Journal of Plant Protection Research ISSN 1427-4345

ORIGINAL ARTICLE

How strongly is rhizobial nodulation associated with bean cropping system?

Leila Tabande[1], Bita Naseri[2]*

[1] Soil and Water Research Department, Fras Agricultural and Natural Resources Research and Education Center, Fars, Iran
[2] Plant Protection Research Department, Kermanshah Agricultural and Natural Resources Research and Education Center, Kermanshah, Iran

Vol. 60, No. 2: 176–184, 2020

DOI: 10.24425/jppr.2020.133307

Received: August 14, 2019
Accepted: October 28, 2019

*Corresponding address:
bitanaseri@yahoo.com

Abstract

Due to inadequate efforts to reinforce nitrogen fixation capability of bean via symbiosis with rhizobia, improvement of bean productivity is still highly dependent on chemical fertilization. An advanced understanding of agro-ecosystem-bean-*Rhizobium* interaction is required to improve symbiosis efficiency. Thus, seasonal development of rhizobial nodulation was characterized according to 20 agro-ecological properties for 122 commercial bean fields. Principal component analysis identified soil texture as a major descriptor of agrosystem-bean-disease-*Rhizobium* interaction. Nonparametric correlation analysis indicated significant associations of root nodulation with bean class, fungicidal treatment of seed and soil, Fusarium root rot index, planting date and depth, soil texture, clay and sand content. Ordinal regression analysis demonstrated that rhizobial nodulation was improved by applying initial drought, heavier soil textures with greater organic matter and neutral pH, using herbicides and manure, growing white beans, irrigating every 7–9 days, later sowing in June, reducing disease and weed, shallower seeding, sowing beans after alfalfa, avoiding fungicidal treatment of seed and soil, and omitting urea application. This large-scale study provided novel information on a comprehensive number of agronomic practices as potential tools for improving bean-*Rhizobium* symbiosis for sustainable legume production systems.

Keywords: legume, multivariate, *Phaseolus vulgaris*, *Rhizobium* spp.

Introduction

Dry (common) bean (*Phaseolus vulgaris* L.) is the most widely cultivated pulse crop worldwide. In 2014, the level of production in Iran was 226,369 t, 15% of which was concentrated in Zanjan province, in northwestern Iran. It is much desired by local bean growers to lower the environmental costs of intensive agriculture through sustainable agronomy practices and to improve crop productivity. Although the ability of bean crops to provide nitrogen-fixing symbiosis with rhizobial bacteria has been known for many years, nitrogen deficiency still limits bean productivity and increases demands for chemical fertilizers. A well-established rhizobial nodulation in bean crops reduced root rot diseases caused by soil-borne pathogens and enhanced seed production (Naseri 2013a, 2014a; Kalantari *et al.* 2018). Efficient rhizobial nodulation can measurably decrease the demand for chemical N-fertilizers. In Brazil, cost savings of $1.3 billion per year have been achieved in soybeans inoculated with rhizobia (Coutinho *et al.* 2000). The effectiveness of this crop management practice depends on providing agro-ecological conditions favorable to symbiotic N fixation and plant productivity (Naseri 2019). For instance, it is believed that greater soil clay content for rhizobia protection and moisture retention, greater soil residues maintained following no-tillage, sowing in field soil with neutral, to above-neutral pH levels, regular cultivation of rhizobia-inoculated chickpea and avoiding saline soils allow for the development of soil rhizobial communities in chickpea cropping systems (Elias and Herridge 2015). Furthermore, the associations of soil moisture (Slattery *et al.* 2001), clay content (Howieson and Ballard 2004), pH (Elias and Herridge 2015), seed treatment with benlate and mancozeb (Naidu 2000), soil depth (Rupela *et al.* 1987), sowing date (Elias and Herridge 2015), N-fertilizer (Chemining'wa and KevinVessey 2006), growth stage and temperature (Rennie and Kemp 1983), and crop rotation (Kucey and Hynes 1989) with legume-rhizobia symbiosis have been previously reported. However, a joint comparison of agro-ecological traits associated with seasonal patterns of root-nodulation by rhizobia under commercial bean production is still needed. A systematic understanding of agro-ecological features influencing rhizobial nodule-development during the growing season will assist in optimizing the effectiveness of bean-*Rhizobium* symbiosis for agricultural sustainability. Furthermore, large-scale findings can extend the applicability of outcomes to cropping systems different from those studied. Therefore, the major objective of this study was to characterize relationships between rhizobial nodulation and a comprehensive set of agro-ecological variables examined over two growing seasons in 122 commercial bean fields.

Materials and Methods

To provide a comprehensive, useful and reliable description of the agro-ecosystem-bean-nodulation interplay, the study included a total of 122 commercial fields (experimental sites) across Zanjan's main bean growing regions (Fig. 1) and were assessed during two growing seasons. The date of planting ranged from the second week of May to the third week of June across the commercial fields studied during the two years of research. Harvest time also varied from early September to early October. The averages of mean monthly air-temperature and total monthly rainfall over the two growing seasons for May, June, July and August were 14.2°C and 21.2 mm, 18.3°C and 19.0 mm, 22.6°C and 8.9 mm, 21.1°C and 0.6 mm, respectively. This research framework was conceived to involve highly diverse agro-ecosystems in interaction with rhizobial nodulation in bean crops in order to achieve

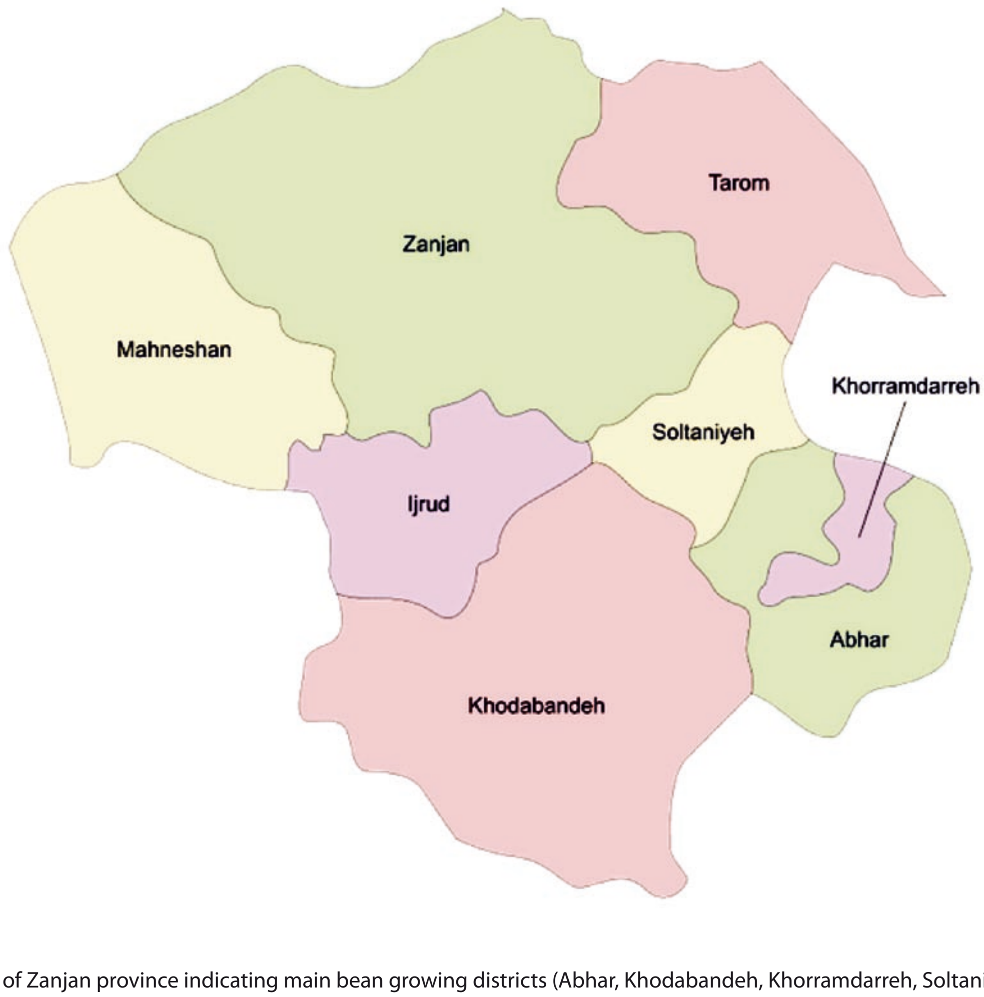

Fig. 1. A map of Zanjan province indicating main bean growing districts (Abhar, Khodabandeh, Khorramdarreh, Soltaniyeh) studied

a systematic understanding of the bean-*Rhizobium* symbiosis. All the farmers' fields were examined by the second author at V3 (opening of the first trifoliate leaf in full), R6-7 (flowering-podding) and R9 (pod maturation) stages of bean growth (van Schoonhoven and Pastor-Corrales 1987). A full description of the method for measuring rhizobial nodulation on bean roots has been documented previously (Naseri 2013a). The status of nodule formation on the root was based on a modified scale as follows to simplify symbiosis assessments across a large number of bean fields: 0 – no nodule was formed on the root system, 1 – low nodulation with one to three small nodules < 1 mm diameter per rootlet, and 2 – high nodulation with more than four medium to large-sized nodules > 1 mm diameter per rootlet (Naseri 2013a). In each field, nodulation status was determined for three to four bean plants at three randomly chosen spots of 0.25 m squares, which included about six plants in relation to planting density. Soil samples collected from the fields at V3 were analyzed to determine organic matter, pH and texture fractions based on standard methods as described previously (Naseri 2013a).

Farmers were interviewed for details of their agronomic practices regarding the date of planting, irrigation frequency, the crop grown before bean, and applications of fertilizers and herbicides. As presented in Table 1, bean fields were classified into the three groups of bean market class (red, pinto and white), fertilization (not applied, chemical fertilizer and manure), herbicide use (not applied, trifluralin and others: paraquat or bentazon), and rhizobial nodulation on roots (absent, low and high). There were two classes of fungicidal treatment of bean seed and field soil, and initial drought (not applied and applied). The initial drought variable was defined as the period of 15–45 days from seeding to the first irrigation of the field. The field soils were classified into four main texture groups: light (sand, loamy sand and sandy loam), medium (loam, silt loam and silt), heavy (sandy clay loam, clay loam, silty clay loam and sandy clay), and highly heavy (silty clay and clay). The seven classes of preceding crops were described as follows: alfalfa, barley, bean, maize, potato, tomato, and wheat.

The plant and weed densities (the number of plants or weeds per quadrat), and seeding depth were also recorded at V3, R6–7 and R9. In addition, the incidence (the percentage of symptomatic plants per quadrat) and severity of Fusarium root rot, caused by *Fusarium solani*, were examined on the three sampling dates. To rate disease severity, the percentage of symptomatic root tissues (discoloration, lesions, or necrosis) was determined for three diseased plants per quadrat. Then, for each quadrat, a root disease index was determined as follows: disease incidence × disease severity/5 (Naseri 2008).

To facilitate interpretation of associations between rhizobial nodulation, Fusarium root rot and 19 agro-ecological variables, a principal component analysis (PCA) based on correlation matrix was used to estimate the loading values for variables. A loading value ≥ 0.35 was considered significant (Kranz 1974). An eigenvalue > 1.0 was used for interpretation (Sharma 1996). To examine the significance of agro-ecosystem-disease-symbiosis interactions, nonparametric correlations between the rhizobial nodulation, Fusarium root rot and agro-ecological variables assessed at V3, R6-7 and R9 growth stages of bean crops were performed with SPSS software for Windows (2016). Furthermore, an ordinal regression analysis of the large-scale-study data simplified interpreting a large number of significant relationships. In this study, multiple regressions defined the contribution of agro-ecological traits to the status of rhizobial nodulation in commercial bean fields. The contribution of an independent descriptor to the bean-*Rhizobium* symbiosis during the growing season was regarded as significant if $p < 0.10$ for the parameter estimated.

Table 1. Frequencies of commercial bean fields classified into categories of agro-ecological traits studied at vegetative (V3), flowering--podding (R6-7) and pod maturity (R9) growth stages

<table>
<tr><th>Variables</th><th colspan="7">Categories/Frequencies</th></tr>
<tr><td>Bean class</td><td colspan="3">red/180</td><td colspan="2">pinto/69</td><td colspan="2">white/117</td></tr>
<tr><td>Initial drought</td><td colspan="4">not applied/170</td><td colspan="3">applied/196</td></tr>
<tr><td>Fertilization</td><td colspan="3">not applied/142</td><td colspan="2">chemical/209</td><td colspan="2">manure/15</td></tr>
<tr><td>Growth stage</td><td colspan="3">V3/122</td><td colspan="2">R6-7/122</td><td colspan="2">R9/122</td></tr>
<tr><td>Herbicide</td><td colspan="3">not applied/117</td><td colspan="2">trifluralin/219</td><td colspan="2">bentazon/paraquat/30</td></tr>
<tr><td>Previous crop</td><td>alfalfa/18</td><td>barley/18</td><td>bean/72</td><td>maize/18</td><td>potato/15</td><td>tomato/13</td><td>wheat/210</td></tr>
<tr><td>Rhizobial nodulation</td><td colspan="3">absent/145</td><td colspan="2">low/146</td><td colspan="2">high/75</td></tr>
<tr><td>Seed fungicide treatment</td><td colspan="4">not applied/339</td><td colspan="3">applied/27</td></tr>
<tr><td>Soil fungicide treatment</td><td colspan="4">not applied/324</td><td colspan="3">applied/42</td></tr>
</table>

Results

The seasonal development of bean-*Rhizobium* symbiosis was characterized in a total of 122 commercial fields by using 20 agro-ecological traits at vegetative, flowering-podding, and pod maturity stage of bean growth (Table 1). From the PCA, six principal components, accounting for 62% of the total variance, characterized the development of rhizobial nodules over two growing seasons in highly diverse bean cropping systems (Table 2). The first principal component accounted for 17.6% of the variance in agro-ecosystem-disease--*Rhizobium* data. This factor showed moderate positive loadings for the two soil descriptors, soil texture and clay content, and also a significantly negative loading for the sand content. The second principal component, which accounted for 14.7% of the data variance, concerned only the silt content. The significant contribution of these four soil-textural descriptors to the first and second principal components, accounting for 32.4% of the total variance, recognized soil texture as the superior descriptor of the agro-systems-bean--*Fusarium-Rhizobium* interaction studied. These loading values also verified the same or similar contributions of the soil texture and textural fractions (clay, sand and silt) into the bean farming systems examined at vegetative, flowering-podding, and pod maturity stages over two growing seasons and on a large scale. Therefore, only the texture variable was used for the remainder of the multivariate analysis.

The third principal component, which accounted for 10.2% of the data variance, can be interpreted as an overall description of the extent of Fusarium root rot development over time depending on planting depth (Table 2). This principal component gave similar moderate positive loadings for the disease index and planting depth. The fourth principal component, accounting for 7.8% of total variance, gave significantly positive and negative loadings for planting-date and soil-pH variables, respectively. This factor was highlighted considering the date of planting and pH of field soil in order to examine the bean-*Fusarium-Rhizobium* interaction. The fifth principal component, explaining 6.0%

Table 2. Principal component analysis results for bean symbiosis data collected over two growing seasons according to Fusarium root rot and agro-ecological variables

Variables	Principal components					
	1	2	3	4	5	6
Bean class	0.06	–0.16	0.16	0.31	–0.12	**0.46**
Fertilization	0.23	0.30	0.05	–0.24	–0.01	0.07
Fusarium root rot index	–0.23	–0.08	**0.47**	0.01	–0.07	0.04
Herbicide	0.25	0.32	0.01	–0.06	–0.06	0.07
Initial drought	0.20	0.25	0.25	–0.16	0.07	**–0.41**
Irrigation interval	0.05	–0.28	–0.30	–0.09	0.14	**0.37**
Planting date	0.15	0.12	–0.11	**0.41**	–0.14	–0.21
Planting density	0.21	0.25	–0.10	0.07	0.01	0.23
Planting depth	–0.13	–0.02	**0.49**	–0.14	–0.19	0.08
Previous crop	–0.20	–0.12	0.09	0.05	0.26	**–0.39**
Rhizobial nodulation	0.12	0.02	–0.11	0.28	**–0.46**	–0.20
Seed fungicide treatment	–0.01	0.23	0.13	0.14	**0.56**	–0.05
Soil fungicide treatment	–0.02	0.19	0.23	0.06	**0.35**	0.34
Soil clay	**0.44**	–0.19	0.22	0.05	–0.01	–0.00
Soil sand	**–0.40**	0.31	–0.17	0.02	–0.08	0.05
Soil silt	0.22	**–0.40**	0.06	–0.12	0.18	–0.10
Soil organic matter	0.09	–0.23	–0.25	–0.31	0.12	–0.18
Soil pH	0.12	0.02	–0.00	**–0.54**	–0.15	0.14
Soil texture	**0.39**	–0.13	0.24	0.18	–0.03	–0.02
Urea use	–0.16	0.01	0.20	–0.25	–0.33	–0.03
Weed density	–0.25	–0.31	0.10	0.12	0.02	–0.03
Eigenvalues	3.71	3.09	2.13	1.63	1.27	1.18
Variation [%]	17.6	14.7	10.2	7.8	6.0	5.6
Accumulated variation [%]	17.6	32.4	42.5	50.3	56.3	61.9

Bold numbers refer to significant loading values equal to or above 0.35

of the variance, identified the significance of fungicidal treatment of bean seed and the soil in estimating the development of bean-*Rhizobium* symbiosis during the growing season. In fact, this factor provided moderate positive loadings for the treatment of seed and soil with fungicides, and a moderate negative loading for the rhizobial nodulation descriptor. Finally, the sixth principal component demonstrated the close relationships of bean class, initial drought, irrigation interval, and previous crop. Our PCA showed that the development of rhizobial nodulation in bean crops was dependent mainly on the soil texture (factors 1 and 2), Fusarium root rot index and planting depth (factor 3), soil pH and planting date (factor 4), fungicidal treatment of seed and soil (factor 5), bean market class, irrigation program and previous crop (factor 6).

According to nonparametric correlation analysis, the strongest associations between the nodulation and agro-ecological variables were determined for Fusarium root rot index [correlation coefficient – (–0.19)], the planting date (correlation coefficient – 0.21), and soil texture (correlation coefficient – 0.20; Table 3). There were positive associations of root nodulation with bean class, planting date, soil clay and texture. Negative linkages of the nodule-formation status to *Fusarium* root rot index, fungicidal treatment of seed and soil, planting depth and soil sand were determined.

The multivariate description of rhizobial nodulation according to the bean class, initial drought, irrigation intervals, fertilizer and herbicide applications, fungicide-treated seed and soil, Fusarium root rot index, plant and weed density, planting date and depth, previous crop, seed and soil treatments with fungicides, soil organic matter, pH, texture and urea usage was significant (ordinal regression: $n = 355$; *Chi* Prob. Model < 0.001). Based on the ordinal regression analysis, the above-mentioned agro-ecological and disease descriptors (except the plant-density variable) contributed significantly to nodule formation by rhizobial bacteria across the 122 bean cropping systems studied (Table 4). The market class of bean crops, planting date, and the two-way interaction of Fusarium root rot index with planting depth contributed the most to the variability of bean-*Rhizobium* symbiosis. The two-way interaction of weed density and herbicide descriptors

Table 3. Non-parametric correlations of rhizobial nodulation with agro-ecological traits studied in 122 commercial bean fields

Variables	Correlation coefficient	Sig.* (2-tailed)	No. of observations
Bean class	0.116	0.027	366
Fertilization	0.005	0.928	366
Fusarium root rot index	–0.188	0.0001	364
Herbicide	0.098	0.061	366
Initial drought	0.041	0.435	366
Irrigation interval	–0.030	0.566	366
Planting date	0.207	0.0001	366
Planting density	0.027	0.607	366
Planting depth	–0.125	0.017	366
Previous crop	–0.068	0.197	364
Seed fungicide treatment	–0.108	0.039	366
Soil fungicide treatment	–0.132	0.012	366
Soil clay	0.134	0.011	366
Soil sand	–0.111	0.033	366
Soil silt	0.019	0.723	366
Soil organic matter	–0.068	0.196	366
Soil pH	–0.086	0.100	366
Soil texture	0.198	0.0001	366
Urea use	–0.063	0.229	365
Weed density	–0.024	0.652	362

*significance

Table 4. Ordinal regression analysis of rhizobial nodulation according to Fusarium root rot (FRR) index and agro-ecological traits in 122 commercial bean fields

Variables	Estimate	Standard error	t prob.*	Antilog of estimate
Bean class	0.469	0.123	0.001	1.598
Fertilization × previous crop	–0.058	0.035	0.097	0.944
FRR index × planting depth	–0.002	0.009	0.001	0.999
Herbicide × weed density	0.050	0.022	0.022	1.051
Initial drought × irrigation interval	0.106	0.049	0.029	1.112
Organic matter × soil texture	–0.233	0.132	0.078	0.792
Planting date	0.034	0.009	0.001	1.035
Planting density	–0.049	0.041	0.226	0.952
Seed fungicide treatment	–0.776	0.462	0.093	0.460
Soil fungicide treatment	–0.652	0.353	0.065	0.521
Soil pH × urea use	0.000	0.000	0.067	1.000

*t-test probability

was the second most important contribution to nodulation variability. Multivariate analysis also indicated that there was a minor contribution of the plant-density variable to the over-season development of bean-*Rhizobium* symbiosis varying among the farming systems investigated.

Based on the correlation coefficients provided by nonparametric correlations and parameters estimated by ordinal regression, higher rhizobial nodulation levels were associated with applying initial drought, choosing heavier soil textures with greater organic matter content and neutral pH, herbicide application, fertilizing with manure, growing white beans, irrigating at 7–9 day intervals, later sowings in June, a lower *Fusarium* root rot index, lower weed density, shallower seeding, sowing beans after alfalfa, the lack of fungicidal treatment of bean seeds and field soil, and the lack of a urea application.

Discussion

The cultivation of legume crops is a well-established strategy for increasing soil fertility and decreasing the need for chemical N-fertilizers without serious environmental hazards. Therefore, optimization of N fixation through rhizobial nodulation is a major goal in common bean cultivation. Certain agricultural practices and environmental conditions have been known to be effective in improving the efficiency of rhizobial N fixation for sustainable farming systems. The present findings extend our understanding of agro-ecological factors controlling bean-*Rhizobium* symbiosis over the growing season that will be useful for maximizing symbiotic effectiveness for economical and sustainable production. The ranking of 26 cultivars of common beans for rhizobial nodulation was highly dependent on the bean growth stage at the evaluation timepoint. For instance, some cultivars established symbiosis better when evaluated at anthesis but not at maturity (Rennie and Kemp 1983). For this reason, a multiple-timepoint assessment of rhizobial nodulation was considered in the present research.

Remans *et al.* (2008) demonstrated the significant interaction of bean genotypes with *Rhizobium*–*Azospirillum* co-inoculation across environments and called for more field-scale experimentation in various seasons and environments to describe relationships between genotype, inoculum and environment (Remans *et al.* 2008). However, the association of bean market class with rhizobial nodulation across different agroecosystems is little understood. The ordinal regression analysis in this study recognized bean class as the most relevant variable to rhizobial nodulation among the 20 agro-ecological traits examined in 122 commercial fields. This suggests that breeding bean crops for high nodulation capacity should be a main part of crop-improvement programs, as favorable alleles are restricted within certain bean classes.

Elias and Herridge (2015) reported planting date (influencing crop water use efficiency) as a major determinant of grain yield in *Rhizobium*-nodulated chickpeas. However, they did not evaluate the impact of sowing date on rhizobial nodulation. In southeastern Australia, an earlier sowing (late April to early May) of peas increased N fixation by 53% compared to late sowing (late June to early July) and improved the total soil N via residue retention (O'Connor *et al.* 1993). O'Connor *et al.* (1993) related the benefits from earlier sowing to greater plant biomass and N concentration coming from N fixation. In the present research, later planting of bean crops in June (late spring) favored rhizobial nodulation compared to earlier planting in mid-late May (mid-spring). It has previously been established that cowpea nodulation is significantly dependent on glasshouse temperature with an optimum temperature of 24°C (Dart and Mercer 1965). Furthermore, during the hyacinth bean growing season, lower nodule weights were detected in winter (January) than in summer (June; Habish and Mahdi 1976). Thus, legume nodulation appears to be improved under warmer climatic conditions. In addition to appropriate climate, the present large-scale findings may be partially attributed to more severe root rot infections caused by *F. solani*, *Rhizoctonia solani*, *Macrophomina phaseolina*, and *F. oxysporum* in beans sown earlier under colder and moister climatic conditions in May (Naseri 2013b; 2014a, b, c). It should be added that the present findings recognized *Fusarium* root rot as one of the most important indicators of rhizobial nodulation in beans among the 20 agro-ecological variables studied. To the best of our knowledge, this is the first report of the bean-*Fusarium*-*Rhizobium*-sowing-date interaction. Such information advances our understanding of sowing date modification in ways that can be applied in the control of bean root rot and improvement of nodule formation for sustainable agriculture purposes.

In this study, farmers widely treated bean seeds and field soils with benomyl and mancozeb. Mårtensson (1992) observed the ability of *R. leguminosarum* b.v. *trifolii* to multiply in the presence of benomyl and mancozeb at rates equal to or higher than standard doses. However, these two fungicides reduced rhizobial-induced root deformations essential for nodule development and mancozeb inhibited nodulation at high doses (Mårtensson 1992). Although treatment of bean seeds with both benomyl and rhizobia decreased drastically nodulating inoculants, the same fungicide treatment followed by seed-furrow-inoculation of rhizobia had no effect on inoculant survival (Ramos and Ribeiro Jr. 1993). In India, pre- and post-inoculation of seeds treated with benomyl and mancozeb at label doses were non-toxic to rhizobia and for benomyl, they were even stimulatory (Naidu 2000). Elsewhere, single or mixed applications of benomyl and bradyrhizobia in laboratory, greenhouse and field trials in Brazil decreased nodulation by up to 87% (Campo *et al.* 2009). The present large-scale findings revealed that fungicidal treatment of bean seeds and field soils reduced nodulation under commercial production conditions. Thus, this study agrees with Campo's *et al.* (2009) advice on fungicide applications with only pathogen-contaminated seeds.

Soil populations of rhizobia detected in research stations and farmers' fields in diverse Indian chickpea growing regions decreased with increasing soil depth and the largest populations were observed at a 5 cm depth (Rupela *et al.* 1987). However, information on the impact of planting depth on bean-*Rhizobium* symbiosis is still lacking. Thus, the present finding on the significantly negative association of nodulation with this agronomic practice in commercial bean crops appears to have been reported for the first time. Further plot-scale investigation on the bean-depth-*Rhizobium* interaction is required.

Clay content positively influences rhizobial survival, presumably due to physical protection of the bacteria, improved substrate availability and increased soil moisture (Revellin *et al.* 1996; Elias and Herridge 2015). In agreement with previous reports on legume nodulation, in this study, greater soil clay contents (range 14–53% in 122 Iranian bean farming systems) and heavier soil textures corresponded to higher rhizobial nodulation under commercial production conditions. In addition, sand content was negatively linked to root nodulation.

Populations of naturally nodulating rhizobia in legumes differed notably across diverse soil environments in southeastern Australia (Slattery *et al.* 2001). Chickpea rhizobia populations in Indian soils of various geographic areas were unrelated to soil nitrate-nitrogen status (Rupela *et al.* 1987). Elsewhere, nodulation of bean cultivars was highly relevant to mineral N levels and the application of N-fertilizer reduced N fixation in most cultivars (Rennie and Kemp 1983). In southern Manitoba, application of N-fertilizer reduced pea nodulation by resident rhizobia or commercial inoculants (Chemining'wa and KevinVessey 2006). According to another study, cowpea nodulation in a glasshouse differed with ammonium nitrate levels depending on temperature and light intensity (Dart and Mercer 1965). In this study, there was a significant negative impact of urea applied to commercial bean fields within the 0–500 kg · ha^{-1} range on root nodulation by natural rhizobia.

Elias and Herridge (2015) reported crop-available water as a major determinant of grain yield in chickpea-*Rhizobium* symbiosis systems. They advised farmers to develop soil rhizobia via zero tillage and residue retention on the soil surface for moisture maintenance. A positive linkage of soil moisture with rhizobial-nodule numbers was reported by Slattery *et al.* (2001). Although soil moisture is primarily affected by the soil and climatic conditions, agronomic practices, in particular irrigation or initial drought, can influence the development of rhizobial nodulation. Irrigation frequency affected the number and dry weight of nodules in hyacinth bean fields and irrigating at 7 day intervals improved nodulation (Habish and Mahdi 1976). In this research, the irrigation interval, ranging from 2 to 9 days, had a significant impact on root nodulation over the bean growing season in 122 commercial fields. Although moisture stresses reduced bean nodulation on a field scale (Sangakkara 1994), the initial drought stress due to the lack of irrigation early in the season improved bean nodulation in this regional-based study. Local farmers believe that this practice lowers weed density and improves root system expansion.

In Australia, effective N-fixation in legume crops responded to soil pH depending on soil type and location, and large resident populations were found on alkaline soils (Slattery *et al.* 2001). In contrast, soil pH within the ranges of 6.3–8.9 in Australia and 6.4–8.8 in India was unrelated to rhizobial-nodule numbers in chickpea farming systems (Rupela *et al.* 1987; Elias and Herridge 2015). Elias and Herridge (2015) advised Australian farmers to develop rhizobial communities by maintaining ≥ 7 soil pH. Although the association of soil pH with rhizobial populations is well-known, the nodulation-pH interaction deserves further consideration. A survey of 20 sites across geographical regions in eastern Canada showed reductions in nodulation rates with decreasing soil pH from 8 to 6 (Chemining'wa and Kevin Vessey 2006). In the present study of 122 field-sites, soil pH within the range of 7–8 was inversely related to bean nodulation.

Larger populations of chickpea rhizobia in highly diverse Indian soils were detected after growing chickpea compared to pigeonpea, groundnut and corn, while rice showed a nearly 100-fold smaller rhizobial population (Rupela *et al.* 1987). Soil populations of rhizobia in five bean, five pea, and two wheat fields in southern Alberta indicated that legume cultivation and use of commercial inoculants maintained large populations of rhizobia after 4 years of fallow or non-legume cultivation (Kucey and Hynes 1989). However, the interaction of bean nodulation with the preceding crop has received little consideration. The significant association of rhizobial nodulation with legumes such as alfalfa grown before bean in this study may be explained with the help of findings from Kucey and Hynes (1989) on the long lasting beneficial impact of legume cultivation on soil rhizobial populations. It should be noted that legumes like beans are major crops used for rotation in the regions studied.

In southern Manitoba, the influence of soil electrical conductivity on pea nodulation rates following field application of commercial inoculants was attributed to the effects of soil texture and organic matter (Chemining'wa and KevinVessey 2006). Amending soil with corn leaf residues had no impact on bean rhizobia, while alfalfa residues decreased populations of these beneficial bacteria (Pena-Cabriales and Alexander 1983). The present study revealed for the first time the significant relevance of organic matter (which ranged from 0.4 to 1.8% in agricultural soils across the major bean growing area in Zanjan) to bean rhizobial development.

In a study carried out by Bertholet and Clark (1985), nodulation was not significantly decreased due to trifluralin applications in faba bean fields. Although high rates of trifluralin, which was non-toxic to *Rhizobium* sp. in a disc inhibition study, decreased nodule numbers in soybeans and lima beans, the recommended rates decreased soybean but not lima bean nodulation (Yueh and Hensley 1990). According to Mårtensson (1992), the development of nodules by *R. leguminosarum* b.v. *trifolii* was inhibited at concentrations of bentazone higher than label rates. In this study, the application of herbicides had a remarkable association with rhizobial nodulation in bean fields where trifluralin was the most commonly used herbicide followed by bentazone and paraquat. This positive impact of herbicide application may be explained with the significant linkage of bean nodulation to lower weed density evidenced in our study.

In South Australia, lucerne nodulation by *Sinorhizobium meliloti* naturalized in 50 cropping systems was unrelated to plant density (range 3–66 plants · m^{-2}; Ballard *et al.* 2003). Similarly, this study evidenced the lack of a significant association between root nodulation and plant density (range 4–68 plants · m^{-2}) in commercial bean fields.

Acknowledgements

The authors gratefully acknowledge the Iranian Ministry of Agriculture, for funding project no. 4-47-16-86143.

References

Ballard R.A., Shepherd B.R., Charman N. 2003. Nodulation and growth of pasture legumes with naturalised soil rhizobia. 3. Lucerne (*Medicago sativa* L.). Australian Journal of Experimental Agriculture 43 (2): 135–140. DOI: https://doi.org/10.1071/ea02047

Bertholet J., Clark K.W. 1985. Effect of trifluralin and metribuzin on faba bean growth, development, and symbiotic nitrogen fixation. Canadian Journal of Plant Science 65 (1): 9–21. DOI: https://doi.org/10.4141/cjps85-002

Campo R.J., Araujo R.S., Hungria M. 2009. Nitrogen fixation with the soybean crop in Brazil: Compatibility between seed treatment with fungicides and bradyrhizobial inoculants. Symbiosis 48 (1–3): 154–163. DOI: https://doi.org/10.1007/bf03179994

Chemining'wa G.N., KevinVessey J. 2006. The abundance and efficacy of *Rhizobium leguminosarum* bv. *viciae* in cultivated soils of the eastern Canadian prairie. Soil Biology and Biochemistry 38 (2): 294–302. DOI: https://doi.org/10.1016/j.soilbio.2005.05.007

Coutinho H.L., DeOliveira V.M., Moreira F.M.S. 2000. Systematics of legume nodule nitrogen fixing bacteria. In: "Applied Microbial Systematics" (F.G. Priest, M. Goodfellow, eds). Springer, Dordrecht. DOI: https://doi.org/10.1007/978-94-011-4020-15

Dart P.J., Mercer F.V. 1965. The effect of growth temperature, level of ammonium nitrate, and light intensity on the growth and nodulation of cowpea (*Vigna sinensis* Endl. ex Hassk.). Australian Journal of Agricultural Research 16 (3): 321–345. DOI: https://doi.org/10.1071/ar9650321

Elias N.V., Herridge D.F. 2015. Naturalised populations of mesorhizobia in chickpea (*Cicer arietinum* L.) cropping soils: effects on nodule occupancy and productivity of commercial chickpea. Plant and Soil 387 (1–2): 233–249. DOI: https://doi.org/10.1007/s11104-014-2298-z

Habish H.A., Mahdi A.A. 1976. Effect of soil moisture on nodulation of cowpea and hyacinth bean. Journal of Agricultural Science 86 (3): 553–560. DOI: https://doi.org/10.1017/s0021859600061098

Howieson J., Ballard R. 2004. Optimising the legume symbiosis in stressful and competitive environments within southern Australia – some contemporary thoughts. Soil Biology and Biochemistry 36 (8): 1261–1273. DOI: https://doi.org/10.1016/j.soilbio.2004.04.008

Kalantari S., Marefat A.R., Naseri B., Hemmati R. 2018. Improvement of bean yield and Fusarium root rot biocontrol using mixtures of *Bacillus*, *Pseudomonas* and *Rhizobium*. Tropical Plant Pathology 43 (6): 499–505. DOI: https://doi.org/10.1007/s40858-018-0252-y

Kranz J. 1974. Comparison of epidemics. Annual Review of Phytopathology 12: 355–374.

Kucey R.M.N., Hynes M.F. 1989. Populations of *Rhizobium leguminosarum* biovars *phaseoli* and *viceae* in fields after bean or pea in rotation with nonlegumes. Canadian Journal of Microbiology 35 (6): 661–667. DOI: https://doi.org/10.1139/m89-107

Mårtensson A.M. 1992. Effects of agrochemicals and heavy metals on fast-growing rhizobia and their symbiosis with small-seeded legumes. Soil Biology and Biochemistry 24 (5): 435–445. DOI: https://doi.org/10.1016/0038-0717-(92)90206-d

Naidu P.H. 2000. Effect of pre- and post-inoculation seed treatment with chemicals on *Rhizobium* in groundnut. Indian Journal of Plant Protection 28: 152–155.

Naseri B. 2008. Root rot of common bean in Zanjan, Iran: major pathogens and yield loss estimates. Australasian Plant Pathology 37 (6): 546–551. DOI: https://doi.org/10.1071/ap08053

Naseri B. 2013a. Epidemics of Rhizoctonia root rot in association with biological and physicochemical properties of field soil in bean crops. Journal of Phytopathology 161 (6): 397–404. DOI: https://doi.org/10.1111/jph.12077

Naseri B. 2013b. Linkages of farmers' operations with *Rhizoctonia* root rot spread in bean crops on a regional basis. Journal of Phytopathology 161 (11–12): 814–822. DOI: https://doi.org/10.1111/jph.12140

Naseri B. 2014a. Bean production and Fusarium root rot in diverse soil environments in Iran. Journal of Soil Science and Plant Nutrition 14: 177–188. DOI: https://doi.org/10.4067/s0718-95162014005000014

Naseri B. 2014b. Charcoal rot of bean in diverse cropping systems and soil environments. Journal of Plant Disease and Protection 121 (1): 20–25. DOI: https://doi.org/10.1007/bf03356486

Naseri B. 2014c. Sowing, field size, and soil characteristics affect bean-*Fusarium*-wilt pathosystems. Journal of Plant Disease and Protection 121 (4): 171–176. DOI: https://doi.org/10.1007/bf03356506

Naseri B. 2019. The potential of agro-ecological properties in fulfilling the promise of organic farming: a case study of bean root rots and yields in Iran. p. 361–389. In: "Organic Farming, Global Perspectives and Methods" (M.R Unni, C. Sarathchandran Veloormadom, S. Thomas, eds). Elsevier, USA, 407 pp.

O'Connor G.E., Evans J., Fettell N.A., Bamforth I., Stuchberry J., Heenan D.P., Chalk P.M. 1993. Sowing date and varietal effects on the N_2 fixation of field pea and implications for improvement of soil nitrogen. Australian Journal of Agricultural Research 44 (1): 151–163. DOI: https://doi.org/10.1071/ar9930151

Pena-Cabriales J.J., Alexander M. 1983. Growth of *Rhizobium* in soil amended with organic matter. Soil Science Society of America Journal 47 (2): 241–245. DOI: https://doi.org/10.2136/sssaj1983.03615995004700020013x

Ramos M.L.G., Ribeiro Jr.W.Q. 1993. Effect of fungicides on survival of *Rhizobium* on seeds and the nodulation of bean (*Phaseolus vulgaris* L.). Plant and Soil 152 (1): 145–150. DOI: https://doi.org/10.1007/bf00016344

Remans R., Ramaekers L., Schelkens S., Hernandez G., Garcia A., Reyes J.L., Mendez N., Toscano V., Mulling M., Galvez L., Vanderleyden J. 2008. Effect of *Rhizobium-Azospirillum* coinoculation on nitrogen fixation and yield of two contrasting *Phaseolus vulgaris* L. genotypes cultivated across different environments in Cuba. Plant and Soil 312 (1–2): 25–37. DOI: https://doi.org/10.1007/s11104-008-9606-4

Rennie R.J., Kemp G.A. 1983. N_2-fixation in field beans quantified by 15N isotope dilution. II. Effect of cultivars of beans. Agronomy Journal 75 (4): 645–649. DOI: https://doi.org/10.2134/agronj1983.00021962007500040016x

Revellin C., Pinochet X., Beauclair P., Catroux G. 1996. Influence of soil properties and soya bean cropping history on the *Bradyrhizobium japonicum* population in some French soils. European Journal of Soil Science 47 (4): 505–510. DOI: https://doi.org/10.1111/j.1365-2389.1996.tb01850.x

Rupela O.P., Toomsan B., Mittal S., Dart P.J., Thompson J.A. 1987. Chickpea *rhizobium* populations: Survey of influence of season, soil depth and cropping pattern. Soil Biology and Biochemistry 19 (3): 247–252. DOI: https://doi.org/10.1016/0038-0717(87)90005-8

Sangakkara U.R. 1994. Growth, yield and nodule activity of *Phaseolous vulgaris* L. as affected by soil moisture. Journal of Agronomy and Crop Science 172 (1): 62–68. DOI: https://doi.org/10.1111/j.1439-037x.1994.tb00159.x

Sharma S. 1996. Applied Multivariate Techniques. Wiley, New York, USA, 512 pp.

Slattery J.F., Coventry D.R., Slattery W.J. 2001. Rhizobial ecology as affected by the soil environment. Australian Journal of Experimental Agriculture 41 (3): 289–298. DOI: https://doi.org/10.1071/ea99159

van Schoonhoven A., Pastor-Corrales M. 1987. Standard System for the Evaluation of Bean Germplasm. Centro Internacional de Agricultura Tropical, Cali, Colombia, 53 pp.

Yueh L.Y., Hensley D.L. 1990. Pesticide influence on nitrogen fixation and modulation by soybean and lima bean. Hortscience 25 (9): 1145. DOI: https://doi.org/10.21273/hortsci.25.9.1145f