivars, growth stages and environments

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ABSTRACT

Biological N₂-fixation underpins the role of pulse crops in the development of sustainable cropping systems, but it is uncertain how nodulation and N accumulation may differ with pulse species, cultivars and environments. This 3-year field study investigated nodulation at the early and late flowering stages and seed and straw N uptake for chickpea (*Cicer arietinum* L.), dry bean (*Phaseolus vulgaris* L.), faba bean (*Vicia faba* L.), field pea (*Pisum sativum* L.) and lentil (*Lens culinaris* Medik.). At early flowering, all pulses except dry bean had more nodules in the wetter (2010) than drier year (2009). Faba bean had the most nodules followed by field pea and chickpea, while the nodulation varied with plant growth stages and environments. For both pea and lentil, more nodules were observed at early flowering, but higher nodule biomass was obtained at late flowering. Chickpea had higher nodule biomass at late than early flowering but number of nodules varied with year. Seed N uptake was highest in field pea, while straw N uptake was highest in faba bean. Our results suggest a possibility of improving pulse N₂-fixation by targeting nodule numbers and nodule biomass, although the outcome of plant N uptake will vary with environments.

Key words: nodule number, nodule biomass, nitrogen uptake, nitrogen concentration, pulses, dryland cropping systems

INTRODUCTION

Globally, pulses are a group of crops primarily grown for their protein-rich seeds that are used as human food, animal feeds, or in industrial products (Siddique et al. 2012). Pulse crops deliver a physiologically favourable matrix in human nutrition because of their seed structure and composition and are important in balancing the diet. Pulses can symbiotically fix atmospheric N₂, thus, they play an important role in the development of sustainable cropping systems by reducing fertilizer nitrogen (N) use (Miller et al. 2003), mitigating nitrogenous greenhouse gas emissions (Lupwayi and Kennedy 2007), and reducing the carbon footprint of grain products (Gan et al. 2014).

The long-term use of pulses in crop rotation can improve soil physical (Peoples et al. 2009; Williams et al. 2014), chemical (Bazghaleh et al. 2016; St. Luce et al. 2016) and biological properties (Borrell et al. 2016; Yang et al. 2012), as well as reduce soil-borne pathogens (Bainard et al. 2017). Pulse plants meet their N demand through assimilating soil and fertilizer N (Gan et al. 2015) and fixing atmospheric N through symbiosis. The N accumulated in different plant tissues varies with many factors, including soil N availability (Yang et al. 2015), N₂-fixation efficiency (Hossain et al. 2016), plant genotypes (Bazghaleh et al. 2015), and cropping sequences in rotation systems (Borrell et al. 2016). During seed filling, the N requirements of developing seeds are generally higher than fixed N₂ and soil N uptake, so the N accumulated in vegetative tissues is translocated to the filling seeds (Salon et al. 2001; Schiltz et al. 2005). Remobilisation of N from vegetative tissues to reproductive organs plays an important role in seed yield. However, N remobilisation varies largely among pulses and can be dependent on their N₂-fixation efficiency (Pampana et al. 2016). Knowledge of N accumulation or uptake in seed and straw are needed in efforts to quantify N removal from the soil and to determine N-use

efficiency from different sources. Such quantification would be useful in the establishment of more productive and effective N fertilizer management programs. Furthermore, N uptake in pulse plants is of importance for grain quality such as seed protein (Lithourgidis et al. 2011; Uddin et al. 2014).

Seed protein content in pulses is dependent on various factors, including biotic and environmental stresses (Bourion et al. 2010). Lecoecur and Sinclair (2001) reported seed N concentration of field pea averaging 35 mg g^{-1} , but may reach 46 mg g^{-1} depending on environmental conditions. However, no systematic study has been reported that evaluated N concentration and accumulation or the effect of environmental conditions on these traits in different pulse species and/or cultivars on the semiarid Canadian Prairie.

Pulse N_2 -fixation is largely dependent on the formation of nodules initiated by rhizobial strains (Abdellatif et al. 2016; Gan et al. 2010b). Nodules are the specialized organ formed on the roots wherein the rhizobia-induced symbiosis fixes atmospheric N_2 . Parameters such as the earliness of nodulation, nodule number, mass and colour, distribution on the root systems and longevity of the nodules are important for N_2 -fixation. Nodule formation begins approximately 2-3 weeks after sowing, but it may take longer depending on environmental conditions. Nodule numbers and N_2 -fixation rates generally peak during early- to mid-flowering stage (Voisin et al. 2003) and slow down with the onset of pod filling (Bethlenfalvay and Phillips 1977; Salon et al. 2001). However, information on nodulation for different pulse crops throughout the flowering stage is limited and warrants detailed investigation. From a developmental perspective, N is remobilized from vegetative parts to seeds during the seed filling stage to meet the N demand of the developing seeds. The remobilization of N induces plant senescence, which shortens the seed filling period (Salon et al. 2001), limiting seed yield.

Increased N supply from exogenous sources or by prolonged biological N₂-fixation (BNF) reduces the demand for leaf N remobilization to seeds which would delay plant senescence and increase the grain filling period. The life span of nodules and the contribution of N₂-fixation to the seed may be related to the metabolic activity of the relevant plant organs until senescence. In the scientific literature, there is a lack of systematic studies on the abundance of nodules at the early- and late-flowering stages, and thus the duration of nodulation in pulses is hard to predict. We designed the study with the objectives to (i) determine N accumulation in seed and straw of different pulse crops, and (ii) the dynamics of nodulation between early and late flowering stages in different pulse species and cultivars. We hypothesized that (i) nodulation pattern and N₂-fixation vary with pulse species and cultivars and (ii) nodulation changes with plant growth stages in the semiarid growing conditions.

MATERIALS AND METHODS

Field experiments were conducted at the Agriculture and Agri-Food Canada Research Centre, Swift Current (50°25'N, 107°44'W), Saskatchewan, in 2008, 2009 and 2010. The soil is 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 the three years of the study, the experiments were established at the same research farm but the plots were on different field locations. The plot area did not have any pulse grown within the five years before the initiation of the experiment.

Plant Material

Eight market classes of pulse crops, each with two or three cultivars, were included in the experiments, resulting in 23 species-cultivar combinations: (1) field pea with green cotyledons

(cvs. 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). One cultivar of each of dry bean (cv. Pintium) and faba bean (cv. Blitz) were also included in the study.

Experimental Design

Pulse crops were seeded into standing wheat (*Triticum aestivum* L.) stubble with an eight-row plot seeder equipped with side-band openers, and fertilizer and inoculant boxes. Plot size was 8 m × 2 m. Pulse seeds were inoculated with commercial *Rhizobium* inoculants following manufacturer's recommendation. Seeds were sown on 6, 12 and 14 May of 2008, 2009 and 2010, respectively, when noon soil temperature at the 10-cm depth was greater than 9°C. The experiment was organized in a randomized complete block design with four repetitions.

Plot Management

Initially 11-51-0 (N-P₂O₅-K₂O) fertilizer was applied to each plot with the seed to supply a total of 9.5 kg N (start-N) and 20 kg P ha⁻¹ as a basal dose. Weeds were managed using herbicide Trifluralin 10G (0.85 kg a.i., ethalfluralin ha⁻¹) applied 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 when necessary.

Data Collection

In each plot, the number of days from seeding to first flowering was recorded as the vegetative growth (VG) period. First flowering was determined when 15% of plants in a plot had started flowering and the late flowering stage was determined when 85% of plants completed flowering. Reproductive growth (RG) was recorded as the number of days from first flowering to full maturity. In each plot, the number of nodules per plant was counted at the early- and late-flowering stages each year except 2010 when nodule data were recorded only at early flowering. The plant-root-soil matrix of ten plants per plot were excavated from the top 60 cm soil layer, placed in containers, and brought to the laboratory. The soil on the plant-root-soil matrix was carefully removed, rinsed with running water, and dried with paper towels. The number of nodules on the roots of these plants was counted and the samples were oven-dried at 70°C to a constant weight for nodule dry weight determination.

At maturity, aboveground plant biomass was sampled from a 1-m² area in each plot. The entire plant samples were oven-dried to a constant weight in a drying oven at 70°C, and then separated into seed and straw. Seed and straw samples were ground into fine powder, weighed and stored into glass bottles until chemical analysis. The N concentrations in the seed and straw 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). The seed or straw N uptake was determined as the product of the dry weight and the N concentration in the respective tissues and area-scaled to kg N ha⁻¹.

Statistical Analysis

Analysis of variance on different traits, including nodule number and biomass plant⁻¹, N uptake in seed and straw, were performed using Proc ANOVA in SAS 9.3 (SAS Institute Inc., Cary, NC). Crop species and cultivars were considered as fixed effects, and block the random effect.

Due to significant year $\times$ treatment interactions, the treatment effect was analyzed separately for each year. Means comparisons were conducted with the Tukey HSD test at $P < 0.05$.

RESULTS AND DISCUSSION

Weather

Rainfall varied considerably during the study period; it was highest (560 mm) in 2010, intermediate (356 mm) in 2008 and lowest (214 mm) in 2009. The 30-year (1983-2012) average rainfall during the growing season (April – Sep) was 288 mm, as recorded by our weather station at the Research Centre. Thus, the 2009 rainfall was about 74% of the normal.

Nodulations

Nodule numbers varied significantly among the years, species and cultivars, and growth stages (Table 1). In 2008, chickpea, faba bean, field pea and lentil had similar nodule numbers at early flowering (Table 2). The variation among the pulses was not significant in 2009 and the number of nodules was generally low in 2009 compared with other years (Table 2). In 2010, large variation was observed among the species, with faba bean producing the most nodules at 50.3 plant⁻¹, followed by field pea and chickpea at 40.7 and 37.9 nodules plant⁻¹, respectively. Averaged across years, faba bean, field pea and chickpea had the most nodules at early flowering (31.3, 27.9 and 27.5 nodules plant⁻¹, respectively), while dry bean had the fewest nodules at 4.2 plant⁻¹ (Table 2). Highly significant effects of year and year $\times$ species interaction were observed for this trait (Table 1), suggesting that nodule formation highly depended on water availability and pulse genotype. We also compared nodule number plant⁻¹ at early flowering with BNF (Tables 2 and 3) and seed yield (Hossain et al. 2016). Like nodule number and nodule weight, BNF varied significantly among the pulse species and across the years (Hossain et al. 2016).

Overall, dry bean fixed the lowest amount of N (9.3 kg ha^{-1}) and faba bean fixed the highest amount (67.5 kg ha^{-1}), a trend that matched with the nodule number of the pulses at early flowering (Table 2 and 3).

Across the years, all the pulses except dry bean fixed the highest amount of N in 2010 (Table 3) (Hossain et al. 2016), and nodule number plant^{-1} at early flowering also showed similar trends (Table 2). However, greater nodulation or nodule biomass across pulse species did not necessarily lead to greater N_2 -fixation. For example, chickpea had $25.9 \text{ nodules plant}^{-1}$ compared to 28.2 plant^{-1} of field pea in 2008 (Table 2) but the amount of N fixed by field pea was almost double to that of chickpea (Table 3). This apparent discrepancy can be explained by the number of plant m^{-2} and N_2 -fixation efficiency. In 2008, chickpea had $39.5 \text{ plants m}^{-2}$ compared to $58.7 \text{ plants m}^{-2}$ of field pea that contributed much higher number of nodules on field pea plants m^{-2} and had a positive impact on the BNF of field pea. The BNF efficiency or the amount of N fixed by various pulse species differs widely. Among the pulse crops, faba bean is reported to be the most efficient in BNF followed by field pea and lentil while the capability of chickpea is relatively low (Jensen et al. 2010; Peoples et al. 2009; Rose et al. 2016). However, despite having more plants m^{-2} and a greater number of nodules, field pea fixed less N_2 compared to chickpea in 2010 (Tables 2 and 3). Straw yield of chickpea was much higher compared to field pea in 2010 and this higher straw yield was supported by higher BNF in chickpea (Hossain et al. 2016). Nodule numbers at early flowering were also positively related with seed yield in field pea and lentil (Hossain et al. 2016). This relationship suggests that there is opportunity to improve seed yield and N_2 -fixation in pulses by optimizing the number of nodules plant^{-1} .

The pulses showed significant variation in nodule numbers at late flowering in 2008, with chickpea producing the highest number of nodules, 36.7 plant^{-1} , a number which was not

statistically different from those found in field pea, faba bean and lentil (Table 2). Averaged across years, the effect of pulse species was highly significant (Table 1) and chickpea produced the most nodules (26.0 plant^{-1}) and dry bean produced the least (4.1 plant^{-1}). Nodule numbers at late flowering were also highly dependent on the year, highlighting the importance of environmental conditions in effecting nodule formation. The study also underscored the importance of selecting plant genotypes that maximize nodule formation through optimal use of available environmental resources such as rainfall. Water restriction can drastically decrease nodulation in chickpea and faba bean (Kurdali et al. 2002). Drought tolerant genotypes might overcome such negative impact.

Chickpea did not show variation among the cultivars in a specific year but the effect of growing year and the year $\times$ cultivar interaction on nodule numbers at early flowering was significant (Table 1). Significant differences in nodule numbers at early flowering were found among the field pea cultivars in 2008, with cultivar CDC Handel having the most nodules plant^{-1} (48.5). A significant year effect was observed in field pea and chickpea in the combined analysis (Table 1). Lentil cultivars showed significant differences in nodule numbers only in 2010 with cultivar CDC Impact having the highest nodule production ($35.3 \text{ nodules plant}^{-1}$) and CDC Rouleau, the lowest ($13.4 \text{ nodules plant}^{-1}$). In general, all the cultivars of chickpea, field pea and lentil produced higher number of nodules in 2010 while most of the cultivars produced lower number of nodules in 2009, a year with 74% of normal growing-season precipitation (Fig. 1).

Cultivars of chickpea, field pea or lentil had similar nodule numbers at late flowering in any specific year or when averaged across years (Table 1). However, the effect of year on nodule numbers at late flowering was highly significant. In general, cultivars produced more nodules in 2008 and fewer in 2009, with the exception of the chickpea cultivar Venture (Fig. 2a). Venture

had nearly equal numbers of nodules plant⁻¹ in 2008 and 2009, suggesting that this cultivar is tolerant to drier growing seasons, at least at the level of nodulation. In a study conducted in the USA, Abi-Ghanem et al. (2011) showed significant genotypic effect on number of nodules plant⁻¹ in pea and lentil. Wide difference in nodule numbers among the cultivars within a species or the significant effect of growing conditions underscore the importance of selecting the appropriate cultivar for specific environments to optimize the nodulation for N₂-fixation in pulses.

Nodule Biomass

Nodule biomass plant⁻¹ was generally lower in 2009, the driest growing season, than in the two other years (Table 2). The pulses showed significant variation in nodule biomass at early flowering in 2008 and 2010, but not in 2009 (Table 1), which is consistent with nodule counts presented above. In 2008, chickpea had greater nodule biomass at early flowering (118 mg plant⁻¹), statistically similar to those of field pea and faba bean (Table 2). In 2010, a wide variation was observed among the species. Chickpea was superior to other pulses with a nodule biomass of 122 mg plant⁻¹, while dry bean had lowest nodule biomass at 3.0 mg plant⁻¹ (Table 2). In the analysis with years combined, pulses showed significant variation in nodule biomass at early flowering and chickpea had the highest value (data not shown). Field pea and faba bean had moderate values for this trait with 72.5 and 51.0 mg plant⁻¹, respectively, while the nodule biomass of lentil and dry bean were significantly lower, at 22.7 and 7.3 mg plant⁻¹, respectively. Significant effects of growing year and the year × species interaction (Table 1) were observed for this trait.

Nodule biomass at late flowering varied significantly among pulses in 2008, but not in 2009 (Table 1). Among the pulses, chickpea had highest nodule biomass (203 mg plant⁻¹) in 2008 followed by field pea (157 mg plant⁻¹), while the nodule biomass of lentil and dry bean

were the lowest (Table 2). Averaged across years, chickpea had the greatest nodule biomass ($141 \text{ mg plant}^{-1}$) and followed by field pea ($110 \text{ mg plant}^{-1}$). The effect of year and the year $\times$ cultivar interaction was also highly significant for this trait (Table 1) indicating that like early flower nodule biomass, late flower nodules biomass also was dependent on growth conditions. Saha et al. (2016) reported lower nodule mass plant^{-1} (87.4 mg) at 50% flowering than at the pod filling stage ($104.4 \text{ mg plant}^{-1}$) in chickpea. Drought stress has been reported to negatively affect pulse nodule biomass. Water stress was reported to significantly reduce nodule biomass in field pea (Mahieu et al. 2009) and a similar trend was also observed in chickpea and faba bean (Kurdali et al. 2002).

At early flowering, in 2008, nodule biomass varied significantly among chickpea cultivars with Amit showing the highest ($160 \text{ mg plant}^{-1}$) and CDC Anna, the lowest nodule biomass (79 mg plant^{-1}) (Fig. 3a). A significant effect of year and year $\times$ cultivar interaction was observed on chickpea cultivars in the combined analysis (Table 1). For field pea, variation among cultivars was not significant either in a specific year or in the combined analysis, but year significantly impacted nodule biomass plant^{-1} at early flowering. A similar trend was also observed for lentil cultivars (Table 1).

At late flowering, the cultivars of chickpea, field pea or lentil did not show significant variation in nodule biomass in any specific year or in the combined analysis (Table 1; Fig 4). However, the effect of growing year on the cultivars of each of chickpea, field pea and lentil was highly significant. A significant year $\times$ cultivar interaction was also observed on chickpea cultivars for this trait. In general at the late flowering stage, nodule biomass and number showed the same patterns for each respective cultivar (Table 2). The significant effect of year or year $\times$ cultivar interaction on early or late flowering nodule biomass suggests that this trait depends on

growing conditions. Bourion et al. (2007) showed significant genotypic variations in nodule dry matter accumulation in field pea. With spring-sown chickpea, Koutroubas et al. (2009) obtained an average of 119 – 184 mg nodule biomass plant⁻¹ in five cultivars, whereas nodule biomass plant⁻¹ was 87 – 120 mg for early and 126 – 163 mg for late flowering stages in our study. Growing conditions and cultivar background may contribute to these differences.

Difference in Nodulation at Early and Late Flowering Stages

We compared nodule numbers and nodule biomass at the late and early flowering stages to determine the nodulation pattern during the flowering period. In chickpea, nodule number increased from early to late flowering in 2008, but decreased in 2009 (Fig. 1a and 2a). It appears that water shortage inhibited nodulation or led to nodule shedding. The nodule dry weight at late flowering was higher than at early flowering; this was largely because of higher biomass accumulation in the nodules. It is possible that nodules we recorded at late flowering stage persisted longer on the roots than early flowering nodules and therefore, accumulated more biomass. The biomass of nodules was larger at late than early flowering, despite that there were fewer nodules at late flowering (Table 2). However, there was a wide difference among the cultivars for both nodules plant⁻¹ and nodule biomass plant⁻¹. For example, CDC Nika showed an increase of 0.2 nodules plant⁻¹ while it was 19.3 nodules plant⁻¹ for CDC Frontier in 2008. A similar trend was observed for nodule biomass, with CDC Frontier being highest at 130.7 mg plant⁻¹ and CDC Nika the lowest (Fig. 3a and 4a). In 2009, the response of cultivars was mixed whereby cultivars CDC Frontier and CDC Vanguard had the most nodules at late flowering, but remaining cultivars had more nodules at early flowering. These contrasting results between nodule numbers and nodule biomass in 2009 support our hypothesis that nodules at late flowering accumulated more dry matter than those at the early flowering stage. Łotocka et al.

(2012) reported the expansion of nodule size with time in *Lupinus luteus*. In chickpea, Saha et al. (2016) reported 26.1 nodules plant⁻¹ at the 50% flowering stage which was lower than 36.2 nodules plant⁻¹ obtained at pod filling.

In field pea, nodule numbers increased from early to late flowering in both 2008 and 2009, but the magnitude of the change varied with cultivars. The pea cultivars Cooper, CDC Meadow and Striker had the most nodules at late flowering while the other cultivars had more nodules at the early than late flowering stage (Fig. 1b and 2b). Nodule biomass increased with the development of pea plant in both years, with nodule biomass being higher in 2008 than in 2009 (Fig. 3b and 4b).

Lentil showed a slight decline in nodule number from early to late flowering in 2008 and 2009, whereas nodule biomass moderately increased in both years (Fig. 3c and 4c). At the cultivar level, mixed responses were observed in both years with some cultivars showing increased nodule numbers with plant development, and the others showing a decrease in nodule numbers. Concurrently, most cultivars had higher nodule biomass at late than early flowering stage in both years. Only two cultivars (CDC Meteor and CDC Richlea) in 2008 and two cultivars (CDC Glamis and CDC Impact) in 2009 had higher nodule biomass plant⁻¹ at the early than late flowering stage and in both cases, differences in nodule numbers were high (Fig. 1c and 2c). Dry matter accumulation in lentil nodules was much lower than chickpea and field pea at both the late and early flowering stages in 2008 and differences between the stages were small in lentil (Fig. 3 and 4). Photosynthesis is higher in favourable environmental conditions, which might have contributed to the higher dry matter accumulation in the nodules in 2008 when rainfall was well above normal.

N Uptake and Partitioning

For a given genotype, the N uptake in plant tissues is an indicator of the soil fertility level. In cereals, the shoot is the main contributor to the yield and N uptake can be divided into two main fractions: N in the seed and N in the straw. Seed N is removed at harvest while straw N is returned to the soil. Nitrogen partitioning depends on the N concentration and distribution among different organs and tissues. Patterns of N partitioning or uptake significantly impacts N removal from agricultural systems (Grant et al. 2016). Detailed assessment of the N uptake in seed and straw allowed the determination of the impact of plant genotypes on soil N level.

The N uptake varied widely among the pulse species and cultivars evaluated in the study. In general, the N uptake was higher in 2010 relative to the other years and was the lowest in 2009 (Table 3; Fig. 5 and 6). From an environmental perspective, 2009 was drier which led a lower biomass yield (Hossain et al. 2016) and less plant N uptake. Water availability has been reported to affect N uptake in the straw and seed of pulses (Gan et al. 2010a; Mahieu et al. 2009). Nitrogen is an integral component of proteins and the observation of an effect of growth conditions on plant tissue N concentration suggests an environmental impact on protein concentration and content in pulses. In agreement with this assumption, Bourion et al. (2010) concluded that seed protein content in pulses depended on various factors, including environmental stresses.

Seed N Uptake

In general, seed N uptake was higher than straw, with the exception of chickpea in 2010 when straw N uptake was higher than seed (Table 3). The N uptake in seed and straw varied with pulse species and growth conditions. In 2008, seed N uptake of field pea, lentil and chickpea was significantly greater than dry bean and faba bean. In contrast, seed N uptake was comparatively low in 2009 in most of the pulses because of significantly lower seed yield (Hossain et al. 2016)

and low N concentration (data not shown). However, seed yield of faba bean was higher in 2009 than 2008 and it showed significantly higher seed N uptake than other pulses (Table 3). In 2010, faba bean had the highest seed N uptake (115.9 kg ha^{-1}), which was statistically similar to field pea (97.5 kg ha^{-1}), while chickpea had lowest N uptake (37.2 kg ha^{-1}) (Table 3), and the trend was similar to seed yield (Hossain et al. 2016). In combining years, the pulses significantly differed in seed N uptake. Field pea had the highest seed N uptake (80.9 kg ha^{-1}) followed by faba bean (78.8 kg ha^{-1}), while dry bean had the lowest seed N uptake (37.3 kg ha^{-1}). The effect of year and the year $\times$ pulse interaction was also highly significant (Table 1). An effect of pulse cultivars on seed N uptake was also observed by other research groups. In a Brazilian study, the seed N uptake of 18 dry bean cultivars ranged from 42 to 55 kg ha^{-1} (Araujo and Teixeira 2008). The importance of environmental stress and nutrient availability on seed N concentration and uptake was also reported by other groups (Gan et al. 2010a; Lenssen et al. 2007; Mahieu et al. 2009; Voisin et al. 2002). Our results on the Canadian prairie support these observations found elsewhere on the planet.

Chickpea cultivars did not show significant variation in seed N uptake in any year, but the effects of year and the year $\times$ cultivar interaction were significant (Table 1). Field pea cultivars showed significant variation in seed N uptake only in 2010 when seed N uptake of CDC Handel, CDC Meadow and Striker were significantly greater than the other cultivars (Fig. 5b). In the combined analysis, the N uptake of cultivars varied significantly; CDC Handel (89.6 kg ha^{-1}) and Venture (88.2 kg ha^{-1}) had the highest seed N uptake and CDC Golden (71.8 kg ha^{-1}) had lowest N uptake, and the trend coincided with the seed yield of the cultivars (Hossain et al. 2016). The effect of year on the seed N uptake of field pea cultivars was also highly significant. In lentil, cultivars showed significant variation only in 2009 (Table 1); CDC Meteor had highest

seed N uptake (56.1 kg ha^{-1} ; Fig. 5c) while CDC Sedley had lowest (34.9 kg ha^{-1}). Cultivars showed significant variation for this trait in the combined years analysis, with CDC Meteor (88.9 kg ha^{-1}) ranking first for seed N uptake, and seed yield (Hossain et al. 2016).

Straw N Uptake

Straw N uptake was significantly affected by pulse species and varied across years without consistent patterns (Table 1). In 2008, field pea straw N uptake was the highest followed by lentil and faba bean (Table 3). In 2009, total N uptake was low due to low straw N concentration (data not shown) and low straw yield (Hossain et al. 2016); faba bean had highest straw N uptake among species. In 2010, straw N uptake was highest in chickpea followed by faba bean and both had a similar straw yield (Hossain et al. 2016). In each year, dry bean had the lowest straw N uptake due to low straw N concentration and yield. In the analysis combining years, the variation among the pulses was highly significant (Table 1). Faba bean had the highest straw N uptake (47.1 kg ha^{-1}) followed by chickpea and lentil. Although field pea straw yield was significantly higher (3270 kg ha^{-1}) than that of faba bean (2894 kg ha^{-1}) (Hossain et al. 2016), overall straw N uptake was higher in faba bean because of higher straw N concentration (data not shown). In Australia, significantly higher N concentration and uptake in faba bean than chickpea was reported (Rose et al. 2016). The effect of year and the year $\times$ species interaction on this trait was highly significant emphasizing the importance of growing conditions on straw N concentration (Lenssen et al. 2007; Mahieu et al. 2009).

Chickpea cultivars differed in straw N uptake in 2009 (Fig. 6a) and CDC Frontier exhibited significantly higher straw N uptake (19.8 kg ha^{-1}) than the other cultivars, which corresponded with straw yield (Hossain et al. 2016). In the analysis combining years, only year significantly impacted the straw N uptake of chickpea cultivars. Field pea cultivars had

significantly greater straw N uptake in 2008 than 2009. In 2008, Cooper had highest N uptake (72.8 kg ha^{-1}) and CDC Meadow had the lowest (43.7 kg ha^{-1}). Straw N uptake was lower in 2009 than in 2008, but a similar trend was observed (Fig. 6b). The cultivars varied significantly in the analysis combining years (Table 1); Cooper had highest straw N uptake (50.8 kg ha^{-1}), which also yielded highest amount of straw (Hossain et al. 2016). The effect of year was highly significant for this trait. Bourion et al. (2007) also observed a significant genotypic effect on N uptake in the shoots of field pea cultivars. In lentil, cultivars showed significant variation for straw N uptake in 2008 and 2009 (Table 1). In 2008, CDC Robin had the highest straw N uptake (59.4 kg ha^{-1}), but in 2009, the straw N uptake of CDC Glamis, CDC Sedley and Laird were significantly higher than the other cultivars (Fig. 6c). The N concentration (data not shown) as well as seed yield (Hossain et al. 2016) of these three cultivars was higher in 2009, which contributed to the significantly higher N uptake. In the analysis combining years, the significant effect of year and the year $\times$ cultivar interaction for this trait was observed. High straw biomass for all of the pulses except field pea in 2010 (Hossain et al. 2016) contributed to the high N uptake in the straw.

Large variation in the N uptake in pulse straw has been reported. In Canadian semiarid conditions, the straw N uptake ranged from 11.8 to 17.2 for chickpea, 29.4 to 56.2 for field pea, and 31.1 to 51.3 kg ha^{-1} for lentil (Gan et al. 2010a; Walley et al. 2007). In semiarid Australia, the straw N uptake of field pea, chickpea, lentil and faba bean was 148, 108, 83 and 153 kg ha^{-1} , respectively (Jensen et al. 2010). Similarly high straw N uptake in field pea, chickpea and faba bean were obtained in another Australian group (Peoples et al. 2009). The higher straw N uptake in the Australian pulses may be attributable to their longer growing season compared to the Canadian prairie (Mauromicale et al. 2005; Yang et al. 2010). These results indicate that N

uptake in pulses is highly dependent on genotype and the genetic expression of this trait depends on experimental conditions such as soil moisture and nutrient management.

Cereal crops grown in rotation with pulses improve their yield and quality compared to cereal monoculture (Campbell et al. 1992; Irvine et al. 2013; Lupwayi and Soon 2016). Also, cereals following a pulse in rotation requires less N-fertilizer (Campbell et al. 1992; Lupwayi and Soon 2016). Pulse straw or residues add more N to the soil compared to cereal residues. For example, lentil straw may supply about 50% more N to the soil organic matter pool than a well-fertilized wheat crop (Campbell et al. 1992). Overall, straw N uptake was highest in faba bean. Field pea and faba bean had greater seed N uptake than lentil and chickpea (Table 3). The greater N contribution of the pulse crop residues may build up an active N pool in the soil over time, which would increase the soil N supply and lower fertilizer-N requirements of subsequent cereals in the rotation (Campbell et al. 1992). The beneficial effects of pulses on subsequent crops in the long-term rotational systems are partly due to improved soil N availability (Ross et al. 2015). Seed yield of field pea was much higher than lentil and chickpea (Hossain et al. 2016), whereas lentil or chickpea could contribute more N to the soil when straw was decomposed.

We compared the N uptake (seed + straw) with the amount of N fixed by respective pulse species to shed light on the relative contribution of BNF/soil source N on shoot N uptake. The relative contribution of BNF varied widely among the pulses and the extent of the variation differed over the years. In 2008, the contribution of BNF to N uptake was 30, 2, 65, 41 and 31% for chickpea, dry bean, faba bean, field pea and lentil, respectively (Table 3). In 2009, the contribution was 37, 41, 40, 45 and 38% for the same pulses while it was 85, 18, 57, 49 and 58% for chickpea, dry bean, faba bean, field pea and lentil, respectively, in 2010. The BNF of

chickpea in 2010 was much higher than the other two years and higher straw biomass was mostly attributed to high BNF (Hossain et al. 2016).

We also compared the BNF contribution to seed and straw N uptake separately which showed that BNF contribution was much higher to straw N uptake than seed (Table 3). The overall contribution of BNF to N uptake of the pulses except dry bean was about 40-54% which underscores the importance of BNF/pulses in the cropping systems in reducing reliance on inorganic N fertilizer use. The BNF contribution to N uptake of faba bean and field pea was comparatively stable over the years but was more variable in chickpea and dry bean. Variation of BNF contribution to shoot/plant N uptake was also observed in previous studies. For example, BNF contribution to shoot N uptake was 26-77% in chickpea and 36-85% in faba bean under variable N fertilizer rates (Rose et al. 2016), while BNF accounted for about 60% of total N in chickpea at physiological maturity (Kurdali 1996). However, the contribution of soil N to shoot N uptake was higher than BNF in our study, so there may be opportunities to increase BNF efficiency of pulse crops on the prairies.

CONCLUSION

Nodule number and nodule biomass of pulse crops on the Canadian prairie differed significantly among species and cultivars evaluated, and the magnitude of the differences varied among years. Overall, nodule biomass was higher at late flowering than at early flowering, but the influence of plant growth stage on nodule number varied with years. Among the pulse species evaluated, faba bean and field pea had highest seed N uptake; this was attributable to their higher seed yield and greater N_2 -fixation ability compared to the others. Field pea had higher seed N uptake, while lentil and chickpea contributed more straw N to the soil, and thus, these three pulses may play an

equally important role in developing cropping systems for enhancing farm productivity and system sustainability. Water stress reduced nodule number and N uptake in seed and straw significantly. Pulse genotypes with improved water-stress tolerance may be developed to improve the N₂-fixation trait.

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Table 1: Effect of pulse species, cultivars and growing season on nodulation and nitrogen uptake expressed by *P* values

Effect	Early flower nodule number plant ⁻¹	Early flower nodule biomass (mg plant ⁻¹)	Late flower nodule number plant ⁻¹	Late flower nodule biomass (mg plant ⁻¹)	Seed N uptake (kg ha ⁻¹)	Straw N uptake (kg ha ⁻¹)
Species comparison ^a						
2008	0.0064	0.0007	0.0285	0.0024	<.0001	<.0001
2009	0.1613	0.3132	0.1043	0.1401	0.0003	<.0001
2010	<.0001	0.0013	-	-	0.0001	<.0001
Species combined	<.0001	<.0001	0.0031	<.0001	<.0001	<.0001
Year	<.0001	0.0509	0.0017	0.0018	<.0001	<.0001
Year × species interaction	<.0001	0.0141	0.0837	0.0053	<.0001	<.0001
Cultivar comparison ^b						
Chickpea 2008	0.3565	0.0107	0.2596	0.0859	0.2892	0.0599
Chickpea 2009	0.3603	0.4664	0.4526	0.2843	0.1072	0.0360
Chickpea 2010	0.1369	0.0685	-	-	0.0704	0.4765
Chickpea combined	0.5537	0.4369	0.3393	0.5337	0.2688	0.2047
Year	0.0002	<.0001	<.0001	<.0001	<.0001	<.0001
Year × cultivar interaction	0.0396	0.0194	0.2852	0.0144	0.0235	0.7774
Field pea 2008	0.0273	0.4496	0.2731	0.2881	0.1037	0.0030
Field pea 2009	0.2895	0.6180	0.4822	0.5128	0.4303	0.0005
Field pea 2010	0.2657	0.7108	-	-	0.0489	0.1388
Field pea combined	0.0946	0.2443	0.3346	0.1589	0.0162	<.0001
Year	<.0001	0.0083	0.0002	0.0002	<.0001	<.0001
Year × cultivar interaction	0.1361	0.9166	0.2631	0.7559	0.0945	0.0718
Lentil 2008	0.3152	0.0659	0.3559	0.1853	0.6144	0.0003
Lentil 2009	0.3817	0.3790	0.5798	0.2326	0.0437	<.0001
Lentil 2010	0.0169	0.0792	-	-	0.2877	0.0644
Lentil combined	0.1165	0.4894	0.4028	0.2247	0.0171	0.0599
Year	<.0001	<.0001	<.0001	0.0015	<.0001	<.0001
Year × cultivar interaction	0.1533	0.1850	0.4941	0.1275	0.8925	0.0022

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

^b Comparison of cultivars within the chickpea, field pea, or lentil species.

Table 2: Nodulation in different pulse species measured at the early and late flowering stages^a

Pulse	Early flower nodule number plant ⁻¹			Early flower nodule wt. (mg plant ⁻¹)			Late flower nodule number plant ⁻¹		Late flower nodule wt. (mg plant ⁻¹)	
	2008	2009	2010	2008	2009	2010	2008	2009	2008	2009
Chickpea	25.9 ± 2.1 ^a	18.8 ± 3.3	37.9 ± 3.9 ^b	118 ± 8 ^a	59 ± 12	122 ± 10 ^a	36.7 ± 2.8 ^a	15.4 ± 2.0	203 ± 18.4 ^a	80 ± 16.5
Dry bean	0.1 ± 0 ^b	8.3 ± 1.9	4.3 ± 1.6 ^d	1 ± 0 ^b	18 ± 6	3 ± 0.6 ^c	0.9 ± 0.7 ^b	7.3 ± 2.0	16 ± 6.3 ^c	20 ± 15.2
Faba bean	35.2 ± 7.9 ^a	8.4 ± 4.8	50.3 ± 3.8 ^a	80 ± 27 ^a	29 ± 24	44 ± 28 ^{bc}	22.0 ± 14.1 ^a	7 ± 3.1	90 ± 53 ^{bc}	29 ± 14.8
Field pea	28.2 ± 3.2 ^a	14.8 ± 2.8	40.7 ± 3.8 ^b	100 ± 7 ^a	54 ± 14	63 ± 6 ^b	28.1 ± 2.5 ^a	13.5 ± 2.5	157 ± 12.7 ^{ab}	64 ± 16.7
Lentil	21.7 ± 2.4 ^a	12.0 ± 1.5	24.0 ± 1.7 ^c	14 ± 0.9 ^b	42 ± 8	12 ± 0.9 ^c	21.3 ± 1.2 ^{ab}	10.6 ± 1.4	18 ± 1.3 ^c	51 ± 10.4
<i>P</i>	0.0064	0.1613	<.0001	0.0007	0.3132	0.0013	0.0285	0.1043	0.0024	0.1401

^a Data are means of three replicates, ten plants from each replicate ± standard error of mean. Means within a column not sharing a lowercased italic letter differ significantly at the $P < 0.05$ level according to the Tukey HSD test.

Table 3: N uptake in the seed and straw and the percent contribution of biological N₂-fixation to the N uptake^a

Pulse	Seed N uptake (kg ha ⁻¹)			Straw N uptake (kg ha ⁻¹)			Biological N ₂ -fixation (kg ha ⁻¹) ^b					
							Seed			Straw		
	2008	2009	2010	2008	2009	2010	2008	2009	2010	2008	2009	2010
Chickpea	74.5 ± 2.9 ^a	42.0 ± 1.5 ^c	37.2 ± 3.4 ^c	26.1 ± 1.1 ^c	15.1 ± 0.7 ^b	84.5 ± 4.9 ^a	12.8 ± 1.5 ^b (17.1)	9.1 ± 0.9 ^b (21.7)	18.0 ± 2.0 ^b (48.4)	17.8 ± 2.2 ^c (68.2)	11.9 ± 1.1 ^b (78.8)	85.6 ± 5.9 ^a (101.3)
Dry bean	33.5 ± 5.7 ^b	28.7 ± 4.0 ^c	49.6 ± 5.5 ^c	10.2 ± 1.3 ^d	8.6 ± 1.1 ^c	16.3 ± 2.6 ^d	0.2 ± 0.1 ^c (0.6)	5.8 ± 2.0 ^b (20.2)	0.9 ± 0.1 ^c (1.8)	0.6 ± 0.2 ^d (5.9)	9.6 ± 3.3 ^b (111.6)	10.9 ± 2.1 ^d (66.9)
Faba bean	39.2 ± 4.9 ^b	81.9 ± 12.2 ^a	115.5 ± 13.9 ^a	37.2 ± 7.2 ^b	30.9 ± 3.8 ^a	73.1 ± 9.1 ^{ab}	14.8 ± 3.0 ^b (37.8)	16.1 ± 2.6 ^a (19.7)	35.8 ± 6.8 ^a (31.0)	34.7 ± 4.3 ^a (93.3)	29.3 ± 8.3 ^a (94.8)	71.9 ± 9.6 ^{ab} (98.4)
Field pea	83.3 ± 4.0 ^a	62.1 ± 2.1 ^b	97.5 ± 3.3 ^{ab}	57.3 ± 2.4 ^a	20.0 ± 1.2 ^b	42.2 ± 1.8 ^c	25.5 ± 2.7 ^a (30.6)	17.1 ± 1.8 ^a (27.5)	30.2 ± 2.3 ^a (31.0)	32.8 ± 3.7 ^{ab} (57.2)	19.5 ± 2.1 ^{ab} (97.5)	38.3 ± 2.7 ^c (90.8)
Lentil	79.4 ± 2.2 ^a	43.3 ± 1.6 ^c	88.6 ± 3.7 ^b	44.5 ± 1.5 ^b	17.7 ± 1.0 ^b	61.5 ± 3.3 ^b	13.2 ± 1.0 ^b (16.6)	8.4 ± 0.8 ^b (19.4)	27.9 ± 1.7 ^{ab} (31.5)	25.0 ± 2.2 ^{bc} (56.2)	14.7 ± 1.4 ^b (83.1)	58.9 ± 4.2 ^b (95.8)
<i>P</i>	<.0001	0.0003	<.0001	<.0001	<.0001	<.0001	<.0001	0.005	0.0009	0.0003	0.0496	0.0001

^a Data are means of four replicates, four observations from each replicate ± standard error of mean. Means within a column not sharing a lowercased italic letter differ significantly at the *P* < 0.05 according to the Tukey HSD test.

^b Adapted from Hossain et al. (2016); % contribution of BNF to seed and straw N uptake is given in the parentheses.

Figure Caption

Fig. 1: Early flower nodule number plant⁻¹ of chickpea (i), field pea (ii) and lentil (iii) cultivars in 2008, 2009 and 2010. Values are presented as mean of three replicates $\pm$ SE. Means followed by different letters are significantly different based on Tukey's test.

Fig. 2: Late flower nodule number plant⁻¹ of chickpea (i), field pea (ii) and lentil (iii) cultivars in 2008 and 2009. Values are presented as mean of three replicates $\pm$ SE.

Fig. 3: Early flower nodule biomass plant⁻¹ of chickpea (i), field pea (ii) and lentil (iii) cultivars in 2008, 2009 and 2010. Values are presented as mean of three replicates $\pm$ SE. Means followed by different letters are significantly different based on Tukey's test.

Fig. 4: Late flower nodule biomass plant⁻¹ of chickpea (i), field pea (ii) and lentil (iii) cultivars in 2008 and 2009. Values are presented as mean of three replicates $\pm$ SE.

Fig. 5: Seed N uptake (kg ha⁻¹) of chickpea (i), field pea (ii) and lentil (iii) cultivars in 2008, 2009 and 2010. Values are presented as mean of four replicates $\pm$ SE. Means followed by different letters are significantly different based on Tukey's test.

Fig. 6: Straw N uptake (kg ha⁻¹) of chickpea (i), field pea (ii) and lentil (iii) cultivars in 2008, 2009 and 2010. Values are presented as mean of four replicates $\pm$ SE. Means followed by different letters are significantly different based on Tukey's test.

Fig. 1.

190x254mm (300 x 300 DPI)

Fig. 2.

190x254mm (300 x 300 DPI)

190x254mm (300 x 300 DPI)

Fig. 4.

190x254mm (300 x 300 DPI)

Fig. 5.

190x254mm (300 x 300 DPI)

Fig. 6.

190x254mm (300 x 300 DPI)