

Variation of chickpea nodulation in a Mediterranean agroecosystem: relationship with soil characteristics and thresholds for significant contribution to plant growth

Y. Chenene^{1,*}, D. Blavet², M. Belalmi¹, G. Kaci^{1,3}, M. Teffahi¹ and S.M. Ounane¹

¹High National School of Agronomy, Plant Production Department, Laboratory for Vegetal Production, El Harrach, Algiers, Algeria

²Research Institute for Development-IRD, UMR Eco&Sols, Functional Ecology and Biogeochemistry of Soils and Agro-Ecosystems, INRA-IRD-CIRAD-SupAgro, University of Montpellier, Montpellier, France

³University M'Hamed Bougara of Boumerdes, Department of Agronomic Sciences, Avenue de l'indépendance, Boumerdes, Algeria

*Correspondence: cheneneyacine@gmail.com

Received: January 19th, 2021; Accepted: February 28th, 2021; Published: March 22nd, 2021

Abstract. This study was designed to investigate the influence of some environmental constraints on chickpea (*Cicer arietinum* L.) growth and nodulation in a reference agroecosystem. This multi-local field experiment, realized in the agroecosystem of Chlef in northern Algeria, involved 24 sites where the local well-adapted genotype Ain temouchent is grown. Determination of soil properties allowed the identification of three clusters of sites. Plant biomass (SDW) varied significantly among sites from 6.7 to 39.4 g SDW plant⁻¹ and was highly correlated with nodule biomass (NDW). The slope of the regression function between NDW and SDW, defined as the efficiency in use of the rhizobial symbiosis (EURS) inside clusters ranged from 67 to 200 g SDW g⁻¹ NDW. Analysis of the EURS highlighted the existence of a low nodulation threshold of 0.1 g NDW plant⁻¹ below which the contribution of nodulation to the host plant growth was not significant and a high threshold above which the nodule biomass increase was not linked with an increase in shoot biomass. Thus, the significant regression of shoot growth as a function of nodulation ranged from 0.10 to 0.35 g NDW plant⁻¹ ($R^2 = 0.54$, $P < 0.001$). Moreover, nodule biomass was positively correlated with soil Olsen-P, more particularly in cluster A ($R^2 = 0.50$, $P < 0.05$) and B ($R^2 = 0.61$, $P < 0.01$). This approach proved to be a rapid and efficient way to identify the major factors affecting nodulation in order to develop strategies to optimize nodule contribution to chickpea growth and yield.

Key words: chickpea; efficiency in use of the rhizobial symbiosis; nodulation; phosphorus; plant-soil interaction; symbiotic nitrogen fixation.

INTRODUCTION

Legumes play a crucial role in Mediterranean farms sustainability through their agro-ecological services. Moreover, in many households they are often the main source of protein and the central core of healthy diet. Chickpea is one of the three major legumes

in the world, the second most grown in Algeria after dry faba bean (Abdelguerfi-Laouar et al., 2001b; Rawal & Navarro, 2019) and an important crop for low input rainfed agroecosystems due to its ability to grow on poor and marginal soils. Chickpea is generally cultivated in rotation with cereals for its seeds that have high nutritional value, for its stubbles that provide a good forage and also as break crop and a source of nitrogen to the farming system (Boughrarou, 1992; Herridge et al., 1995; Schwenke et al., 1998; Rawal & Navarro, 2019), nitrogen being the most limiting element of crop growth and yield after water in semiarid areas.

Indeed, chickpea like other legumes have the ability to biologically fix atmospheric nitrogen (SNF) through the establishment of a symbiotic association with specific soil-borne rhizobia species from the genera *Mesorhizobium* and *Ensifer* and the formation of root nodules (Dekkiche et al., 2017). Thus, chickpea can fix up to 81% of their requirements from atmospheric nitrogen, a large part of this fixed N is returned to the soil after harvest benefiting the successive crop (Schwenke et al., 1998).

However the success of chickpea cultivation and the expected benefit of its integration into the cropping system depends on an optimal level of nodulation and SNF, otherwise the plant will depend on root uptake, which will negatively affect the pool of N in the soil and lead to poor growth if the soil is already deficient in N (Schwenke et al., 1998; Lazali et al., 2016). Nodule formation and functioning are tightly controlled by the host plant to achieve optimal N nutrition with minimal energy cost (Voisin et al., 2010). However, at field level, nodulation can be affected by several external factors such as the native rhizobial strains in the soil, environmental variables, genotypes differences, farming practices, nutrient availability and soil characteristics (Evans, 1982; Ben Romdhane et al., 2007; Lazali et al., 2016; Dekkiche et al., 2017) and very little is known about what could be considered as an optimal level of nodulation and how to reach this level, moreover, discerning between whether low nodulation is a result or the main cause of low host plant growth remains difficult (Robson et al., 1981; Israel, 1987).

Among these factors, phosphorus deficiency, which could be one of the main causes of low yields of chickpea and legumes in general due to its highly limiting effect on nodule formation and functioning (N_2 -fixation) (Robson et al., 1981; Israel, 1987; Ribet & Drevon, 1996; Benlahrech et al., 2018). According to Vance (2001), most of the world's arable land is p-deficient; in Algeria due to the calcareous nature of the soil, soluble P is precipitated (Djili et al., 1999; Tunesi et al., 1999). As a result of this soil deficiency and the high needs of symbiosis, nodule biomass and thereafter SNF are often strongly correlated with soil available P (Lazali et al., 2016).

Nodulation and SNF can be assessed by different methods such as the direct measurement of the nitrogen deriving from biological fixation, nitrogenase activity in the nodule, counting and/or measuring the dry weight of nodules; some of these methods are very costly and difficult to set up for large multilocal field trials. However, the measurement of nodule dry weight (NDW) represent a rapid and efficient way to assess SNF contribution to plant growth (Drevon et al., 2015; Lazali et al., 2016). Several authors proved that NDW augmentation is positively correlated with SNF and plant growth (Evans, 1982; Rao et al., 2002; Voisin et al., 2003; Elias, 2009; Lazali et al., 2016; Benlahrech et al., 2018; Kaci et al., 2018). Low nodulation is often considered as the major limiting factor of chickpea growth and yield in Mediterranean areas (Ben Romdhane et al., 2007). Thus the need arises for a proper analysis of chickpea nodulation in order to determine the major factors affecting nodulation and establish thresholds of

optimal nodulation since low NDW was proved to have no significant positive effect on the host plant (Drevon et al., 2015).

In this context, the major objective of this work is to assess the levels of nodulation of winter sown chickpea grown under rainfed conditions in the region of Chlef in the north of Algeria and emphasize the quantitative relationship between plant growth and nodulation in interaction with contrasted soil conditions.

MATERIALS AND METHODS

Study area and diagnostic Sites

The nodular diagnosis was conducted during the 2017–2018 growing season in the semiarid region of Chlef located in the north west of Algeria, in an area of 40 by 30 km extending from 36°26'5"N, 1°6'15"E, altitude 193 m at the northwestern limit, to 36°5'20" N, 1°27'5"E, altitude 517 m at the southeastern limit (Fig. 1). The area was selected for its importance in winter rainfed chickpea production and is characterized by cold wet winter and hot dry summer, with irregular rainfall (603 mm during the growth 2017–2018 season). The 24 sites were chosen on the basis of a previous farm survey to cover a large diversity in agro-ecological conditions. In each location the diagnostic site consisted in a 20 by 20 m homogeneous area.

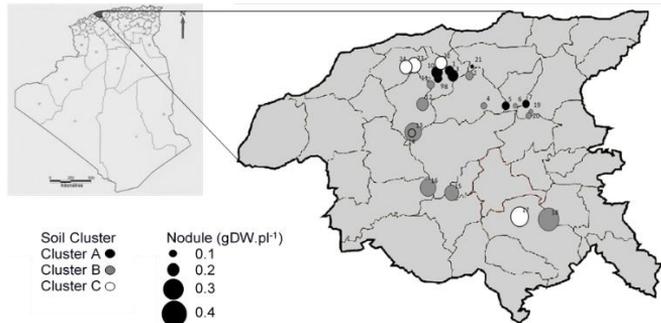


Figure 1. Map of the study area showing the locations of the sampled sites and the spatial variation of nodulation, the size of the dots corresponds to the mean NDW of each site and the color of the dot indicates to which cluster the site belongs.

Plant material and growth condition

In each site chickpea was grown according to conventional agricultural practices, as recommended by local authorities. Weed, pests and diseases were controlled by appropriate pesticides. All sites were rainfed without irrigation, and sown at 30–35 seeds per m² without rhizobial inoculation. Before sowing, the topsoil was mechanically prepared using a disc plough and a harrow. Sowing was performed using a seeder followed by a smooth roller, the distances between rows and between plants on the row were 30 and 10 cm, respectively. This experiment was carried out with the well adapted commonly grown cultivar Aïn Témouchent, a vigorous, short-cycle chickpea genotype with erect growth, good culinary and nutritional qualities of grains (Boughrarou, 1992; Abdelguerfi-Laouar et al., 2001a, 2001b).

Soil sampling and analysis

At sowing four random soil samples from each site were collected at 0–30 cm with an auger and conserved at 4 °C before analysis in the laboratory of soil science (ENSA–Algeria). Soil samples were crushed and sieved through a 2 mm sieve to obtain

the fine soil fraction before measuring soil pH in distilled water and granulometry by sedimentation with the pipette method (Robinson, 1922), the obtained values were then used to assign each site with a textural class based on the soil textural triangle. Total nitrogen, CaCO₃ content, available phosphorus, total organic carbon were measured with the methods of Kjeldahl (1883), Bernard Calcimeter, Olsen (Olsen & Dean, 1965) and Anne (1945), respectively.

Assessment of growth, nodulation and mapping

At full flowering stage which corresponds to period with maximal nodule dry weight (NDW), 20 plants per site were randomly harvested from the middle rows of each site by carefully digging around each plant. Shoots were separated from roots at the cotyledonary node. Nodules, if any, were gently separated from roots and the surrounding soil, washed with water and stored at 4 °C for further measurements. All the samples were oven dried for 48 h at 70 °C before being weighted. Mean NDW for each site were used in QGIS software (3.4.4) to generate the map in Fig. 1. Linear regression models were computed between nodules and shoots dry weights ($SDW = a \cdot NDW + b$). The slopes of these models (a) were retained as estimators of the efficiency in use of the rhizobial symbiosis (EURS) for shoot biomass productions with the atmospheric N₂ fixed by the nodules i.e the ratio of N₂-dependent growth/nodule mass, while the intercepts (b) are shoot biomass productions without nodules i.e with soil nitrogen only (Zaman-Allah et al., 2007; Drevon et al., 2015).

Statistical analysis

The statistical analyzes were performed with R software version 3.5.2 (R Core team, 2018). Means, standard errors (s.e.) and standard deviations (s.d.) were computed with 20 plants and 4 soil samples by site. Analyzes of variance were performed and means among sites were compared using Tukey's multiple comparison tests at $P < 0.05$, $P < 0.01$, and $P < 0.001$.

Hierarchical clustering of sites was performed using the physico-chemical properties of sites with Ward method. The relationships between nodule biomass, shoot biomass and available soil phosphorus (Olsen-P) were assessed by regression analysis. The relationship between nodulation and shoot biomass was also observed with locally weighted scatterplot smoothing (loess method). Bar graphs and scatter plots were also performed using R v 3.5.2.

RESULTS

Soil physico-chemical properties

Table 1 summarizes some physico-chemical properties of the sites, data show highly significant differences between the sites for all parameters. Overall, the diagnostic sites had a basic pH ranging from 8.09 ± 0.15 to 8.56 ± 0.04 corresponding to site 16 and 19 respectively, total organic matter varied from 1.06 ± 0.01 to 2.47 ± 0.52 corresponding to sites 20 and 13 respectively, moreover the total CaCO₃ content ranged from 4.72 ± 0.14 in site 23 to 29.47 ± 6.12 in site 14, the highest values of Olsen-P were observed in sites 24 and 13 with 30.47 ± 2.27 and 32.19 ± 3.03 respectively and the lowest is site 19 with 5.02 ± 0.22 , the total nitrogen ranged from 0.93 ± 0.06 in site 05 to 2.40 ± 0.30 in site 11.

Table 1. Physico-chemical properties of the 24 site of the diagnostic. Data are means \pm standard deviation of 4 soil samples collected before sowing

Site	Clay ***	Silt ***	Sand ***	OM (%) ***	Ph ***	CaCO ₃ (%) ***	Olsen-P (mg kg ⁻¹) ***	Total N (g kg ⁻¹) ***
S01	41 \pm 0.60	34 \pm 1.28	24 \pm 1.04	1.4 \pm 0.33	8.4 \pm 0.32	16.64 \pm 0.55	20.32 \pm 1.65	1.63 \pm 0.15
S02	37 \pm 0.49	38 \pm 0.95	25 \pm 1.08	1.2 \pm 0.01	8.3 \pm 0.00	21.60 \pm 0.96	15.95 \pm 1.17	1.43 \pm 0.23
S03	43 \pm 1.56	36 \pm 0.26	20 \pm 1.80	1.3 \pm 0.04	8.3 \pm 0.07	17.91 \pm 0.48	23.14 \pm 1.10	1.87 \pm 0.21
S04	31 \pm 0.91	39 \pm 0.56	30 \pm 1.46	1.1 \pm 0.10	8.4 \pm 0.15	24.88 \pm 0.60	19.28 \pm 2.28	1.17 \pm 0.25
S05	38 \pm 0.70	35 \pm 0.85	27 \pm 0.95	1.2 \pm 0.05	8.4 \pm 0.08	16.32 \pm 0.42	14.02 \pm 0.57	0.93 \pm 0.06
S06	30 \pm 0.31	32 \pm 0.78	38 \pm 0.47	1.2 \pm 0.03	8.3 \pm 0.04	15.60 \pm 0.24	6.89 \pm 0.71	1.00 \pm 0.26
S07	36 \pm 0.06	33 \pm 0.06	31 \pm 0.00	1.3 \pm 0.05	8.4 \pm 0.03	13.12 \pm 0.73	7.80 \pm 1.90	1.40 \pm 0.30
S08	44 \pm 0.40	36 \pm 1.25	20 \pm 0.87	1.4 \pm 0.07	8.3 \pm 0.01	12.32 \pm 0.37	14.11 \pm 3.35	1.53 \pm 0.21
S09	40 \pm 1.72	37 \pm 0.72	22 \pm 1.11	1.2 \pm 0.03	8.3 \pm 0.06	14.56 \pm 2.18	13.40 \pm 1.37	1.67 \pm 0.25
S10	39 \pm 1.37	35 \pm 0.31	26 \pm 1.37	1.1 \pm 0.07	8.3 \pm 0.01	11.28 \pm 2.44	21.17 \pm 4.62	1.83 \pm 0.06
S11	32 \pm 0.26	36 \pm 0.83	32 \pm 0.85	2.1 \pm 0.22	8.4 \pm 0.07	12.32 \pm 0.14	21.48 \pm 0.94	2.40 \pm 0.30
S12	36 \pm 0.70	41 \pm 1.63	23 \pm 2.32	1.2 \pm 0.05	8.3 \pm 0.01	21.12 \pm 0.83	18.90 \pm 1.77	1.37 \pm 0.35
S13	34 \pm 0.15	31 \pm 0.40	34 \pm 0.49	2.5 \pm 0.52	8.4 \pm 0.12	20.88 \pm 0.48	32.19 \pm 3.03	2.30 \pm 0.10
S14	32 \pm 1.12	35 \pm 1.57	34 \pm 2.68	1.5 \pm 0.63	8.4 \pm 0.02	29.47 \pm 6.12	10.51 \pm 3.96	1.27 \pm 0.12
S15	31 \pm 1.51	40 \pm 0.53	29 \pm 1.35	1.4 \pm 0.09	8.5 \pm 0.08	24.80 \pm 3.50	26.07 \pm 2.59	1.93 \pm 0.32
S16	33 \pm 0.44	37 \pm 1.35	30 \pm 0.95	1.3 \pm 0.07	8.1 \pm 0.15	24.16 \pm 0.55	22.62 \pm 0.54	2.27 \pm 0.31
S17	35 \pm 0.67	36 \pm 2.57	29 \pm 3.12	1.7 \pm 0.14	8.4 \pm 0.02	8.32 \pm 0.28	28.92 \pm 2.66	2.30 \pm 0.20
S18	30 \pm 0.36	34 \pm 0.45	35 \pm 0.12	1.6 \pm 0.06	8.1 \pm 0.08	20.16 \pm 0.96	24.10 \pm 2.19	1.96 \pm 0.13
S19	31 \pm 1.75	33 \pm 0.70	36 \pm 1.31	1.1 \pm 0.03	8.6 \pm 0.04	20.72 \pm 0.50	5.02 \pm 0.22	1.33 \pm 0.50
S20	33 \pm 0.29	39 \pm 0.95	28 \pm 0.86	1.1 \pm 0.01	8.2 \pm 0.08	26.63 \pm 0.39	15.76 \pm 2.96	1.47 \pm 0.23
S21	42 \pm 0.84	38 \pm 0.42	19 \pm 1.15	1.3 \pm 0.09	8.1 \pm 0.14	12.64 \pm 0.60	6.38 \pm 0.65	1.77 \pm 0.31
S22	36 \pm 0.17	40 \pm 2.89	24 \pm 2.79	1.4 \pm 0.02	8.4 \pm 0.13	5.52 \pm 0.00	23.13 \pm 0.88	2.20 \pm 0.26
S23	35 \pm 1.13	39 \pm 0.55	26 \pm 1.64	1.3 \pm 0.05	8.3 \pm 0.07	4.72 \pm 0.14	25.19 \pm 3.14	1.93 \pm 0.06
S24	37 \pm 1.30	37 \pm 0.38	26 \pm 1.42	1.4 \pm 0.06	8.3 \pm 0.11	8.41 \pm 1.49	30.47 \pm 3.27	1.43 \pm 0.31

*** indicate significant difference at $p < 0.001$.

The highest values of clay (44%), silt (41%) and sand (36%) were found in sites 8, 12, and 19 respectively. Based on soil particle size, we determined sites textural class of each site according to the USDA texture triangle. Following this textural classes; the sites can be divided into 2 groups: clay sites which regroups sites 1, 3, 8, 9 and 21. This sites exhibit the highest percentage of clay (over 40%), the rest of the sites have a clay loam texture. Fig. 1 shows that all these clay sites are located in the north of the study area.

Clustering analysis allowed regrouping the sites into three clusters gathering between four to twelve sites. Table 2 shows the mean values of soil properties for each cluster. Clusters were significantly different in all parameters except CaCO₃ content, pH and total organic matter content. Cluster A regroups 8 sites (1, 3, 5, 7, 8, 9, 10 and 21) and contains all the clay sites. In this cluster mean total N and Olsen-P are also the lowest. Cluster B contains 12 sites (2, 4, 6, 11, 12, 13, 14, 15, 16, 18, 19 and 20) and show the highest CaCO₃ content, but medium levels of Olsen P. On the other hand, cluster C which is characterized by a low CaCO₃ content and the and highest P content contains 4 sites (17, 22, 23 and 24).

Shoot and nodule biomass

Significant differences in shoot and nodule biomass were observed between sites (Fig. 2), plants in site 8 had no nodules and low shoot biomass (7.9 ± 1.74 g DW plant⁻¹). Site 17 and 18 exhibited the highest nodulation (0.32 ± 0.02 and 0.38 ± 0.03 g DW plant⁻¹ respectively). Overall, nodule biomass ranged from 0 to 0.38 g DW plant⁻¹ and shoots biomass from 6.7 to 39.4 g DW plant⁻¹ corresponding to sites 6 and 17 respectively. Moreover Fig. 1 indicates that all the sites in the north east of the study area suffered from low nodulation, while the sites in the north and south had average to high nodulation.

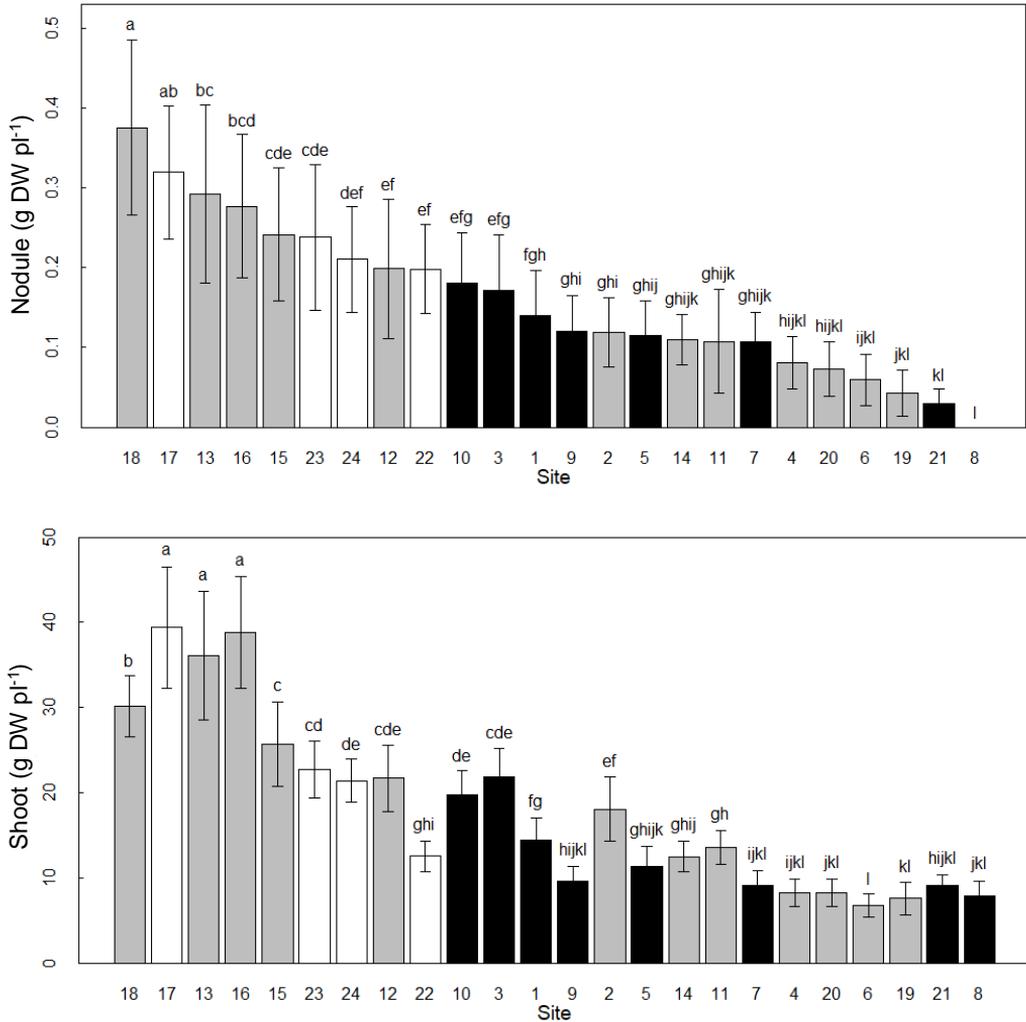


Figure 2. Nodule and shoot biomass of the Ain Témouchent genotype grown in 24 sites of Chlef. Values are means of 20 plants per site harvested at flowering stage. Vertical bars represent standard error. Black, grey and white color corresponds to cluster A, B and C respectively. Different letters indicate significant differences between sites at $P < 0.05$.

Nodulation and growth as a function of soil properties

As shown in Table 2, in cluster A who contains all the clay sites and the lowest values of total N and Olsen-P, shoot and nodule biomass were low. Cluster B who exhibited a high content of CaCO₃ and medium content of Olsen-P is characterized by an average shoot and nodule biomass production. On the other hand, cluster C which is characterized by a low CaCO₃ content and the highest P content exhibited the highest shoot and nodule biomass.

According to Fig. 3 a positive correlation between P augmentation and NDW was found in cluster A and B ($R^2 = 0.5$ at $P < 0.05$ and $R^2 = 0.61$ at $P < 0.01$ respectively). On the other hand, no significant correlation was found for cluster C which corresponds to the cluster with the highest P content in average (26.93 ± 3.80 mg kg⁻¹).

Table 2. Mean shoot biomass, nodule biomass, physical and chemical properties of the three soil clusters. Data are means \pm standard deviation

	A	B	C
Sand (%) *	23.66 \pm 3.92 ^b	31.08 \pm 4.55 ^a	25.96 \pm 2.73 ^b
Silt (%) *	35.75 \pm 1.75 ^c	36.28 \pm 3.29 ^{ab}	38.29 \pm 2.38 ^a
Clay (%) ***	40.59 \pm 2.70 ^a	32.64 \pm 2.29 ^c	35.75 \pm 1.19 ^b
OM (%) (ns)	1.28 \pm 0.14	1.41 \pm 0.45	1.44 \pm 0.19
Total N (g.kg ⁻¹) *	1.58 \pm 0.34 ^b	1.66 \pm 0.53 ^{ab}	1.97 \pm 0.40 ^a
Olsen-P (mg kg ⁻¹)***	15.33 \pm 6.13 ^b	18.23 \pm 7.93 ^{ab}	26.93 \pm 3.80 ^a
CaCO ₃ (%) (ns)	14.35 \pm 2.50	21.86 \pm 4.86	6.74 \pm 1.84
pH (ns)	8.31 \pm 0.14	8.32 \pm 0.16	8.37 \pm 0.09
SDW (g) ***	12.71 \pm 5.32 ^c	19.17 \pm 11.83 ^b	23.65 \pm 10.78 ^a
NDW (g) ***	0.11 \pm 0.07 ^c	0.17 \pm 0.13 ^b	0.24 \pm 0.09 ^a

*, **, *** indicates significant difference at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively; ns: not significant ($P > 0.05$). Values followed by the same letter are not significantly different at $P < 0.05$.

Efficiency in use of the rhizobial symbiosis

In order to better observe how nodulation links with shoot biomass, the mean SDW of each site was plotted as a function of the mean NDW in each cluster and among all sites.

For the three clusters, as shown in Fig. 4, the SDW and NDW were significantly correlated regardless of cluster N and P contents, with the EURS ranging from 67 to 200 g SDW g⁻¹ NDW corresponding to cluster A and C, respectively.

Among all sites (Fig. 5, A), results show a highly significant correlation between shoot and nodule biomasses ($R^2 = 0.81$, $P < 0.001$) with a linear model. On the other hand the loess curve shows three distinct parts, a first part where NDW augmentation is not accompanied by SDW augmentation, a second part that exhibits positive correlation between the two parameters and a third part where SDW is not affected or tends to decrease when NDW increases.

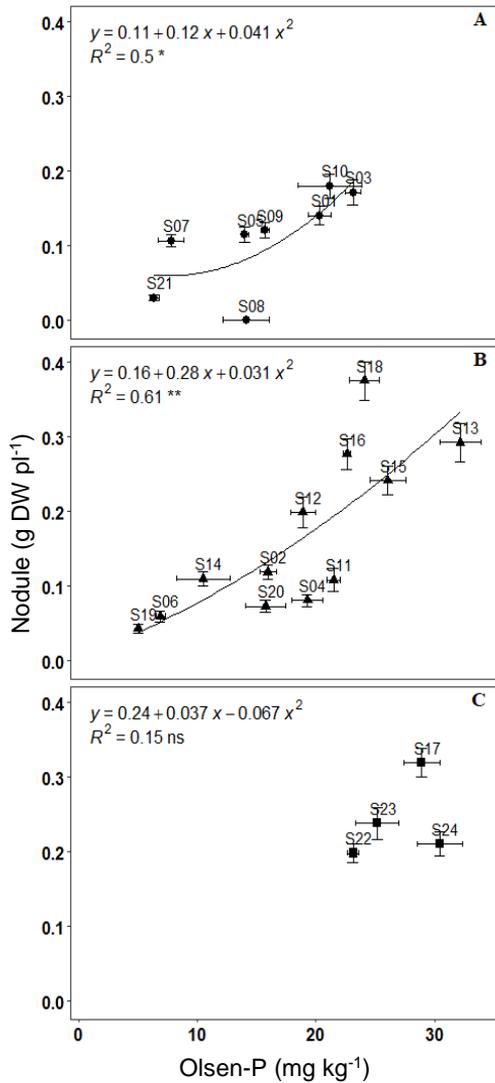


Figure 3. Nodule biomass as related to the concentration of Olsen-P in the sites of the three soil clusters (A, B and C). Each point represents the means calculated on 20 plants per site harvested at flowering stage and 4 soil samples collected before sowing. Vertical and horizontal bars represent standard errors. A curvilinear regression was fitted to the data. *, **, *** indicates significance at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively.

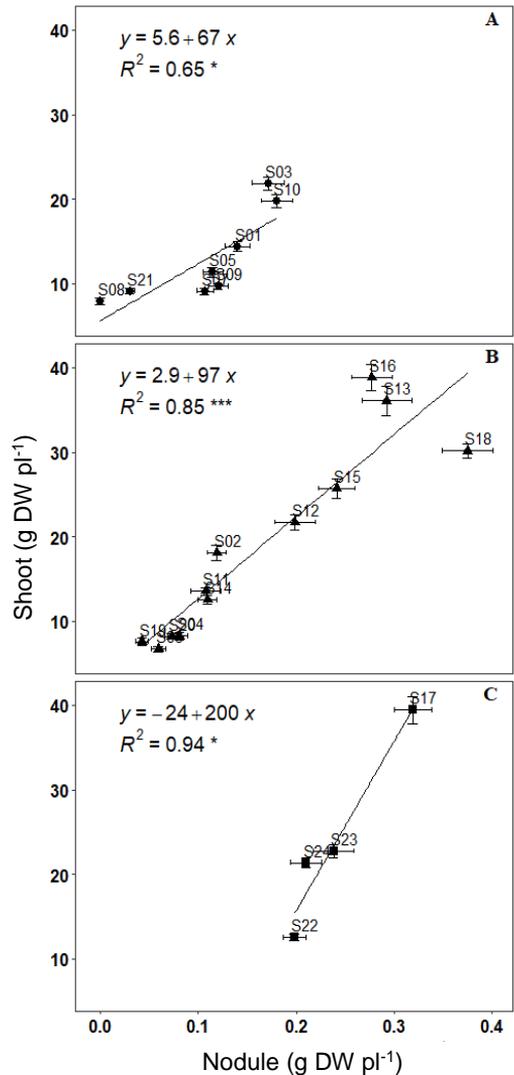


Figure 4. Shoot biomass as related to nodule biomass in the three soil clusters (A, B and C). Each point represents the means calculated on 20 plants per site harvested at flowering stage. Vertical and horizontal bars represent standard errors. A linear regression was fitted to the data. *, **, *** indicates significance at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively.

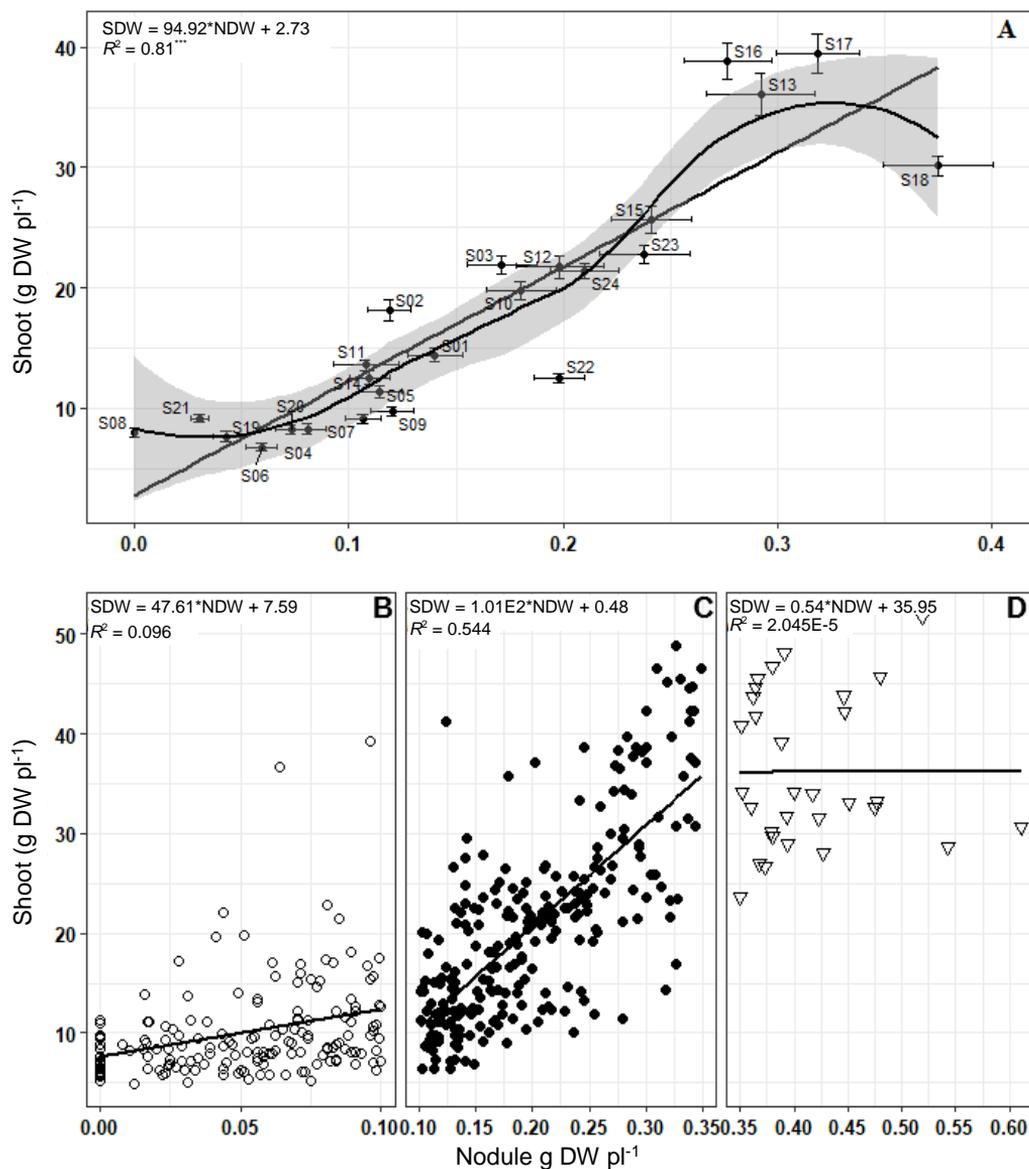


Figure 5. Efficiency in use of rhizobial symbiosis in the 24 site of the study (A); for plants with a NDW less than 0.1 g (B); plants with a NDW between 0.1 and 0.35 g (C) and plants with a NDW above 0.35 g (D). For (A) each point represents the means calculated on 20 plants per site harvested at flowering stage. Vertical and horizontal bars represent standard errors. A linear regression was fitted to the data in (A) and (C) and a loess regression was fitted to the data in (A). *, **, *** indicates significance at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively.

To investigate in more details to what extent the NDW was linked to shoot production, all the sampled plants were divided into three groups based on NDW: the first group of plants with nodulation below 0.1 g NDW plant^{-1} ; the second with NDW between 0.10 and 0.35 g NDW plant^{-1} ; the third with NDW above 0.35 g NDW plant^{-1}

(Fig. 5, B; 5, C & 5, D respectively) based on Drevon et al. (2015) work and the loess regression analysis. Results show no significant correlation between the two parameters for the first and third group (Fig. 5, B & 5, D respectively), on the other hand it shows a highly significant correlation for the second group (Fig. 5, C) ($R^2 = 0.54$ at $P < 0.001$).

DISCUSSION

Our study provided results at several scales: the scale of all the sites, the scale of more homogeneous subsets of sites (soil clusters), and the scale of some particular sites. At all sites scale, we observed a high variability of nodulation and plant growth for chickpea and the correlation between NDW and SDW among all sites was highly significant indicating that growth was dependent on the nitrogen fixed by nodules (except for site 08 which relied entirely on soil nitrogen to grow) in the study area. Indeed Voisin et al. (2003) showed that NDW augmentation was positively correlated with N fixation although Drevon et al. (2015) indicated the existence of a nodulation threshold for significant SNF contribution to the growth of the host plant. Our results in Fig. 2 are in accordance with this author findings because there is no significant differences in SDW for sites 8, 21, 19, 6, 20, 4 which nodulation ranged from 0 to 0.1 g NDW plant⁻¹, showing that below a certain NDW the contribution of nodulation to plant growth is not significant. Moreover our results indicate the existence of a highly significant correlation when NDW ranges from 0.1 g to 0.35 g (Fig. 5, C) proving that below a certain NDW threshold there is no effect of nodules on plant growth and above a certain threshold an increase in NDW does not have positive effect on the host plant. For the upper threshold, it is most likely that plant needs in N were satisfied and growth became limited by other factors which makes NDW augmentation unnecessary especially due to the high energy cost of nodule formation and functioning (Minchin & Pate, 1973; Ryle et al., 1979). As shown by Voisin et al. (2015) the efficiency of the Legume-Rhizobia symbiosis (N benefits for the plant against carbon cost of nodulation) decreases with the augmentation of nodulation, as well as the nodule specific activity (Evans, 1982).

Data also show that among all sites, the increase in nodulation was correlated with P augmentation and no significant inhibitory effect of soil N was observed, in fact the site with the highest SDW and NDW corresponded to site with highest total N. Our results are similar to those of Elias (2009) who indicated that N concentration in 0–30 cm had no significant negative effect on the nodulation of chickpea in Australia. It is well established that nitrogen in the root zone limits nodulation at high levels (Gentili & Huss-Danell, 2002; Namvar et al., 2011; Goh et al., 2016, Bekele et al., 2019), but the presence of adequate levels of N in the soil is proved to not impair nodulation and SNF or even improve it when P levels are optimal (Reinsvold & Pope, 1987; Bekele et al., 2019; Hellsten & Huss-Danell, 2000). It is also possible that the presence of high P content in the soil made the N levels insufficient in absence of other limiting factor which favored nodulation and N fixation. Various authors indicated that having a starter N in the soil before the onset of nodule functioning or at certain stages of plant growth is optimal for nodulation and SNF (Yinbo et al., 1997; Gan et al., 2003, 2004) and Namvar et al. (2011) proved that adding up to 75 kg ha⁻¹ N fertilizer to chickpea had a positive effect on growth, yield and nodulation.

The positive effect of P on legumes nodulation and SNF is often explained by either a positive stimulation of the host plant (Robson et al., 1981) or by a direct effect on

nodules growth and functioning (Israel, 1987; Hellsten & Huss-Danell, 2000; Gentili & Huss-Danell, 2002), indeed Ribet & Drevon (1996) indicated that NDW was more impaired by P deficiency than SDW even though P concentration in nodules remained unaffected and Drevon & Hartwig (1997) also found similar results for soybean and alfalfa.

At this scale, NDW and SDW were also significantly correlated to organic matter content. Moreover, the site with the highest organic matter was also the site with the highest Olsen-P which could be due to a reducing effect of organic matter on P-insolubilisation in calcareous soil (Braschi et al., 2003) or to the general use of P-rich organic inputs like manure in the study area, highlighting simple relationships between soil properties is often difficult due to their highly intercorrelated character (Ige et al., 2007). Alternatively, it could also be assumed that organic matter promotes the development of symbiotic microorganisms.

Considering that plant growth and nodulation are subjected to different factors, notably to soil characteristics, we chose to use a clustering approach (as previously used by Lazali et al. (2016)) to group sites on the basis of their similarities to obtain homogenous groups (Oumenskou et al., 2018). This approach allowed having a more accurate idea of the effect of some parameters on plant such as the relation between available P and nodulation observed in Fig. 3. Cluster C exhibited the highest nodulation which can be linked to the high P content suggesting that the abundance of P and N promoted biomass production and was not limiting for nodulation in this cluster, the absence of correlation between nodulation and soil available P is mainly due to the high P content in this cluster ($26.93 \pm 3.80 \text{ mg kg}^{-1}$) compared to the two other clusters which indicate that plant and nodule requirement in that element were covered (Shukla & Yadav, 1982). On the other hand data also show that, site 18 gave the higher NDW per unit of P which can be an indicator of the presence of P efficient rhizobia strains in the site (Zarrin et al., 2006). P deficiency is known to be the major limiting factor of legumes growth and SNF in low input Algerian farming systems because this crops have higher phosphorus needs compared to plant relying on mineral N (Israel, 1987; Ribet & Drevon, 1996) and because of the price of farming inputs no mineral fertilizer is added, in addition to that, P is often precipitated due to the high CaCO_3 content of soils under alkaline conditions (Tunesi et al., 1999; Mihoub et al., 2016).

Our results also show that clay sites had lesser SDW and NDW compared to clay loam sites, White & Robson (1989) obtained similar results for lupins SDW and NDW on two different soil types and showed that this culture grow poorly on fine-textured soils under alkaline condition, furthermore Msumali & Judith (1998) showed in a jar experiment with inoculated bean that nodulation tends to decline from the sandy through loam to the clay soils and obtained significantly higher shoot weights in the sandy and loam soils than in the clay soil. Our results could be explained by the joined effect of soil type and the native rhizobial population. Indeed Elias (2009) found in a multi-local field trial on chickpea that nodule biomass was largely governed by the size of the rhizobial population in the soil but showed that soil texture and clay content did not affect highly the size of this rhizobial populations.

Soil particle size is known to have a significant effect on plants growth especially legumes which needs adequate soil condition to promote root growth, nodules development and nitrogen fixation, indeed coarse textured soils ensure a better aeration and water infiltration compared to fine-textured soils which tends to puddle and form a

hard soil crust after receiving heavy rains. Indeed the effect of soil type on plants and rhizobia is tightly related to water status, Issa & Wood (1995) confirmed that rhizobia survival and multiplication is dependent on the combined effect of soil type and moisture, although fine textured soil improves rhizobia survival (Heijnen et al., 1992). These soils can limit significantly nodulation and growth when water is in excess and the diagnosis region received in 2018 63% more rains than the 35 years average which could cause waterlogging and affect negatively nodulation and growth (Worku, 2016). Chickpea being more sensitive to waterlogging than other legumes such as faba bean which explains the maintaining of spring chickpea in Algeria (Munir, 2016).

In site 8 plants had no nodules despite the fact that the site had average phosphorus content and no high inhibitory nitrogen content, the absence of nodulation in this site can be explained by the absence of rhizobia in the soil or by the presence of other inhibitory factors. Overall, the augmentation of shoot biomass was correlated with the augmentation in nodule biomass (Fig. 5, A) suggesting that plants relied mostly on nitrogen deriving from biological fixation to grow. The low nodulation observed in some site can be explained by a sufficient N supply covering plant needs. Voisin et al. (2002) and (2010) showed that nodule initiation occurs only under situations of N limitation when the uptake by roots appeared to be insufficient to fully satisfy plant N demand. These authors also demonstrated that NDW augmentation is correlated with SDW, which is consistent with our results, indicating that the presence of limiting factors restricting plant growth would restrict nodulation also. The absence of nodulation can also be linked to low population of native rhizobia in the soil or to the ineffectiveness of the indigenous strains of rhizobia (Elias, 2009; Benlahrech et al., 2018).

Finally and although we could not provide a precise quantification of the spatialization of nodulation and plant growth, the map shown in Fig. 1 suggests the existence of a regionalization of nodulation and the associated plant growth, with areas grouping the lowest values and areas grouping the highest values. This regionalization could be due to a regionalization of soil properties, farming practices or also certain environmental factors (climatic or pedoclimatic factors for example) whose impact could be verified in the future.

CONCLUSION

The nodular diagnosis realized in the semiarid region of Chlef emphasized the strong relationship between chickpea growth and nodulation, the latter is mainly controlled by soil Olsen-P as well as by other physical and chemical properties of the soil. The major finding of this work is the highlight of thresholds for optimal nodulation which could be used by farmers as a benchmark to enhance yield and benefits from incorporating chickpea in the farming system. This work proved the necessity to study in more details the factors impairing the symbiotic system of chickpea to profit more from SNF benefits and accomplish a sustainable improvement of production.

ACKNOWLEDGEMENTS. The authors thank the company semence du Chéiff for their technical support of this work. The authors also thank the local authorities and chickpea growers for their participation in the multilocal trial realized in this study.

REFERENCES

- Abdelguerfi-Laouar, M., Bouzid, L., Zine, F., Hamdi, N., Bouzid, H. & Zidouni, F. 2001a. Evaluation of some local chickpea genotypes in the Bejaia region. *Recherche Agronomique* **9**, 31–42 (in French).
- Abdelguerfi-Laouar, M., Hamdi, N., Bouzid, H., Zidouni, F., Laib, M., Bouzid, L. & Zine, Z. 2001b. Food legumes in Algeria: situation, state of the genetic resources and case of chickpea in Béjaia. *Proceedings of the 3èmes Journées Scientifiques de l'INRAA*, Bejaia, Algerie, pp. 171–189 (in French).
- Anne, P. 1945. The rapid measurement of soils organic carbon. *Ann. Agron* **2**, 161–172 (in French).
- Bekele, G., Dechassa, N., Tana, T. & Sharma, J.J. 2019. Effects of nitrogen, phosphorus and vermicompost fertilizers on productivity of groundnut (*Arachis hypogaea* L.) in Babile, Eastern Ethiopia. *Agronomy Research* **17**(4), 1532–1546.
- Ben Romdhane, S., Tajini, F., Trabelsi, M., Aouani, M.E. & Mhamdi, R. 2007. Competition for nodule formation between introduced strains of *Mesorhizobium ciceri* and the native populations of rhizobia nodulating chickpea (*Cicer arietinum* L.) in Tunisia. *World. J. Microb. Biot.* **23**, 1195–1201.
- Benlahrech, S., Kaci, G., Teffahi, M. & Ounane, S.M. 2018. Influence of inoculation and phosphorus regimes on symbiotic nitrogen fixation and phosphorus use efficiency of Algerian cowpea (*Vigna unguiculata* L.(Walp.)) landraces. *Agronomy Research* **16**(2), 337–348.
- Boughrarou, F. 1992 Contribution to the study of Algerian genotypes of *Cicer arietinum* L. for their improvement. Thèse de Magister, INA El-Harrach, Algérie (in French).
- Braschi, I., Ciavatta, C., Giovannini, C. & Gessa, C. 2003. Combined effect of water and organic matter on phosphorus availability in calcareous soils. *Nutr. Cycl. Agroecosys.* **67**, 67–74.
- Dekkiche, S., Benguedouar, A., Sbabou, L., Taha, K., Filali-Maltouf, A. & Béna, G. 2018. Chickpea (*Cicer arietinum*) is nodulated by unexpected wide diversity of *Mesorhizobium* species in Eastern Algeria. *Arch. Agron. Soil. Sci.* **64**, 285–297.
- Djili, K., Daoud, Y. & Ayache, N. 1999. Analysis of the vertical and spatial distribution of limestone in the soils of north Algeria. *Etude et gestion des sols* **6**, 201–213 (in French).
- Drevon, J.J., Abadie, J., Alkama, N., Andriamananjara, A., Amenc, L., Bargaz, A. & Ounane, S.M. 2015. Phosphorus use efficiency for N₂ fixation in the rhizobial symbiosis with legumes. in: Ed. F.J. de Bruijn (eds.), *Biological Nitrogen Fixation*. Wiley, Sons, Inc., 455–464.
- Drevon, J.J. & Hartwig, U.A. 1997. Phosphorus deficiency increases the argon-induced decline of nodule nitrogenase activity in soybean and alfalfa. *Planta* **201**, 463–469.
- Elias, N. 2009. *Optimizing nodulation in chickpea for nitrogen fixation and yield in the northern grains belt of NSW*. PhD Thesis, The University of Western Sydney, Australia, 228pp.
- Evans, J. 1982. Symbiosis, nitrogen and dry matter distribution in chickpea (*Cicer arietinum*). *Exp. Agr.* **18**, 339–351.
- Gan, Y., Stulen, I., van Keulen, H. & Kuiper, P.J. 2003. Effect of N fertilizer top-dressing at various reproductive stages on growth, N₂ fixation and yield of three soybean (*Glycine max* (L.) Merr.) genotypes. *Field. Crop. Res.* **80**, 147–155.
- Gan, Y., Stulen, I., van Keulen, H. & Kuiper, P.J. 2004. Low concentrations of nitrate and ammonium stimulate nodulation and N₂ fixation while inhibiting specific nodulation (nodule DW g⁻¹ root dry weight) and specific N₂ fixation (N₂ fixed g⁻¹ root dry weight) in soybean. *Plant. Soil.* **258**, 281–292.
- Gentili, F. & Huss-Danell, K. 2002. Phosphorus modifies the effects of nitrogen on nodulation in split-root systems of *Hippophaë rhamnoides*. *New. Phytol.* **153**, 53–61.

- Goh, C.H., Nicotra, A.B. & Mathesius, U. 2016. The presence of nodules on legume root systems can alter phenotypic plasticity in response to internal nitrogen independent of nitrogen fixation. *Plant. Cell. Environ* **39**, 883–896.
- Heijnen, C.E., Hok-A-Hin, C.H. & Van Veen, J.A. 1992. Improvements to the use of bentonite clay as a protective agent, increasing survival levels of bacteria introduced into soil. *Soil. Biol. Biochem.* **24**, 533–538.
- Hellsten, A. & Huss-Danell, K. 2000. Interaction effects of nitrogen and phosphorus on nodulation in red clover (*Trifolium pratense* L.). *Acta. Agr. Scand. B-S. P.* **50**, 135–142.
- Herridge, D.F., Marcellos, H., Felton, W.L., Turner, G.L. & Peoples, M.B. 1995. Chickpea increases soil-N fertility in cereal systems through nitrate sparing and N₂ fixation. *Soil. Biol. Biochem.* **27**, 545–551.
- Ige, D.V., Akinremi, O.O. & Flaten, D.N. 2007. Direct and indirect effects of soil properties on phosphorus retention capacity. *Soil. Sci. Soc. Am. J.* **71**, 95–100.
- Israel, D.W. 1987. Investigation of the role of phosphorus in symbiotic dinitrogen fixation. *Plant. Physiol.* **84**, 835–840.
- Issa, S. & Wood, M. 1995. Multiplication and survival of chickpea and bean rhizobia in dry soils: the influence of strains, matric potential and soil texture. *Soil. Biol. Biochem.* **27**, 785–792.
- Kaci, G., Blavet, D., Benlahrech, S., Kouakoua, E., Couderc, P., Deleporte, P., Desclaux, D., Latati, M., Pansu, M., Drevon, J. & Ounane, S.S. 2018. The effect of intercropping on the efficiency of faba bean– rhizobial symbiosis and durum wheat soil-nitrogen acquisition in a Mediterranean agroecosystem. *Plant Soil Environ* **9**, 138–146.
- Kjeldahl, C. 1883. A new method for the determination of nitrogen in organic matter. *Z. Anal. Chem.* **22**, 366.
- Lazali, M., Brahimi, S., Merabet, C., Latati, M., Benadis, C., Maougal, R.T. & Ounane, S.M. 2016. Nodular diagnosis of contrasting recombinant inbred lines of *Phaseolus vulgaris* in multi-local field tests under Mediterranean climate. *Eur. J. Soil. Biol.* **73**, 100–107.
- Mihoub, A., Daddi Bouhoun, M. & Saker, M.L. 2016. Phosphorus adsorption isotherm: A key aspect for effective use and environmentally friendly management of phosphorus fertilizers in calcareous soils. *Commun. Soil. Sci. Plan.* **47**, 1920–1929.
- Minchin, F.R. & Pate, J.S. 1973. The carbon balance of a legume and the functional economy of its root nodules. *J. Exp. Bot.* **24**, 259–271.
- Msumali, G.P. & Judith, A.K. 1998. Bean nodulation patterns in soils of different texture at Morogoro, Tanzania. *Tanzania J. Agric. Sc.* **1**, 181–186.
- Munir, R. 2016. *Comparative physiology of chickpea and faba bean tolerance to low O₂, high CO₂ and ethylene in the root-zone*. PhD Thesis, University of Western Australia, Australia, 143 pp.
- Namvar, A., Sharifi, R.S., Sedghi, M., Zakaria, R.A., Khandan, T. & Eskandarpour, B. 2011. Study on the effects of organic and inorganic nitrogen fertilizer on yield, yield components, and nodulation state of Chickpea (*Cicer arietinum* L.). *Commun. Soil. Sci. Plan.* **42**, 1097–1109.
- Olsen, S.R. & Dean, L.A. 1965. Phosphorus. In A.G. Norman (eds), *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties*. The American Society of Agronomy, Inc, 1035–1049.
- Oumenskou, H., El Baghdadi, M., Barakat, A., Aquit, M., Ennaji, W., Karroum, L.A. & Aadraoui, M. 2018. Multivariate statistical analysis for spatial evaluation of physicochemical properties of agricultural soils from Beni-Amir irrigated perimeter, Tadla plain, Morocco. *Geology, Ecology, and Landscapes* **3**, 83–94.
- R Core Team. 2018. R. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.

- Rao, D.L.N., Giller, K.E., Yeo, A.R. & Flowers, T.J. 2002. The effects of salinity and sodicity upon nodulation and nitrogen fixation in chickpea (*Cicer arietinum*). *Ann. Bot-London*. **89**, 563–570.
- Rawal, V & Navarro, D.K. 2019. *The Global Economy of Pulses*. FAO, Rome, 176 pp.
- Reinsvold, R.J. & Pope, P.E. 1987. Combined effect of soil nitrogen and phosphorus on nodulation and growth of Robinia pseudoacacia. *Can. J. Forest. Res.* **17**, 964–969.
- Ribet, J. & Drevon, J.J. 1996. The phosphorus requirement of N₂-fixing and urea-fed Acacia mangium. *New. Phytol.* **132**, 383–390.
- Robinson, G.W. 1922. A new method for the mechanical analysis of soils and other dispersions. *The Journal of Agricultural Science* **12**, 306–321.
- Robson, A.D., O'hara, G.W. & Abbott, L.K. 1981. Involvement of phosphorus in nitrogen fixation by subterranean clover (*Trifolium subterraneum* L.). *Funct. Plant. Biol.* **8**, 427–436.
- Ryle, G.J.A., Powell, C.E. & Gordon, A.J. 1979. The respiratory costs of nitrogen fixation in soyabean, cowpea, and white clover: II. comparisons of the cost of nitrogen fixation and the utilization of combined nitrogen. *J. Exp. Bot.* **30**, 145–153.
- Schwenke, G.D., Peoples, M.B., Turner, G.L. & Herridge, D.F. 1998. Does nitrogen fixation of commercial, dryland chickpea and faba bean crops in north-west New South Wales maintain or enhance soil nitrogen?. *Aust. J. Exp. Agr.* **38**, 61–70.
- Shukla, U.C. & Yadav, O.P. 1982. Effect of phosphorus and zinc on nodulation and nitrogen fixation in chickpea (*Cicer arietinum* L.). *Plant. Soil.* **65**, 239–248.
- Tunesi, S., Poggi, V. & Gessa, C. 1999. Phosphate adsorption and precipitation in calcareous soils: the role of calcium ions in solution and carbonate minerals. *Nutr. Cycl. Agroecosys.* **53**, 219–227.
- Vance, C.P. 2001. Symbiotic nitrogen fixation and phosphorus acquisition. Plant nutrition in a world of declining renewable resources. *Plant. Physiol.* **127**, 390–397.
- Voisin, A.S., Salon, C., Munier-Jolain, N.G. & Ney, B. 2002. Quantitative effects of soil nitrate, growth potential and phenology on symbiotic nitrogen fixation of pea (*Pisum sativum* L.). *Plant. Soil.* **243**, 31–42.
- Voisin, A.S., Salon, C., Jeudy, C. & Warembourg, F.R. 2003. Symbiotic N₂ fixation activity in relation to C economy of *Pisum sativum* L. as a function of plant phenology. *J. Exp. Bot.* **54**, 2733–2744.
- Voisin, A.S., Munier-Jolain, N.G. & Salon, C. 2010. The nodulation process is tightly adjusted to plant growth. An analysis using environmentally and genetically induced variation of nodule number and biomass in pea. *Plant. Soil.* **337**, 399–412.
- Voisin, A.S., Prudent, M., Duc, G. & Salon, C. 2015. Pea nodule gradients explain N nutrition and limited symbiotic fixation in hypernodulating mutants. *Agron. Sustain. Dev.* **35**, 1529–1540.
- White, P.F. & Robson, A.D. 1989. Effect of soil pH and texture on the growth and nodulation of lupins. *Aust. J. Agr. Res.* **40**, 63–73.
- Worku, W. 2016. Waterlogging effects on growth, nodulation and productivity of Desi and Kabuli chickpea (*Cicer arietinum* L.). *Ethiopian Journal of Biological Sciences* **15**, 55–77.
- Yinbo, G., Peoples, M.B. & Rerkasem, B. 1997. The effect of N fertilizer strategy on N₂ fixation, growth and yield of vegetable soybean. *Field. Crop. Res.* **51**, 221–229.
- Zaman-Allah, M., Sifi, B., L'Taief, B., El Aouni, M.H. & Drevon, J.J. 2007. Symbiotic response to low phosphorus supply in two common bean (*Phaseolus vulgaris* L.) genotypes. *Symbiosis* **44**, 109–113.
- Zarrin, F., Zia, M. & Chaudhary, M.F. 2006. Effect of Rhizobium strains and phosphorus on growth of soybean *Glycine max* and survival of Rhizobium and P solubilizing bacteria. *Pak. J. Bot.* **38**, 459–464.