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Symbiosis of selected *Rhizobium leguminosarum* bv. *viciae* strains with diverse pea genotypes: effects on biological nitrogen fixation

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Abstract

Biological nitrogen fixation (BNF) can be improved by optimizing the interaction between the rhizobial inoculant and pea (*Pisum sativum* L.), leading to increased productivity and reduced nitrogen (N) fertilizer use. Eight *Rhizobium leguminosarum* bv. *viciae* strains were used to inoculate super-nodulating pea mutant Rondo-*nod3*(fix+), hyper-nodulating pea mutant Frisson P88 *Sym29*, CDC Meadow commercial control, and a non-nodulating mutant Frisson P56 (*nod*-) to evaluate BNF in a greenhouse assay. Significant differences in strain×cultivar interactions were detected for shoot and root dry weight which ranged from 1.8 to 4.7 g and 0.27 to 0.73 g per plant, respectively, for nodule number on lateral roots which ranged from 25 to 430 per plant, for amount of fixed N, which ranged from 15 mg to 67 mg, and 4 mg to 15 mg per plant for shoot and root tissue, respectively, and for percentage of nitrogen derived from atmosphere (%*Ndfa*) which ranged from 37% to 61%, and 35% to 65% for shoot and root tissue, respectively. Strain×cultivar interactions in this study could contribute to identifying superior

strains and pea breeding lines with genetic superiority in BNF. Nodule production in pea plants was not necessarily correlated with the amount of fixed N, suggesting nodule activity is more important to BNF than nodule number.

Key words: *R. leguminosarum* bv. *viciae*, rhizosphere, nitrogen fixation, *Pisum sativum* L.,

Introduction

Nitrogen is one of the most limiting factors for crop growth and nitrogen fertilizer represents one of the major costs in crop production (Hardarson *et al.* 1993). In 2016, the world nitrogen fertilizer demand was expected to reach 116 million tonnes per year, a 5.2% increase from 2012, and a 5.4% increase in North America (FAO 2012) and the price of nitrogen fertilizers has more than tripled since 2005 (IFA 2009).

Biological nitrogen fixation (BNF) can reduce the need for artificial N fertilizer, reduce the cost of crop production, and enhance sustainable agriculture (Silva and Uchida 2000). A specific rhizospheric interaction occurring between rhizobia and legumes leads to root-nodule formation which is an essential step for the BNF process. A nodule primordium is formed from cortical cells in the host roots and rhizobia are guided to this primordium in a plant-controlled manner during nodule formation. This process is induced by Nod factors (NF) that are secreted by rhizobia. NFs are lipochitooligosaccharides that contain species-specific substitutions, which can trigger subsequent steps in signal transduction during the BNF process (Geurts *et al.* 2005). The study of BNF can help to improve cropping systems involving N₂-fixing crops in rotations, reduce greenhouse gas emissions, and diminish the environmental impact of agriculture (Gan *et*

al. 2011). Denitrification of nitrogenous fertilizer residues into N_2O was identified as the main source of greenhouse gas emissions from farming activities (Dyer et al. 2010; van Groenigen et al. 2010). Therefore, N_2 -fixing bacteria and BNF in cultivated pulse crop fields are triggering much research interest.

Pulse crops are grown worldwide as a source of high quality proteins (Abi-Ghanem *et al.* 2011). In addition, the seeds provide slowly digestible starch, dietary fiber and many important micronutrients. Globally, dry pea (*Pisum sativum* L.) was grown on 6.6 million ha in 2005 and was estimated to fix about 0.57 Tg of N (Herridge *et al.* 2008). Improving nitrogen fixation in major food crops like pea will increase plant-based protein for human consumption, enhance the growth of subsequent crops, and reduce synthetic N fertilizer use (Abi-Ghanem *et al.* 2011). Research has been conducted on the legume/rhizobium symbiosis, legume inoculation, and quantification of biological nitrogen fixation, but only limited research has been conducted on breeding pulse crops for enhanced nitrogen fixation (Hardarson 1993; De La Peña and Pueyo 2012).

BNF can be improved by optimizing rhizobial infection and efficiency through repeated rhizobial inoculation (Vessey 2004), high efficiency strains (Hynes *et al.* 1995) or co-inoculation with other organisms such as arbuscular mycorrhizal fungi (Mishra *et al.* 2009). Crop varieties and rhizobial strain specificities are both significant factors that affect BNF. Strain×cultivar interaction effects on BNF have been reported in lentil (Abi-Ghanem *et al.* 2011), soybean (Israel 1981), common bean (Valverde and Otabbong 1997) and peanut (Wynne *et al.* 1980), but little research has been conducted on pea-related rhizobia-plant interactions (Abi-Ghanem *et al.* 2011). Evaluation of strain×cultivar interaction on current pea varieties would be beneficial for identifying both superior strains and pea breeding lines with genetic superiority in BNF. Thus,

the objective of this research was to determine the efficiency of nodulation and BNF of selected pea lines with different strains of *Rhizobium leguminosarum* bv. *viciae* selected in the northern Great Plains region.

Materials and methods

Rhizobia strains

Seven *R. leguminosarum* bv. *viciae* strains, designated as NB-R10 to NB-R16, isolated in the northern Great Plains, were obtained from Novozymes Biotechnology (Saskatchewan, Canada). A commercial inoculant (Nodulator XL®, BASF, Saskatoon, Canada) was used as a positive control. Strains were stored in 20% glycerol and 80% yeast extract mannitol broth at -80 °C. Strains were grown in yeast extract mannitol broth at 22 °C for 5 days. Cells were collected by centrifugation at 1,000 g for 4 min, diluted in sterile distilled water to 10^6 cfu ml⁻¹ using a cuvette spectrophotometer (Cole-Parmer, USA) and optical density of 640 nm wavelength, according to Vega (2007).

Pea germplasm

Based on results of our preliminary field experiments (Yang *et al.* 2017), two pea mutants with high BNF potential were used in this study to evaluate their suitability as parents in breeding for improved nitrogen fixation in pea. In particular, a super-nodulating pea mutant Rondo-*nod3* (fix+) (Jacobsen and Feenstra, 1984) from cultivar Rondo that carries the *nod3* gene produced substantially greater nodule numbers and accumulated 3-5 times more N in roots than cultivars

Sparkle and Frisson, while producing comparable biomass and pods to these cultivars. The second pea mutant Frisson P88 *Sym29* (Sagan and Duc, 1996) which was a hyper-nodulating mutant from cultivar Frisson that carries the *sym29* gene and produced larger nodule mass than the progenitor cultivar, but had similar biomass. Seeds of these two lines were provided by Plant Gene Resources of Canada (PGRC) and obtained from John Innes Centre Norwich, UK. In addition, the widely-grown cultivar CDC Meadow (Warkentin *et al.* 2007), and a non-nodulating mutant Frisson P56 (*nod-*) (Sagan *et al.* 1993) were used as controls. Frisson P56 was used as the negative control as a non-fixing control was required to calculate %Ndfa and amount of fixed N.

Greenhouse assay and experimental design

Seeds were surface sterilized with 70% ethanol for 5 min and rinsed 5 times with sterilized water. Surface sterilized seeds were germinated on moist sterile filter paper in the dark at 22 °C for 48 h. Top soil used in this study was steam sterilized by using an Amsco® Century™ SV-136H Prevac Steam Sterilizer (STERIS Corporation, USA) at 121°C for 30 min in order to eliminate cross-contamination from natural microbes in the soil. The standard soil pasteurizing protocol from Department of Soil Science, University of Saskatchewan was utilized as this protocol has previously been used successfully, for example, Ellouze *et al.* 2015. For each 7 litre pot, 2.5 kg sterilized soil was used. Soil samples were sent to ALS Laboratory Group Agricultural Services Saskatoon (<http://www.alsglobal.com>) for soil nutrient analysis. Details of soil nutrient content are shown in Table 1. Two seeds were sown per pot, and each pot was inoculated with one strain of *R. leguminosarium* using 1 ml of inoculum containing 10⁶cfu ml⁻¹. Negative control pots received 1 ml of distilled water without inoculum. After seedling emergence, all pots were

thinned to one plant per pot. For each treatment, 5 replicates were applied, and the whole experiment was conducted twice (December 2014-February 2015 and May 2015-July 2015) under the same greenhouse conditions. Harvest was conducted when pods were fully filled but before dry-down to facilitate harvest of roots and nodules. The temperature in the greenhouse was 22°C in the daytime and 18°C at night, with approximately 54% relative humidity. Photoperiod in the greenhouse was 17/7 (day/night) with light intensity of 250 $\mu\text{E m}^{-2}\text{s}^{-1}$ under 1000W high pressure sodium lights. Watering was conducted every two or three days as needed.

Isotope measurement

The ^{15}N dilution technique was used to measure the percentage of nitrogen fixed by pea plants. Ten millilitres of $^{15}\text{NH}_4^{15}\text{NO}_3$ solution (10 atom%, ICON ISOTOPES, www.iconisotopes.com) was applied to each pot for all pea lines at two weeks after seeding. After 8 weeks of growth, whole plants were removed from the pots and cleaned with tap water to remove attached soil. Nodules on tap and lateral roots were counted, plants were cut at the root crown, and shoot and root tissues were dried at 60 °C for 24 h. Weights of shoot and root tissues were recorded, then the plant tissues were finely ground using a Cyclone sample mill (UDY Corporation, Colorado, USA). Plant ^{15}N -to- ^{14}N ratio was measured by mass spectrometry (V.G. Isotech, Aston Way, Middlewich, Cheshire, CW10 OHT, United Kingdom). The amount and percentage of nitrogen derived from air were calculated as follows (Voisin *et al.*, 2002; Fried and Middelboe 1977):

$$\text{N}_{2\text{fixed}} = \%N_{\text{dfa}} \times \text{total N}_{\text{legume}} / 100$$

$$\%N_{\text{dfa}} = 100 \times (\delta^{15}\text{N}_{\text{legume}} - \delta^{15}\text{N}_{\text{reference plant}}) / (\epsilon_{\text{fix}} \times \delta^{15}\text{N}_{\text{reference plant}})$$

Where $\delta^{15}\text{N}_{\text{legume}}$ is ^{15}N isotope measured in tested pea tissues, $\delta^{15}\text{N}_{\text{reference plant}}$ is ^{15}N isotope measured in tissues of negative control (non-fixing mutant). $\%Ndfa$ is percentage of nitrogen derived from atmosphere, and total N_{legume} is total nitrogen measured in tested pea tissues. ϵ_{fix} (-1 for pea) is the isotopic fractionation factor associated with N_2 fixation processes.

Statistical analyses

Pearson correlation analysis was used to describe the correlation between fixed nitrogen and nodulation among treatments using SYSTAT 12 (Systat Software, Inc. Chicago, IL, USA. <http://www.systat.com/>). Difference in plant dry weights, nodule numbers, amount of fixed nitrogen and $\%Ndfa$ were detected by ANOVA using MYSTAT. A mixed model analysis was applied to test for any significant systemic variation between the two repeated experiments. The Shapiro-Wilk test was used to verify the normality of the data, and the Wilks' Lambda test, to detect significant effects of explanatory variables at the 5% level. After statistical analysis, significant effects between the two repeated experiments were not detected, therefore averaged means of the two experiments were used to make all graphs.

Results

Symbiosis effects on shoot and root growth of pea

In a preliminary study, CDC Dakota and CDC Meadow were tested in pasteurized soil and no visible nodulation was detected, thus the pasteurization method was considered effective. The N

fixation capability of Frisson P56 was tested after inoculation with test strains in the same manner as the other pea lines, and no significant N fixation was detected in this control plant.

Compared with the non-nodulating mutant, all three pea lines (two mutants and one commercial check) inoculated with eight different rhizobia strains (seven test strains plus one commercial strain) showed significantly greater shoot dry weight and root dry weight (Fig. 1 and Table 2, $P < 0.0001$). CDC Meadow had greater shoot dry weight than the two mutants when inoculated with the commercial inoculant. However, significant interaction effects between pea lines and rhizobia strains were detected for both shoot and root dry weight (Fig. 1a and 1b). In particular, Rondo-*nod3*(fix+) had higher shoot dry weight when inoculated with rhizobia strains NB-R11 and NB-R14 compared with the other two pea lines inoculated with the same strains, and Frisson P88 *Sym29* showed greater shoot dry weight when inoculated with strain NB-R10 compared with the other two pea lines inoculated with the same strain (Fig. 1a). For CDC Meadow, inoculation with strain NB-R12 and commercial inoculant Nodulator XL® led to higher shoot dry weight than other tested rhizobia strains (Fig. 1a).

For all plant lines and plant-rhizobia combinations, the shoot dry weight was greater than the root dry weight. Rondo-*nod3*(fix+) had significantly greater root dry weight per plant than CDC Meadow when inoculated with rhizobia strain NB-R14, while Frisson P88 *Sym29* showed significantly greater root dry weight than CDC Meadow when inoculated with strain NB-R16 (Fig. 1b). CDC Meadow had significantly greater root dry weight after inoculation with strain NB-R12 compared with the two mutant lines (Fig. 1b). These results indicate that symbiosis with the same rhizobia strain could differently impact the growth of different pea lines, while the

same pea line showed different growing performance when colonized by different rhizobia strains.

Symbiosis effects on nodulation and nodule production of pea

As high-nodulating mutants, Rondo-*nod3*(fix+) and Frisson P88 *Sym29* showed higher nodulation potentials than CDC Meadow. The average number of nodules on the tap root and lateral roots for Rondo-*nod3*(fix+) : Frisson P88 *Sym29* : CDC Meadow in this study were 29:18:4 and 249:116:27, respectively (Fig. 2). For each pea line, colonization by rhizobia strains led to significant differences in nodulation on the tap and lateral roots (Fig. 2 and Table 2, $P = 0.045$ and $P < 0.0001$ respectively). In particular, rhizobia strain NB-R10 resulted in significantly more tap and lateral root nodules when colonizing Frisson P88 *Sym29* compared with commercial strain symbiosis, and rhizobia strain NB-R11 resulted in significantly more nodules on tap and lateral roots compared to the commercial strain when they colonized Rondo-*nod3*(fix+). Although tap root nodule number of Rondo-*nod3*(fix+) was not significantly greater after inoculation with strains NB-R10, NB-R12, NB-R14 and NB-R15 compared with the commercial inoculant Nodulator XL®, the nodule number on lateral roots of Rondo-*nod3*(fix+) was much greater after inoculation with these four strains compared with commercial inoculant Nodulator XL®. These results suggest that symbiosis of different rhizobia strains may influence the N₂-fixation of pea plants due to their effects on nodulation progression and distribution in the pea root system.

Symbiosis effects on N fixation of pea

Interaction effects between strains and cultivar on BNF per plant were detected. In above ground tissue (Fig. 3a and Table 2, $P < 0.0001$), fixed N in Rondo-*nod3*(fix+) was significantly greater by 42%, 121%, 34% and 150% when colonized by rhizobial strains NB-R10, NB-R11, NB-R13 and NB-R14, respectively, compared with commercial strain symbiosis. Rhizobial strain NB-R11 and NB-R12 showed similar effects as colonized by commercial inoculant in Frisson P88 *Sym29* compared with other tested strains. In root tissue (Fig. 3b and Table 2, $P < 0.0001$), both Rondo-*nod3*(fix+) and Frisson P88 *Sym29* showed highest amount of fixed N after inoculation with NB-R11 compared with other strain symbiosis.

The %*Ndfa* in shoot and root tissue was calculated (Figure 4 and Table 2, $P < 0.0001$). %*Ndfa* in shoots ranged from 35% to 60%, and %*Ndfa* in roots ranged from 37% to 63%. All three tested pea lines showed highest %*Ndfa* in shoot tissue after inoculation with NB-R11 and NB-R12, and higher %*Ndfa* in root tissue after inoculation with NB-R11, compared to inoculation with other rhizobia strains. Pea plants with relatively high %*Ndfa* do not necessarily produce high total fixed N per plant, such as inoculation of NB-R16 with Frisson P88 *Sym29*. Growth characteristics of pea plants including shoot and root biomass and morphology, or hormones and exudates produced from different pea lines, may also impact N fixation.

Correlation between nodule numbers and fixed N in above ground tissue

Significant correlations between nodule number and fixed N in above ground tissue were only detected in Frisson P88 *Sym29* and Rondo-*nod3* (fix+) after inoculation with rhizobium strain NB-R14 (Table 3, $P = 0.045$ and 0.019 , respectively). This positive correlation was not

significant in CDC Meadow or inoculation with other rhizobia strains, indicating that nodule number is not necessarily related to N fixation.

Discussion

Strain effects on fixed N

Legumes can reach their N requirement through BNF only through symbiosis with effective rhizobia strains. Symbiotic N₂-fixing bacteria trigger the formation of root nodules and can fix considerable amounts of N₂. Symbiotic N₂-fixing bacteria are largely associated with leguminous plants (Lindström et al. 2010) and N₂-fixing leguminous crops are widely used to input BNF in agro-ecosystems throughout the world. Previous studies have reported strain effects on BNF in many legume crops; for example, Somasegaran and Bohlool (1990) reported that fixed N of chickpea, soybean and dry bean were affected by inoculating with different rhizobia strains, however, the strain effect was low. Hardarson et al. (1984) found effectiveness of 20 rhizobia strains in symbiosis with soybean was quite variable, and differences in amount of fixed N per ha between their tested strains 61A76 and 61A150 was up to 67%. Abi- Ghanem et al. (2011) reported significant strain effects on fixed N and nodulation in lentil; they found strain effects on nodulation in pea, but not on BNF.

In this research, rhizobium strain had a large effect on both nodulation and BNF among three tested pea lines. For example, in Rondo- *nod3* (fix+) and Frisson P88 *Sym29*, the nodule numbers on tap and lateral roots after inoculation with *R.leguminosarum* bv. *viciae* stains NB-R11 and NB-R13 differed by 2-fold, and the amount of fixed N in shoot and root tissues differed by 1.5-2 fold. These results confirm that N fixation capabilities of legume plants differ when

inoculated with different rhizobia strains.

In this study, %*Ndfa* in shoot and root tissue generally ranged from approximately 40-60% which was lower than the >80% reported by Abi-Ghanem *et al.* (2011). However, experimental protocols differed somewhat between the Abi-Ghanem *et al.* (2011) study and that reported here, i.e, they used sterilized sand and perlite mix (1:1 volume) instead of top soil, and the *Rhizobium* strains differed. The current research was conducted using top soil with optimized nutrients and favorable conditions for release of available N from soil organic matter (3.5%) by mineralization, along with additional N applied as isotope. Hu *et al.*, 2016 showed that %*Ndfa* in pea could reach above 60% under field conditions. In general, higher available N in the soil limits N-fixation, for example, %*Ndfa* in pea was restricted when ammonium concentration increased above 0.1 mM (Fei *et al.*, 2006).

Strain effects were detected on N-fixation among tested pea lines. In particular, three pea lines showed greater %*Ndfa* in shoot and root tissues after inoculation with NB-R11 compared to those inoculated with other rhizobia strains suggesting good N-fixation potential of this strain, even under relatively high soil N availability.

Correlation between nodulation and fixed N

Correlations were detected between fixed N and root nodule numbers on tested pea plants, which confirmed previous research (Hartwig 1998; Abi-Ghanem *et al.* 2011). However, nodule numbers are not always positively correlated with fixed N in plants, as we only found significant correlation between fixed N and nodule number after inoculation with certain rhizobium strains. Hartwig (1998) reported that low nodule numbers were not always related to low fixed N, as

nodules usually have a very good potential to adjust nitrogenase activity, which could lead to higher N₂ fixation with low nodulation. In this study, positive correlations between nodulation and fixed N were found after inoculation with strain NB-R14. Positive correlations between plant biomass and fixed N were also found by other researchers (Bourion *et al.* 2007), but we didn't detect significant correlations between these two parameters, suggesting the capability of N₂ fixation may not be positively correlated to host plant biomass, thus it can be selected separately from the biomass phenotype, as previously reported (Abi-Ghanem *et al.* 2011).

Effects of legume cultivar on BNF

Pea breeding efforts are focusing on improving grain yield, grain quality, and disease resistance (Micke 1993; Warkentin *et al.* 2015). These traits must be maintained while new traits such as high N fixation capability should also be included in order to improve environmental and economic benefits (Herridge and Rose 2000) which highlight the study of interactions between legume crops and their related rhizobium. The Food and Agriculture Organization (FAO) documented that faba bean (*Vicia faba*), lupin (*Lupinus* spp.) and pigeon pea (*Cajanus cajan*) are very efficient in BNF, while common bean (*Phaseolus vulgaris*) is rather poor in fixing nitrogen from the atmosphere (Hardarson 1993), and reports on BNF in pea are not consistent (Abi-Ghanem *et al.* 2011; McCauley *et al.* 2012; Ruisi *et al.* 2012; Schwenke *et al.* 2015). Previous studies have reported cultivar effects on BNF in different legume crops. In soybean, cultivar effects on fixed N per ha was up to 70% (Bello *et al.* 1980). In lentil, the difference in the amount of fixed N among tested cultivars was as high as 81% (Hafeez *et al.* 2000). In pea research based on a greenhouse assay, differences of fixed N among 5 tested pea cultivars varied from 379 mg to 578 mg per plant, with the average value of 475 mg per plant (Abi-Ghanem *et al.*

2011). In this research, significant cultivar effects were detected among three tested pea lines. For example, fixed N in shoot and root tissue of Rondo-*nod3*(fix+) was more than two-fold greater than CDC Meadow when inoculated with rhizobia strain NB-R11, suggesting a good possibility to use this mutant in a breeding program for increased N fixation in pea. Cultivar effects on fixed N in pea plants may also be due to their influence on the composition of *R. leguminosarum* bv. *viciae* in the rhizosphere. Specific flavonoids produced by legumes can attract specific rhizobia to their root hairs, and the rhizobia in turn, produce the *nod* factors that induce morphological changes in their host plant root system (Geurts et al. 2005). Therefore, differences in the signaling system of different pea genotypes could result in differences in the recognition pattern between the plants and associated bacterial communities in their rhizosphere. In the pea lines used in this study, the *nod3* gene carried by Rondo-*nod3* (fix+) could regulate systemic signals involved in nodule production in the root system (Postma et al. 1988), whereas Frisson P88 *Sym29* carries gene *sym29* which is the pea orthologue of the HAR1 gene involved in a putative receptor kinase for shoot-controlled regulation of nodulation and root development (Krusell et al. 2002). As a consequence, root morphogenesis and exudations might differ between mutants carrying these different genes, and signal exchange between the plant and rhizobia may also differ (Yang and Crowley 2000), which can further influence the nodulation and N fixation efficiency by impacting the recognition and symbiosis of rhizobia to their host plants. This can explain the different N fixation performance of the same rhizobial stain among three tested pea lines, and interaction effects between pea lines and rhizobia inoculants on pea growth and nodulation in our study, which were not detected by previous research (Begum et al. 2001; Abi-Ghanem et al. 2011).

In summary, significant rhizobial strain effects were detected on BNF capabilities of tested pea lines. For example, the amount of fixed N in shoot tissue of Rondo-*nod3*(fix+) was more than double when colonized by rhizobia strain NB-R11 and NB-R14 compared with the commercial strain, suggesting a large impact on fixed N in the agroecosystem could be realized by inoculant effects. We also found nodule number in pea is not necessarily correlated to the amount of fixed N, suggesting the activity of nodules rather than the number of nodules contributes more to BNF. These results provide useful information for commercial inoculant selection and pea breeding to benefit producers and the environment.

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Figure 1. Differences of (a) shoot dry weight, and (b) root dry weight of three pea lines after inoculation with eight rhizobia strains. Control: Frisson P56 (nod-); F: Frisson P88 *Sym29*; M: CDC Meadow; R: Rondo-*nod3*(fix+); numbers after capital letters from 10 to 16 indicate different rhizobia strains from NB-R10 to NB-R16. FC, MC and RC are the three tested pea lines inoculated with commercial inoculant. ($n = 10$, $P < 0.0001$ for shoot and root dry weight based on averaged means of two experimental repeats. Standard error bars are indicated).

Figure 2. Differences of nodule number on (a) tap roots and (b) lateral roots of three pea lines after inoculation with eight rhizobia strains. Control: Frisson P56 (nod-); F: Frisson P88 *Sym29*; M: CDC Meadow; R: Rondo-*nod3*(fix+); numbers after capital letters from 10 to 16 indicate different rhizobia strains from NB-R10 to NB-R16. FC, MC and RC are the three tested pea lines inoculated with commercial inoculant. ($n = 10$, $P = 0.045$ and $P < 0.0001$ for nodule numbers on tap and lateral roots respectively based on averaged means of two experimental repeats. Standard error bars are indicated).

Figure 3. Fixed N in (a) shoot tissues and (b) root tissues per plant of three pea lines after inoculation with eight rhizobia strains. Control: Frisson P56 (nod-); F: Frisson P88 *Sym29*; M: CDC Meadow; R: Rondo-*nod3*(fix+); numbers after capital letters from 10 to 16 indicating different rhizobia strains from NB-R10 to NB-R16. FC, MC and RC are the three tested pea lines inoculated with commercial inoculant. ($n = 10$, $P < 0.0001$ for shoot and root tissues based on averaged means of two experimental repeats. Standard error bars are indicated).

Figure 4. Percentage of nitrogen derived from atmosphere (%*Ndfa*) in (a) shoot tissues and (b) root tissues of three pea lines after inoculation with eight rhizobia strains. Control: Frisson P56 (nod-); F: Frisson P88 *Sym29*; M: CDC Meadow; R: Rondo-*nod3*(fix+); numbers after capital letters from 10 to 16 indicating different rhizobia strains from NB-R10 to NB-R16. FC, MC and RC are the three tested pea lines inoculated with commercial inoculant. ($n = 10$, $P < 0.0001$ for cultivar and strain effects in shoot and root tissues based on averaged means of two experimental repeats. Standard error bars are indicated).

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Table 1. Description of soil properties and available nutrients used for potting study

Table 2. *P*-values from ANOVA test of treatment effects on percentage of nitrogen derived from atmosphere (%*Ndfa*), fixed N, and biomass of shoot and root, and nodule number on tap and lateral roots

Table 3. Correlation coefficients between nodule number of three pea lines and fixed N in shoot tissue after inoculation with eight rhizobia strains.

Draft

Table 1. Description of soil properties and available nutrients used for potting study

Soil Type	Texture	pH	E.C.	Organic Matter%	N	P	K	S	Cu	Mn	Zn	B	Fe
					mg available nutrient per kg of soil								
Black	Loam	8.1	0.4	3.5	28	16	600	48	3.1	15	4.1	2.2	186

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Table 2. *P*-values from ANOVA test of treatment effects on percentage of nitrogen derived from atmosphere (%*Ndfa*), fixed N, and biomass of shoot and root, and nodule number on tap and lateral roots

	Root					Shoot		
	% <i>Ndfa</i>	Fixed N	Biomass	Nodules-tap root	Nodules-lateral root	% <i>Ndfa</i>	Fixed N	Biomass
Cultivar	<0.0001	<0.0001	<00001	<00001	<00001	<00001	<00001	<00001
Strain	<0.0001	<0.0001	0.014	<00001	<00001	<00001	<0.0001	<00001
C*S	ns	<0.0001	<0.0001	0.045	<00001	ns	<0.0001	<00001
Repeat	ns	ns	ns	ns	ns	ns	ns	ns

Note: C*S: interaction effects of Cultivar and Strain. Repeat: mixed model test of significance between two repeated experiments.

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Table 3. Correlation coefficients between nodule number of three pea lines and fixed N in shoot tissue after inoculation with eight rhizobia strains.

Pea line	Rhizobia strain							
	NB-R10	NB-R11	NB-R12	NB-R13	NB-R14	NB-R15	NB-R16	Nodulator XL ®
Frisson P88 <i>Sym29</i>	0.109	0.200	-0.404	0.318	0.887*	-0.836	0.393	0.068
CDC Meadow	0.296	0.030	-0.335	0.307	0.262	0.118	-0.646	-0.215
Rondo- <i>nod3</i> (fix+)	0.032	0.181	0.592	0.070	0.937*	0.158	-0.646	0.281

*, significant at the 0.05 level.

Figure 1

Figure 2

Figure 3

Figure 4