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Evaluation of *Lupinus albus* L. nodulation and plant growth in non-inoculated soils collected from different sites in Tunisia

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Abstract -The purpose of this investigation was to assess the nodulation and the plant growth of white lupin (*Lupinus albus* L.) in Tunisian soils. For this, two *L. albus* varieties, Mekna (Tunisian) and Lumen (French), were cultivated in soils sampled from 56 locations without a history of lupin cultivation or rhizobial inoculation. All soils were analyzed for their physical and chemical proprieties. At harvest, nodulation and plant growth parameters were recorded for both varieties.
The results showed that 75% of soils were alkaline and calcareous. Nodulation was often poor, suggesting the absence of host-specific rhizobia. It was absent in 41 soils, abundant in 2 (Sejnen and Mraissa), and scarce but efficient in the remaining 13 soils. Furthermore, white lupin varieties have different responses to native nodulating bacteria since the local variety, Mekna, developed significantly more root nodules and in more soils than the imported variety.
Growth parameters and nitrogen accumulation were also measured as indicators of nitrogen fixation efficiency. Considerable variability was detected among soils. It was found that the imported variety, Lumen, showed high sensitivity to the soil active lime content compared to Mekna. Interestingly, high biomass production and nitrogen accumulation were recorded in soils originating from Madian, Tejerouin, Benouria, and Oued zinga, with a high active lime content ranging from 26% to 31%. This finding suggested that white lupin can be cultivated in Tunisian calcareous soils, especially the most promising local variety, Mekna. Isolation and the selection of efficient bacteria from alkaline calcareous soils are necessary to promote the development of this valuable legume in Tunisia.

Keywords: *Lupinus albus* L., Tunisia, calcareous soils, nodules, Mekna, Lumen

1. Introduction

In Tunisia, animal feeds are mostly based on corn and soybean meal. Soybean meal is used mainly as a protein supply. This feed ingredient is exclusively imported into Tunisia. In addition, the high demand for soybean for human consumption and biofuel production has led to a surge in its price worldwide, consequently increasing feed costs, which account for between 60 and 80% of livestock production costs (Lawrence et al. 2008; Alqaisi et al. 2017). Thus, alternative protein resources that can replace soybean meal need to be found, to decrease its importation and thereby reduce feed and production costs.
Compared to soybean, white lupin (*Lupinus albus* L.) seeds contain large amounts of protein, fiber, oil and sugar, making it a suitable alternative (Erbas et al. 2005; Bahr et al. 2014), particularly for ruminant feeds (Sujak et al. 2006). Furthermore, 70% of global soybean production is a genetically modified organism (GMO). Thus, using *L. albus* as a substitute for soybeans may reduce the risk of GMO contamination of the food chain.
L. albus is traditionally grown in the Mediterranean region and along the Nile valley and has been used in human nutrition and livestock feeding due to its nutritional value, soil fertilization (Suong et al. 2005; Cheng et al. 2011; Cernay et al. 2018), phytoremediation in polluted soils (Vázquez et al. 2006), and also for medicinal purposes (Ragunathan et al. 2009). Despite these advantages, white lupin is not

cultivated in Tunisia, but some lupin species were surveyed by Gammar Grabi et al. (1997) growing naturally in limited areas in the North (Tabarka, Sejnen, El Alia, Mraissa, Borj Hfaidh) and the centre of the country (Sousse). There is an increasing need to cultivate this valuable legume and use it in Tunisian cropping systems. However, the success of white lupin crops in Tunisian soils depends on many factors, including i) soil proprieties and ii) availability of efficient rhizobia compatible with this legume. To our knowledge, there has yet to be a report assessing the potential of cultivating *L. albus* in Tunisian soils. There is therefore a need to identify suitable areas for white lupin cultivation, select varieties adapted to Tunisian soils and investigate the existence of native N_2-fixing bacteria compatible with white lupin. Thus, the aim of this study was to assess i) the ability of native rhizobia to nodulate white lupin, and ii) in a pot experiment the plant growth performance of two white lupin varieties planted in uninoculated soils collected from different locations in Tunisia.

1. Material and methods

1.1. Soil sampling and physico-chemical characterization

The top soil (0-25 cm) with no rhizobial inoculation history was collected in the autumn of 2015/2016 at 56 distant locations (Fig 1). The corresponding GPS data (altitude, latitude, longitude) and province name were recorded (Supplementary Table S1).

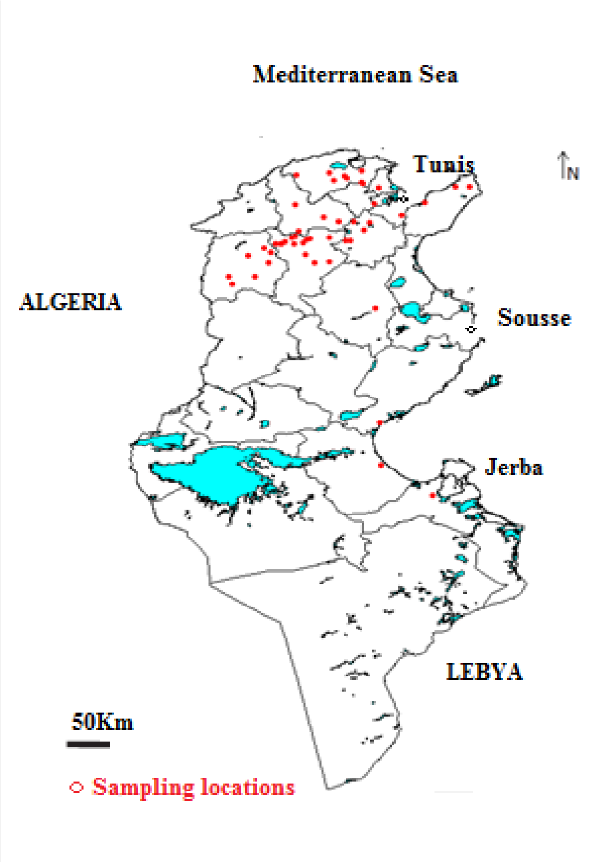

Figure 1. Geographic locations of the 56 sampling sites (Tunisia map)

To measure the initial soil characteristics, sub-samples were dried and sieved at 2 mm in the laboratory, then tested for physico-chemical parameters: soil texture, pH, electrical conductivity (EC), organic carbon (C), organic matter (OM), total and active lime (TL, AL), total nitrogen (NT) and available phosphorus contents (P) were determined using standard methods as described by Pawels et al.(1992).

1.2. Plant material

Two varieties of *L. albus* were studied, as follows: The lupin (*L. albus*) used in the present study consisted of two varieties (1) Mekna, a Tunisian variety collected from Sejnen (northern Tunisia), and (2) Lumen, a commercial variety imported from France.

2.3. Pot experiment

For both varieties, healthy seeds were surface sterilized with 95% ethanol for 1min, then with 25% sodium hypochlorite for 3 min and finally thoroughly rinsed with sterile distilled water ten times and kept in sterile distilled water for 1h. At the same time, soil samples were transferred to sterilized 500 ml plastic pots in four replicates per soil per variety. For each variety, one seed was sown per pot. The pot experiment was arranged in a completely randomized block design. The pots were maintained under semi-controlled conditions and irrigated with sterile distilled water when needed. No fertilizer was applied.

2.4. Determination of growth and nodulation parameters

At the flowering stage, 77 days after sowing, the chlorophyll concentration was estimated using a SPAD meter (Soil Plant Analysis Development, 502, Konica Minolta, Tokyo, Japan). The plants were then harvested. Roots were separated from the shoots and washed with water to remove adhering soil particles. The nodules were recovered from the roots and counted. The plant height (H), number of leaves (NL) and their area (LA) were recorded. Shoots and roots were separately dried in an oven at 65°C until constant weight was achieved. The shoot nitrogen content (Nsh) was determined in 100 mg of ground-dried shoots using the Kjeldahl method (Jones et al.1991).

2.5. Statistical analysis

Descriptive statistics were used to evaluate the physico-chemical proprieties of the sampled soils. Then, Pearson’s correlation was performed to determine the relationships between soil properties using the statistical software XLSTAT (2003) version 5.2.

Growth parameters were subjected to an analysis of variance (ANOVA) using R statistical software version 3.5.1. Pearson’s correlation was also used to determine the relationship between growth parameters and soil physico-chemical traits.

The principal component analysis (PCA) of XLSTAT (2003) was carried out to study variation in the productive performance of the sampled soils depending on shoot and root DW, as well as the Nsh content.

3. Results

3.1. Physico-chemical proprieties of soil samples

The descriptive statistics calculated to investigate soil proprieties are given in table 1 and detailed information is available in Supplementary Table S1. A textural difference was observed within the soils. However, similar contents of clay, silt and sand were recorded.

Electrical conductivity (EC) across the sampled soils ranged from 0.01 dS/m in Testour 1 to 0.593 dS/m in Outhna. Most of the investigated soils (75%: 3rd quartile) were clustered under the non-saline soils with EC values equal to or below 0.159 dS/m. Soils across all the locations showed a wide variation in pH values, ranging from 4.98 in Sejnen to 8.20 in Azmour, but 75% of the soils were grouped under the category of moderately to alkaline soils with a pH over 7.5. The active lime content (AL) of the soils varied from 0 to 49%. Most of the soils were considered calcareous and had AL contents over 8%.

The average organic matter, total nitrogen and available phosphorus contents were rated as relatively high in the majority of soils (with a mean of 1.952%, 0.128% and 52.657 ppm, respectively)

Table 1. Descriptive statistics of soil physico-chemical properties.

	Clay (%)	Silt (%)	Sand (%)	pH	EC (dS/m)	TL (%)	AL (%)	OM (%)	C (%)	NT (%)	P (ppm)
Minimum	15.000	15.000	10.000	4.980	0.010	0.000	0.000	0.270	0.160	0.008	4.200
1st quartile (25%)	27.000	28.000	28.500	7.370	0.078	17.288	11.000	1.380	0.800	0.084	25.000
Median (50%)	30.000	33.500	36.500	7.660	0.132	27.471	18.375	1.863	1.140	0.132	57.000
3rd quartile (75%)	36.000	35.500	37.500	7.920	0.159	44.049	27.000	2.622	1.540	0.168	67.500
Maximum	61.000	55.000	59.000	8.200	0.593	80.519	49.750	3.730	2.160	0.280	110.000
Mean	32.179	32.661	35.161	7.551	0.125	29.688	18.978	1.952	1.156	0.128	52.657
CV	0.314	0.201	0.329	0.071	0.702	0.633	0.572	0.409	0.388	0.469	0.549

EC: electrical conductivity; TL: total lime; AL: active lime; OM: organic matter; C: organic carbon; NT: total nitrogen in soil; P: available phosphorus

cv: coefficient of variation,The1st quartile presented 25% of the soil samples, the median presented 50% of the soil samples, the 3rd quartile, presented 75% of the soil samples

Pearson’s correlation coefficients between the physico-chemical proprieties of the investigated soils are shown in Table 2. The values obtained indicated that the soil texture components were significantly correlated with the lime content. In fact, the clay and silt fractions showed a significant and direct correlation with the soil total and active lime (r = 0.412, r = 0.600 for clay and r = 0.271, r = 0.316 for silt). However, the sand fraction showed a significant negative correlation with the total and active lime by r = -0.522 and r =-0.705, respectively. A positive significant correlation was also observed between the soil pH (r = 0.366 and the total and active lime, r = 0.415).

Table 2. Pearson's correlation coefficients (r) between soil proprieties (n=56)

	Clay	Silt	Sand	pH	EC	TL	AL	OM	C	NT	P
Clay	1										
Silt	-0.090	1									
Sand	**-0.824*****	**-0.491****	1								
pH	0.130	**0.304***	**-0.287***	1							
EC	**0.448****	0.200	**-0.506****	0.242	1						
TL	**0.421****	**0.271***	**-0.522****	**0.366***	**0.463****	1					
AL	**0.600*****	**0.316***	**-0.705*****	**0.415****	**0.646*****	**0.863*****	1				
OM	0.063	0.056	-0.087	**0.350***	0.128	0.218	0.192	1			
C	0.134	0.066	-0.154	**0.277***	0.061	0.150	0.176	**0.929*****	1		
Ns	0.063	0.225	-0.183	**0.365***	-0.046	-0.088	-0.017	0.260	**0.400****	1	
P	0.019	0.042	-0.040	0.105	-0.064	-0.057	-0.103	**0.303***	0.234	0.187	1

EC: electrical conductivity; AL: active lime; TL: total lime; OM: organic matter; C: organic carbon; NT: total nitrogen in soil; P: available phosphorus
Pearson's correlation coefficient r. values in bold letters show significant correlations (α = 0.05)
'*'correlation is significant at P < 0.01.
'**'correlation is significant at P < 0.001.
'***'correlation is significant at P < 0.0001.

3.2. Nodulation *of L. albus* in soil samples

The nodulation potential of the two white lupin varieties, Mekna and Lumen, was examined in all the studied soils (Table 3). Regardless of the variety, *L. albus* nodulation was only observed in 15 out of the 56 locations inspected. In the soils, where nodulation was present, it was very heterogeneous depending on the variety and the location. Mekna developed more root nodules in more soils (15 soils) than Lumen (8 soils).

Table 3. Nodulation potential (nodule number and color) of *L.albus* grown under natural conditions in the sampled soils

Locations	Nodule number (Nodules/plant)		Color of nodules	
	Lumen	Mekna	Lumen	Mekna
Mateur	+	+++	white to pink	pink
Oued beja	+++	+	pink	pink
Sidi thabet	+	+	pink	pink
Borjmassaoudi	+	+	pink	pink
krib 1	+	+	pink	pink
krib 2	-	+	pink	pink
Azmour	++	+	pink	pink
Mraissa	+++	++++	pink to brown	pink to brown
Sejnen	++++	++++	pink to brown	pink to brown
Tejerouine	-	+	pink	pink
Madian	-	+	pink	pink
Zaghouan	-	+	white to pink	pink
Benouria	-	+	pink	pink
jrissa	-	+	pink	pink
Teboursouk	+	+	pink	pink

-: no nodulation, +: nodulation up to 5 nodules/plant,++: nodulation ranging from 5 to 10 nodules/plant,+++: nodulation ranging from 10 to 15 nodules/plant, ++++ nodulation more than 15 nodules/plant

For both varieties, nodulation was adequate (more than 15 nodules/plant) in soils sampled from Sejnen and Mraissa. It was also profuse in those recovered from Mateur and Oued Beja for Mekna and Lumen, respectively. However, it was scarce in the other investigated soils (up to 5 nodules per plant). Irrespective of the location and the variety, a pink color was observed inside the collected nodules.

3.3. Growth of *L. albus* in the sampled soils

The productive performance of the Mekna and Lumen varieties varied according to soils. The statistical analysis (ANOVA) revealed that all studied traits were highly and significantly (P< 0.01) affected by the location (L), the variety used (V), as well as their interaction (L*V) (Table 4). The main factor affecting white lupin growth was the location, with a wide range of variation ranged from 55.47 to 85.74% of the whole sum squares. L*V interaction was substantial and might to be considered in this study. It affected the growth parameters by 10.66-28.08%, while the variety effect contributed 0.18-28.08% (Supplementary Table S2).

Table 4. Analysis of variance of the different growth parameters according to the location, the variety and their interaction

Factors	Df	LN	SPAD	H	LA	SDW	RDW	Nsh	NOD
Location (L)	55	11401***	133318***	13622***	1715396***	116.70***	9.166***	26305***	10177***
Variety (V)	1	386***	291***	2974***	105625***	9.10***	0.075***	231***	80***
L*V	55	1418***	20471***	3138***	456805***	32.94***	2.194***	10376***	2319***
Residuals	336	92	2726	4821	28439	2.47	0.362	33	713

LN: leaf number; SPAD: leaf chlorophyll concentrations; H: plant height; LA: leaf area, SDW: shoot dry weight; RDW: root dry weight; Nsh: nitrogen content in shoot; NOD: nodule number,***The correlation was significant at the 0.01 level

Pearson's correlation coefficients of growth parameters with soil factors were calculated for both varieties and are presented in Tables 5 and 6, respectively. Results showed that the shoot and root dry weight, as well as the nitrogen content of both varieties, were highly and positively correlated with all the measured growth parameters (leaf number and area, chlorophyll content and shoot height).
The results showed that soil factors significantly affected the growth parameters of white lupin. For the French variety, Lumen, the clay and the active lime contents, as well as the electrical conductivity, had significant and negative effects on all the measured growth parameters, except for the leaf number and area. However, the sand and available phosphorus content were significantly and positively correlated with almost all of the growth parameters (Table 5). For its part, the Tunisian variety, Mekna, behaved differently. It was less influenced by the physico-chemical traits of the soil. Indeed, only the root dry weight and the leaf number were significantly and negatively affected by the clay content. Of the measured growth parameters, only the leaf number was significantly and negatively correlated with the active lime content. However, the electrical conductivity was negatively correlated with almost all of the growth parameters (Table 6).

Table 5. Pearson's correlation coefficients (r) between Lumen growth parameters and soil factors (number of observations: 56*4)

	SDW	**Nsh**	**RDW**	**LN**	**SPAD**	**H**	**LA**	**NOD**	**Clay**	**Silt**	**Sand**	**pH**	**AL**	**TL**	**OM**	**C**	**NT**	**P**	**EC**
SDW	1.00																		
Nsh	**0.93*****	1.00																	
RDW	**0.88*****	**0.82*****	1.00																
LN	**0.82*****	**0.77*****	**0.89*****	1.00															
SPAD	**0.85*****	**0.84*****	**0.92*****	**0.97*****	1.00														
H	**0.85*****	**0.76*****	**0.89*****	**0.93*****	**0.93*****	1.00													
LA	**0.87*****	**0.86*****	**0.84*****	**0.85*****	**0.89*****	**0.82*****	1.00												
NOD	**0.41****	**0.59*****	**0.42****	**0.29***	**0.40****	**0.28***	**0.31***	1.00											
Clay	**-0.29***	**-0.29***	**-0.31***	-0.22	**-0.28***	**-0.30***	-0.25	-0.22	1.00										
Silt	-0.25	**-0.29***	-0.26	-0.22	**-0.28***	**-0.31***	**-0.30***	-0.14	-0.09	1.00									
Sand	**0.40****	**0.42****	**0.42****	**0.31***	**0.41****	**0.44****	**0.39***	**0.2*****7**	**-0.82*****	**-0.49****	1.00								
pH	-0.12	-0.25	-0.23	-0.11	-0.21	-0.17	-0.04	**-0.59*****	0.13	**0.30***	**-0.29**	1.00							
AL	**-0.53****	**-0.56****	**-0.44****	**-0.31***	**-0.41****	**-0.43****	**-0.43****	**-0.36***	**0.60*****	**0.32***	**-0.70*****	**0.41****	1.00						
TL	**-0.45****	**-0.53****	**-0.33***	-0.25	**-0.33***	**-0.34***	**-0.38***	**-0.32***	**0.42****	**0.27***	**-0.52****	**0.37***	**0.86*****	1.00					
OM	0.03	-0.03	0.07	0.13	0.11	0.12	0.17	-0.18	0.07	0.05	-0.09	**0.35***	0.19	0.22	1.00				
C	0.12	0.04	0.11	0.20	0.18	0.18	0.23	-0.18	0.14	0.06	-0.16	**0.28***	0.18	0.15	**0.93*****	1.00			
Ns	0.18	0.09	0.01	0.09	0.06	0.03	0.22	**-0.28***	0.06	0.23	-0.18	**0.37***	-0.02	-0.09	0.26	**0.40****	1.00		
P	**0.31***	**0.39****	**0.33***	**0.32***	**0.34***	0.20	**0.36***	0.25	0.02	0.04	-0.04	0.10	-0.10	-0.06	**0.30***	0.23	0.19	1.00	
EC	**-0.42****	**-0.43****	**-0.45****	**-0.39***	**-0.45****	**-0.42****	**-0.38***	-0.23	**0.45****	0.20	**-0.51****	0.24	**0.65*****	**0.46****	0.13	0.06	-0.05	-0.06	1.00

SDW: shoot dry weight, Nsh: nitrogen content in shoot, RDW: root dry weight, LN: leaf number, SPAD: leaf chlorophyll concentration, H: plant height, LA: leaf area, NOD: nodule number, AL: active lime, TL: total lime, OM: organic matter, C: organic carbon, NT: total nitrogen in soil. P: available phosphorus, EC: electrical conductivity. Pearson's correlation coefficient r. values in bold letters show significant correlations ($\alpha = 0.05$),
'*'correlation is significant at $P < 0.01$.
'**'correlation is significant at $P < 0.001$.
'***'correlation is significant at $P < 0.0001$.

Table 6. Pearson's correlation coefficients (r) between Mekna growth parameters and soil factors (number of observation: 56*4)

	SDW	**Nsh**	**RDW**	**LN**	**SPAD**	**H**	**LA**	**NOD**	**Clay**	**Silt**	**Sand**	**pH**	**AL**	**TL**	**OM**	**C**	**NT**	**P**	**EC**
SDW	1.00																		
Nsh	**0.83*****	1																	
RDW	**0.65*****	**0.96*****	1																
LN	**0.75****	**0.66*****	**0.82*****	1															
SPAD	**0.29***	**0.42****	**0.38****	**0.40****	1														
H	**0.8*****	**0.66*****	**0.79*****	**0.92*****	**0.41**	1													
LA	**0.86*****	**0.84*****	**0.64*****	**0.77*****	**0.33**	**0.76*****	1												
NOD	**0.30***	**0.50****	0.21	0.25	0.12	0.20	**0.52****	1											
Clay	-0,21	-0.10	**-0.28*****	**-0.29***	-0.03	-0.26	-0.12	-0.12	1										
Silt	-0.06	-0.08	-0.08	-0.13	0.02	-0.14	-0.15	-0.15	-0.09	1									
Sand	0.22	**0.29***	**0.29***	**0.33***	0.02	**0.31***	0.19	**0.19***	**-0.82*****	**-0.49****	1								
pH	0.11	-0.09	-0.10	-0.01	-0.08	0.03	**-0.06***	**-0.35***	0.13	**0.30***	**-0.29***	1							
AL	-0.19	-0.25	-0.24	**-0.26***	-0.24	-0.24	**-0.17****	**-0.35***	**0.60*****	**0.32***	**-0.70*****	**0.41****	1						
TL	-0.16	-0.24	-0.20	-0.22	-0.18	-0.18	**-0.21***	**-0.34***	**0.42****	**0.27***	**-0.52****	**0.37***	**0.86*****	1					
OM	0.16	0.11	0.21	0.25	0.03	0.18	0.04	-0.22	0.07	0.05	-0.09	**0.35**	0.19	0.22	1				
C	0.20	0.13	0.21	**0.31***	0.04	0.24	0.12	-0.15	0.14	0.06	-0.16	**0.28***	0.18	0.15	0.93	1			
Ns	0.13	0.08	0.08	0.10	-0.06	0.04	0.03	-0.17	0.06	0.23	-0.18	**0.37***	-0.02	-0.09	0.26	**0.40****	1		
P	**0.29***	**0.35***	0.25	0.25	-0.08	0.12	0.25	0.23	0.02	0.04	0.04	0.10	-0.10	-0.06	**0.30***	0.23	0.19	1	
EC	**-0.39****	-0.27	**-0.30***	**-0.41****	-0.15	**-0.45****	**-0.30***	-0.24	**0.45****	0.20	**-0.51****	0.24	**0.65*****	**0.46****	0.13	0.06	-0.05	-0.06	1

SDW: shoot dry weight, Nsh: nitrogen content in shoot, RDW: root dry weight, LN: leaf number, SPAD: leaf chlorophyll concentrations, H: plant height, LA: leaf area, NOD: nodule number, AL: active lime, TL: total lime, OM: organic matter, C: organic carbon, NT: total nitrogen in soil. P: available phosphorus, EC: electrical conductivity
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'***'correlation is significant at $P < 0.0001$.

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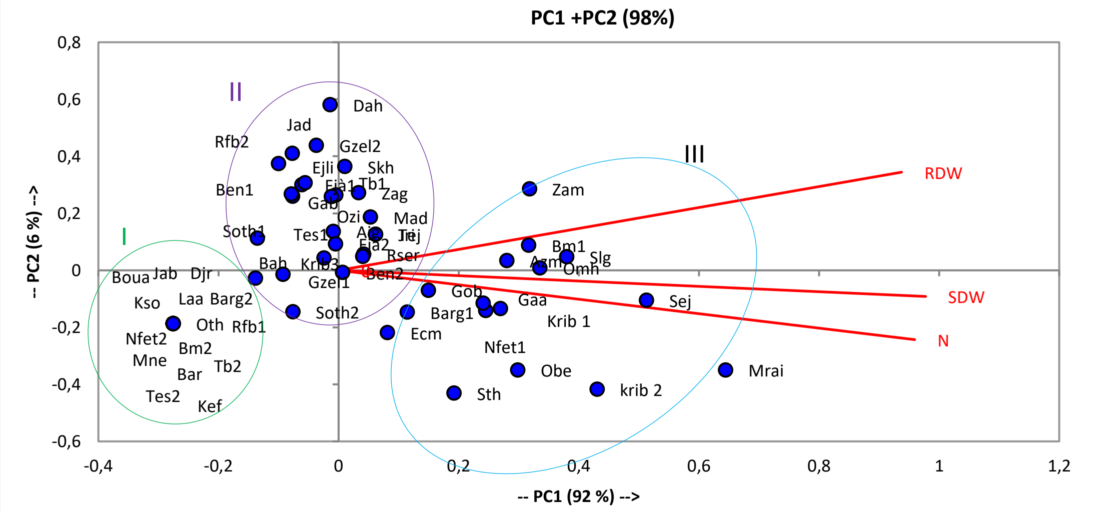
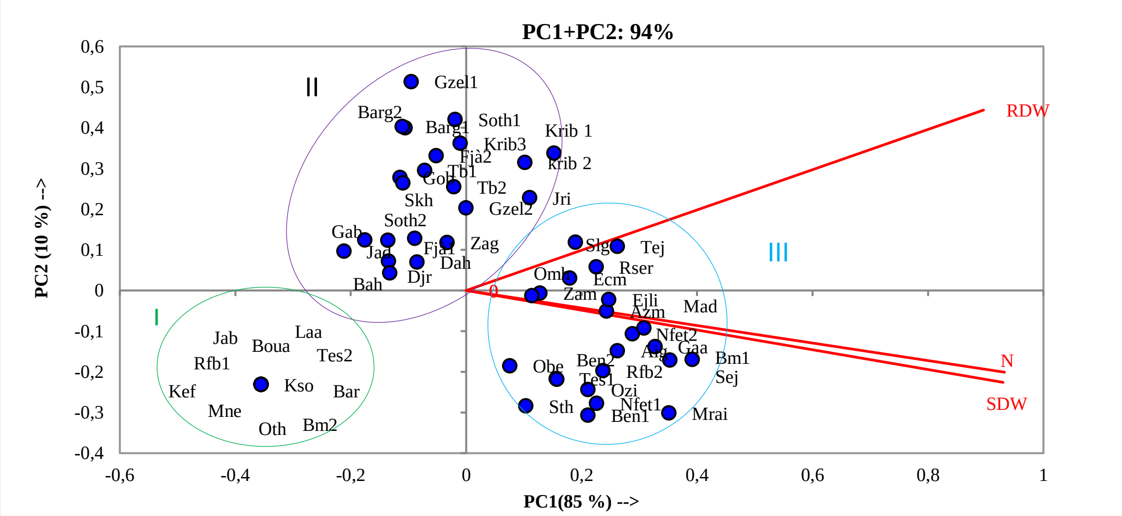
The distribution of soil samples according to Mekna and Lumen biomass productivity and nitrogen content was determined on the main plane of the PCA formed by the first two axes. For the Lumen variety, theses axes accounted a 98% of the total variability (Figure 2A). PCA1 was positively correlated with the growth parameters used. However, PCA2 was weakly correlated with these parameters. For the Mekna variety, PCA 1 and PCA 2 accounted for 94% of the total variability (Figure 2B). For both varieties, three soils groups were identified depending on their productivity potential. The first group contained soils originating from 11 and 15 locations for Mekna and Lumen, respectively. In these soils, *L. albus* was unable to grow. Indeed, senescence of the Lumen and Mekna plants was observed two weeks after emergence.



A



B

Figure 2. Distribution of the 56 soil samples, biomass yields and nitrogen content of the Lumen (A) and Mekna (B) varieties on the main plane of the PCA defined by the first and the second principal axes. The ellipses contain different groups of soil samples with similar *L. albus* shoot and root dry weight and nitrogen content.

The second group included soils recovered from 23 and 25 locations for Mekna and Lumen, respectively, where the two varieties grown showed chlorosis symptoms almost four to six weeks post-seedling, resulting in low shoot and root biomass yields and nitrogen contents. In this group, the Lumen variety displayed SDW and RDW values of 0.47 and 0.25 g/plant, respectively, and contained 2.69 mg/plant of nitrogen (Table 7). In the same group, the Tunisian variety, Mekna, displayed higher productivity with 0.56 g/plant for SDW and 0.28 g/plant for RDW and accumulated 3.56 mg/plant of nitrogen (Table 7).

Table 7. Descriptive statistical analysis for shoot and root biomass yields and nitrogen content of the Lumen and Mekna varieties in the different groups formed

	Number of soils/group	**SDW (g/plant)**			**RDW (g/plant)**			**Nsh (mg/plant)**		
1		**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**
Lumen										
Group 1	15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Group 2	25	0.47	0.21	0.85	0.25	0.12	0.36	2.69	0.36	6.21
Group 3	16	1.17	0.58	1.93	0.38	0.22	0.57	25.83	10.18	36.34
Mekna	2	3	4	5	6	7	8	9	10	11
Group 1	11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Group 2	23	0.56	0.20	1.20	0.28	0.05	0.56	3.56	0.43	12.41
Group 3	22	1.53	0.85	1.97	0.33	0.18	0.60	28.42	15.35	47.86

SDW: shoot dry weight; RDW: root dry weight: Nsh: nitrogen content in shoot

The third group clustered soils sampled from 22 locations for Mekna and 16 locations for Lumen. This group comprised soils in which *L. albus* grew adequately without showing any chlorotic symptoms. In this group, Lumen had 1.17 g/plant of SDW, 0.38 g/plant of RDW and 25.83 mg/plant of nitrogen, while Mekna had 1.53 and 0.33 g/plant of SDW and RDW, respectively and 28.42 mg/plant of nitrogen. When considering the active lime content of the soils contained in this group, we observed for the Lumen variety that 38% of soils had a high active lime content ranging from 12 to 23.75%, while for the Mekna variety, the active lime content of 78% of the soils in this group varied from 10.25 to 31.75%.

4. Discussion

Given the limited data and knowledge on the possibility of cultivating white lupin *L. albus* in Tunisian soils, this study was undertaken to investigate the nodulation and growth potential of two white lupin varieties in soils sampled from 56 different locations. The investigated sites were mainly located in northern Tunisia with no lupin growing or rhizobial inoculation history. The analysis of the physico-chemical proprieties of the surveyed soils indicated that most of them were very heterogeneous in texture and considered to be non-saline, moderately to high alkaline calcareous soils. The dominance of alkaline calcareous soils in northern Tunisia was previously reported by Ben Hassine et al. (2008), who investigated only 20 locations. In our study, more locations were surveyed for further characterization. In terms of richness in organic matter (OM), nitrogen (Ns) and available phosphorus (P), the majority of soils could be classified as moderately rich. It is important to highlight that the richness of the investigated area in organic matter and total nitrogen does not mean that these elements are available to cultivated crops. Indeed, Vimlesh and Giri (2011) observed that more than 90% of N stored in soils were typically in organic form not available to crops and just 1 to 4% were mineralized to obtain the two forms of available nitrogen forms (NH_4^+ and NO_3). In addition, the use of organic N requires prior transformation by soil microbes into inorganic available forms. The correlation among soil traits indicated that the active lime rate increased in soils with a high clay and silt content and a low sand content.

It is known that lupin species grow poorly on alkaline soils, namely those with a high lime content (Tang and Thomson 1996; Liu and tang1999). Thus, Tunisian soils such as the majority of soils surveyed in this study might not be suitable for growing these valuable legumes. Indeed, it has been shown that a high lime content might decrease grain and biomass yield, due to the precipitation of organic acid secreted by roots to mobilize and take up micronutrients and the inhibition of Fe uptake by HCO_3^- (Lopez-Bellido and Fuentes 1986; Tang and Thomson 1996). Furthermore, Duthion et al. (1992) reported that the stressful conditions stated above can contribute to chlorosis symptoms and eventually to complete necrosis. However, some studies suggested that *L. albus* might be less sensitive than yellow and blue lupins (*Lupinus luteus* L. and *Lupinus angustifolius* L.) to calcareous soils and fine texture (Brand 1999, Mahmoud et al. 2014, Gresta et al. 2017).This might possibly be in relation to the root morphology of *L. albus*. For instance, Clements et al. (1993) reported that the roots of white lupin had a more extensive root system when compared to blue lupin. They also found the presence of a structure of proteoid root clusters on the root of white lupins in response to phosphorus starvation, but there is no such formation reported on blue lupin roots. This research was therefore undertaken to assess the growth and nodulation of two *L. albus* varieties, namely an imported variety (Lumen) and a Tunisian variety (Mekna) in the investigated soils, which had no lupin growing history. As a leguminous plant, *L. albus* is able to establish symbiotic relations with soil bacteria, called rhizobia, in order to fix nitrogen from the atmosphere (Barrera et al. 1997). This symbiosis results in the formation of new organs on roots called nodules. Thus, the nodulation of Mekna and Lumen plants was assessed and examined in the 56 soil samples. Nodulation was observed in only 27% of the soils sampled. It was abundant in soils collected from Sejnen and Mraissa, which are characterized by no active lime content (0%) and an acidic or neutral pH (4.98-7.18). However, nodules were scarce but seemed to be able to fix nitrogen from the atmosphere in the remaining soils (nodules showed a pink color). Several reasons may explain the low rate of nodules in this study: (1) lupins had never been cultivated in the soils sampled from the locations investigated, (2) the established legume-rhizobial symbiosis could be ineffective since legumes are generally very selective of their micro-symbionts (Soenens et al. 2018), and (3) the high level of active lime in the majority of the soil samples. In this respect, Mhamdi et al. (2004) and De Aranjo et al. (2016) reported that *Phaseolus vulgaris* and *P. lunatus* were well nodulated in soils routinely cultivated with that legume, but poorly or not nodulated in those newly cultivated with it. For a successful introduction of white lupin in Tunisian soils, our results support the need to inoculate, at least initially, in order to establish the population of a compatible rhizobium able to nodulate this legume and tolerate edaphic conditions.

In this study, nodulation efficiency was assessed by examining shoot nitrogen accumulation and visual leghmoglobin concentration. Indeed, Öütçü et al. (2008) demonstrated that determining the amount of fixed nitrogen might better characterize the nodulation potential of legumes than counting the number of nodules. Accordingly, the most effective nodulation was observed in soils characterized by a high lime content, ranging from 7 to 31%. This finding indicates that rhizobia nodulating *L. albus* are naturally present in these soils and appear to be well adapted to their conditions. Consequently, selecting efficient rhizobial strains could pave the way for cultivating *L. albus* in Tunisian calcareous soils (Tounsi-Hammami et al. 2016).

When comparing the nodulation potential of the two *L. albus* varieties, we found that the Tunisian variety, Mekna, developed significantly more root nodules in more soils than the imported variety, Lumen. Our observation is consistent with that of Mutch and Young (2004), who reported that domesticated crop species tend to have fewer compatible symbionts (higher specificity) than their wild counterparts. In the same context, Li et al. (2012) reported that rhizobial bacteria may show regional endemism that limits the nodulating strain host range at different locations. Similarly, Howieson et al. (2005) showed that geographically distant legume species are usually poorly nodulated when they are introduced to a new area, due to the absence of coevolution with a particular micro-symbiont.

The productive performance of the Mekna and Lumen varieties varied between soils. An analysis of variance revealed that the variety and the location, as well as their interaction, had highly significant effects on all growth parameters. The main factor affecting *L. albus* growth was the location, with a wide range of variation ranging from 55.47 to 85.74%. Consequently, three main soil groups were identified according to their productivity potential. In the first group of soils, *L. albus* was unable to grow since senescence was observed a few days after emergence. This can be explained by several factors, such as a high active lime content, the alkalinity of these soils and their low nutrient availability. Furthermore, it might be related to the absence of symbiotic bacteria specific to *L. albus* in these soils. Indeed, as documented in Kimiti and Odee (2010), specific micro-symbionts would appear to be absent, since nodulation was not observed for white lupin cultivated in the same type of soils.

In the second group of soils, both the Mekna and Lumen varieties had a low growth rate and exhibited yellow colored leaves. This observation indicated that N_2 fixation and N availability to the plant were poor, despite the presence of nodules in some soils. Therefore, as recommended by Date (2001), rhizobial inoculation needs to be carried out to cultivate *L. albus* in these soils and to improve its tolerance of alkaline stress (Tounsi-Hammami et al. 2016).

In the third group of soils, lupin plants displayed better and vigorous vegetative growth, revealed by all the measured growth parameters, in addition to the green color of the leaves, revealing the N availability to the plant. N availability may have been due to the presence of efficient legume-rhizobial symbiosis in some of the soils, or to the nitrogen richness in the others. It is very important to highlight here that,

for Mekna, 78% of the soils included in this group were characterized by a high lime content varying from 10.25% in Sers to 31.75% in Bennouria. However, for Lumen, in this group only 38% of the soils had a high lime content ranging from 12% in Sidi thabet to 23.75% in Bargou. We can conclude that Mekna is able to tolerate higher soil lime contents than those tolerated by the imported Lumen variety.
This result shows that *L. albus* has great potential to grow in Tunisian calcareous soils. For both *L. albus* varieties, the relationship between SDW, RDW, N and growth parameters was positive. However, a negative correlation was detected between growth parameters and some soil physico-chemical traits, such as clay and active lime content and electrical conductivity. Interestingly, this linear correlation revealed that the local type, Mekna, was less sensitive to soil conditions than the imported variety, Lumen. For Mekna, only the leaf number was negatively and significantly affected by active lime. This finding indicates that improving *L. albus* adaptation to Tunisian calcareous soil conditions may be enhanced by selecting the Tunisian variety and improving its growth and nodulation potential. According to Annicchiarico et al. (2010), the adaptability of lupin genotypes seems to depend on their native origin, since varieties originating from Europe, East Africa, West Asia and the Mediterranean region became adapted to specific environmental conditions in Italy and France, while those from Portugal and Spain tended to adapt to wider environments.
The current research provided encouraging results for introducing white lupin in Tunisia. Particularly, for using the Mekna variety, which appeared to be more adapted to Tunisian soils characteristics.The detection of efficient nodulation in alkaline soils, suggests the existence of rhizobia nodulating *L. albus* that are well adapted to the conditions of this category of soils. The exploration of these symbiotic bacteria and selection of the most efficient strains, mainly those tolerant of active lime, could pave the way for the introduction of *L. albus* in Tunisian soils and its expansion in calcareous areas.

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