

Potential of native inoculum to improve the nodulation and growth of white lupin in Tunisia

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ABSTRACT

In order to expand the cultivation of white lupin and improve its nodulation and growth in Tunisian soils, twenty native strains affiliated to the Rhizobium and Agrobacterium genera were used in this study. Phenotypic characterization identified a strain, Lab8, that was highly tolerant of different types of abiotic stress under in vitro conditions, including pH variation (4-11), drought (15-30% PEG600) and salinity (600-1500 mM NaCl). With cultivation in sterilized carrier sand, the nodulation test highlighted the efficiency of this strain in increasing shoot dry weight (+208%) and nitrogen accumulation (+116%) compared to unfertilized control plants. Subsequently, a pot experiment was conducted to elucidate the potential of these selected strains applied as liquid and solid inoculants to promote nodulation and growth, as well as the nitrogen content of white lupin plants grown in non-sterilized soils. The results showed that inoculation with Lab8 significantly increased nodulation in terms of number (+95-118%) and dry weight (213-296%), whether as a solid or liquid inoculant. Significant increases were recorded in inoculated plants for leaf chlorophyll content (+9-11%), plant height (+16-18%), shoot and root dry weight (+38-44% and +51-61% respectively) compared to unfertilized control plants. Interestingly, compared to unfertilized control plants, application of the selected strain induced higher shoot nitrogen accumulation varying from 71% to 79% for liquid and solid inoculants, respectively. Furthermore, the solid inoculant was more efficient than its equivalent, the liquid inoculants gave the best dry nodular biomass and nitrogen content, respectively + (26%) and (+ 5%) respectively than the liquid form.

Keywords: *Lupinus albus* L., nodulation, *Rhizobium* sp., solid inoculants, liquid inoculants, compost

RESUME

Potentialités d'un inoculum autochtone à améliorer la nodulation et la croissance du lupin blanc en Tunisie

Afin d'améliorer la croissance et la nodulation du lupin blanc dans les sols tunisiens, vingt souches autochtones affiliées aux genres *Rhizobium* et *Agrobacterium* ont été utilisées dans cette étude. La caractérisation phénotypique, *in vitro*, a permis d'identifier une souche de *Rhizobium* sp., Lab8, hautement tolérante aux différents stress abiotiques, y compris la variation du pH (4-11), la sécheresse (15-30% PEG600) et la salinité (600-1500 mM NaCl). Le test de nodulation réalisé sur sable stérilisé, a mis en évidence l'effet positif de cette souche sur le poids sec aérien (+208%) et la teneur des feuilles en azote (+116%) en comparaison avec plants témoins non fertilisés. De même, une expérience en pots a été menée sur sol non stérilisé pour évaluer les potentialités de cette souche, sous forme d'inoculum liquide ou solide, sur la nodulation, la croissance et la teneur en azote des plants de lupin blanc. Les résultats ont montré que l'inoculation avec la souche Lab8 a significativement augmenté la nodulation en termes de nombre de nodules (+95-118%) et de poids sec (+213-296%) et ceci quelle que soit l'inoculation liquide ou solide. Comparativement aux plants témoins non fertilisés, l'application de l'inoculum a significativement augmenté la croissance exprimé par la taille des plants (+16-18%), la teneur des feuilles en chlorophylle (9-11%), le poids sec aérien et racinaire (+38-44% and +51-61% respectivement et la teneur en azote (+71%-79%). De plus, l'inoculum solide a été plus efficace que son équivalent liquide et a donné les meilleures biomasses sèches nodulaires et les teneurs en azote les plus élevées, soit respectivement (+26%) et (+5%) que la forme liquide.

Mots clés: *Lupinus albus* L., nodulation, *Rhizobium* sp., Inoculum solide, inoculum liquide, compost

المخلص

إمكانية تحسين نمو نبتة الترمس الأبيض في تونس عبر استعمال لقاح محلي

بغية تحسين نمو نبتة الترمس الأبيض في التربة التونسية وتحسين قدرتها على تثبيت النيتروجين الجوي، تم انتقاء 20 سلالة محلية من بكتيرية الريزوبيا التابعة لجنس *Rhizobium* و *Agrobacterium* لاستخدامها في هذه الدراسة. بينت الدراسات المخبرية أن سلالة *Rhizobium* sp. Lab8 تمتلك قدرة عالية على تحمل العديد من الضغوط المناخية، بما في ذلك تحمل درجات إجهاد الحموضة (4-11) والجفاف (15-30% PEG600) والملوحة (600-1500 mM NaCl). إضافة إلى ذلك، تمت دراسة قدرة كل السلالات على تكوين العقد الجذرية وتحسين نمو النباتات الملحقة، وأثبتت النتائج تميز سلالة Lab8 ومدى فاعليتها في تحسين الوزن الجاف للجزء العلوي للنبتة (+208%) ومحتوى النيتروجين في الأوراق (+116%) مقارنة بنبتات الترمس الغير الملحقة. كما تم اختبار هذه السلالة في شكل لقاح سائل وآخر صلب على نبتات من الترمس الأبيض زرعت في أوعية بلاستيكية تحتوي على تربة غير معقمة للتأكد من كفاءتها والتمكن من انتقاء نوع اللقاح المناسب. أوضحت النتائج أن التلقيح بـ Lab8 أدى إلى زيادة كبيرة في عدد العقد الجذرية (95-118%) وفي وزنها الجاف (213-296%) سواء تم استعماله في شكل لقاح سائل أو صلب مقارنة بنبتات الترمس الغير ملحقة. كما مكن هذا اللقاح من تحسين ارتفاع النبات (16-18%)، ومحتوى الكلوروفيل في الأوراق (9-11%) والوزن الجاف للجزء العلوي (38-44%) وجذور النبتة (51-61%) ومحتوى النيتروجين مقارنة بنبتات الترمس الغير ملحقة. إضافة إلى ذلك فإن اللقاح الصلب سجل أفضل المعدلات للوزن الجاف للعقد الجذرية ومحتوى النيتروجين وحسن على التوالي (26%) و (5%) أكثر من اللقاح السائل.

كلمات مفتاحية: الترمس الأبيض، العقد الجذرية، *Rhizobium* sp.، لقاح سائل، لقاح صلب

INTRODUCTION

White lupin (*Lupinus albus* L.) is an annual legume traditionally cultivated around the Mediterranean regions and along the Nile River, where it greatly appreciated for human and animal nutrition, as well as for green manuring (Jensen *et al.* 2004; Wolko *et al.* 2011). Recently, this legume attracted national and international interest as it offers a successful alternative to soybean due to its high protein, fiber, oil and sugar contents (Wolko *et al.* 2011; Lucas *et al.* 2015).

L. albus has never been cultivated in Tunisia. Its introduction and exploitation in our cropping systems could be a promising management strategy for reducing expensive soybean imports, enhancing soil fertility (Cheng *et al.* 2011), as well as increasing cereal yields when used as a preceding crop (Reeves *et al.* 1984; Trujillo *et al.* 2005; Cernay *et al.* 2018).

White lupin, like other legumes, is able to form a symbiotic relationship with rhizobial bacteria known as slow-growing bradyrhizobia (Velázquez *et al.* 2010), as well as fast-growing bacteria belonging to *Phyllobacterium trifolii* (Valverde *et al.* 2005) and *Ochrobactrum* strains (Trujillo *et al.* 2005). Previously, it was found that rhizobia nodulating white lupin in Tunisia were affiliated to three genera, *Agrobacterium*, *Rhizobium*, and *Neorhizobium*, with large internal diversity, including separate lineages (Hammami-Tounsi *et al.* 2019). There are problems related to the nodulation of white lupin when cultivated in Tunisian soils, suggesting a general lack, or inefficiency of indigenous lupin-nodulating rhizobial populations in Tunisia, which is easily explained by the historic absence of lupin plants in these soils (Tounsi-Hammami *et al.* 2019). Inoculation with efficient strains would therefore be mandatory (Date, 2001). However, symbiosis has been found to be generally affected by various types of environmental stress, such as drought, salinity, acidity, alkalinity and others, which lead to unsuccessful legume-rhizobia symbiosis (Zahran *et al.* 1999; Giller *et al.* 2001). Thus, growing this valuable legume in Tunisian soils calls for the selection of efficient native rhizobial strains that are well adapted to several types of abiotic stress.

In this study, twenty native rhizobial strains previously isolated from nodules of white lupin cultivated in Tunisian soils were screened *in vitro* for their tolerance of abiotic stress (acidity, alkalinity, salinity and drought) (data not shown). They were then assessed for their ability to enhance nodulation and growth under semi-controlled conditions. A selected strain was subsequently applied in liquid and solid formulations and tested for its ability to promote nodulation and growth of white lupin plants cultivated in non-sterilized soils in pots.

MATERIAL AND METHODS

1. Collection of bacteria

A collection of twenty strains previously isolated from root nodules of white lupin plants cultivated in Tunisian soils, identified as rhizobial bacteria belonging to *Rhizobium* sp., and *Agrobacterium* sp. strains (Tounsi-Hammami *et al.* 2019), were used in this study with a view of selecting the most efficient strains that might be tolerant to different types of abiotic stresses, and facilitate the introduction of white lupin in Tunisian soils (Table 1).

2. Tolerance of strains to variations in NaCl, pH and PEG

The tolerance ability of strains to grow under stressful conditions (salinity, drought, acidity, as well as alkalinity) was analyzed under *in vitro* conditions. The salinity tolerance of rhizobia was examined by inoculating rhizobia on YEM agar plates containing different

concentrations of NaCl varying from 0.6 to 1.5 M. Strain tolerance to variations in pH was assessed in YEM agar medium with the pH adjusted within a range of 4 to 11. After 24 h of incubation at 28°C, the plates were examined for bacterial growth.

In addition, the ability of strains to withstand drought stress was tested by growing bacteria on liquid YEM medium with varying PEG6000 concentrations: 15, 20, 25, and 30%. Bacterial growth was then examined by measuring the optical density at 620nm in a spectrophotometer after 72 h of incubation at 28°C. Four replicates were considered for each treatment.

3. Authentication and determination of symbiotic efficiency

All the 20 rhizobial isolates were evaluated for their ability to induce root nodules on white lupin plants. Sandy soil was autoclaved three times (at 121°C for 2h), placed in sterilized plastic pots (500 ml) and used as growing medium. One seed was sown in each pot. After germination, lupin seedlings were inoculated with one milliliter of cell suspension (approx. 10^9 bacterial cells). The experiment also included uninoculated and unfertilized plants used as negative controls (T0) and uninoculated but nitrogen-fertilized plants (equivalent to 90 units of nitrogen applied per ha) served as positive controls (TN). Three replications were used for each treatment. Pots were arranged randomly and irrigated twice a week with a nitrogen-free nutrient solution in order to maintain balanced plant nutrients (Somasegaran and Hoben, 1994).

Forty-seven days after planting, plants were harvested and examined for nodulation. For infective strains, the shoot dry weight and nitrogen content were used as indicators of effectiveness. The data were analyzed for variance and means were compared by the LSD test ($P \leq 0.05$) using R statistical software version 3.5.1.

4. Selection of elite strains and inoculum preparation

Based on the symbiotic effectiveness results of the pot experiment, only the most efficient strain was selected to be assessed for its potential to promote the nodulation, growth and nitrogen content of white lupin.

For the solid inoculants, an indigenous carrier material previously selected for its ability to maintain rhizobium survival for long periods was used in this experiment (Tounsi-Hammami *et al.* 2015). Fifty grams of the carrier was packed in a sterilized bag, heat-sealed and sterilized twice at 121°C for 20 min. Rhizobia were grown in YEM liquid medium up to the late log phase ($>10^8$ cells/ml), and were aseptically injected into the sterilized carrier to obtain a final moisture content of 40%. The inoculant was well mixed then incubated at 28-30°C for six days to allow the development of a maximum number of rhizobia before being stored at 4°C pending use. The liquid inoculants were prepared just before use. The same strains were grown up to the late exponential phase in liquid YEM medium. The bacterial suspension was adjusted to 10^9 cfu ml⁻¹.

5. Pot experiment

The efficiency of the selected native strain LAb8 was assessed under semi-controlled conditions. Plants were grown in plastic pots (7 kg) filled with non-sterilized soil. The latter was characterized as sandy soil with pH 7.8 and contained 0.98% and 77 ppm of total nitrogen and available phosphate, respectively. One pre-sterilized seed was grown in each pot.

Seedlings were inoculated at the two-leaf stage. The solid inoculant was diluted with sterilized distilled water and adjusted to 10^9 cfu ml⁻¹. One milliliter of cell suspension of the

strains was added to each seedling directly in soil. The experiment included two controls: a negative control comprising uninoculated plants and a positive control comprising uninoculated but nitrogen-fertilized plants that had received 90 kg N ha⁻¹.

Pots were arranged in a Randomized Complete Block Design with three replications and irrigated with well water. At the flowering stage, white lupin plants were harvested to assess nodulation (number and dry weight of nodules per plant), as well as growth parameters (leaf chlorophyll content SPAD, plant height, shoot and root dry weights). The efficiency of the selected strain was estimated by analyzing the nitrogen concentration (%N) by the Kjeldahl method (Jones *et al.* 1991).

RESULTS AND DISCUSSION

1. Phenotypic characteristics

In all, twenty native strains previously isolated from nodules of white lupin plants and grown in soils collected from five distant locations were selected to be used in this study (Tounsi-Hammami *et al.* 2019). Phenotypic traits were analyzed in order to assess their ability to tolerate abiotic stress (Table 2). It appeared that all native isolates were classed as fast-growing bacteria forming 1-3 mm rod-shaped, gummy, white colored colonies when grown in YEM plates supplemented with Red Congo dye at 28°C.

For phenotypic traits, the minimum inhibitory concentrations of all the strains were estimated under different salt concentrations ranging from 600 to 1500 mM NaCl. The results showed that 12 strains exhibited a tolerance of 600 to 1000 mM NaCl, while the strains obtained from soil originating from Sejnen could not tolerate more than 900 mM NaCl. The most tolerant strains were LAb8, LAb30, and LAb32 with a minimum inhibitory concentration of 1500 mM NaCl. For tolerance to PEG6000 concentrations, the minimum inhibitory concentrations varied from 25 to 30%. All the tested strains were tolerant of a wide range of pH from 5 up to 11, while 60% of the strains were highly acidic-tolerant up to pH 4.

Our findings confirmed the results obtained by Youssef *et al.* (2014), who reported that fast-growing rhizobia could tolerate high variations of pH and exhibited tolerance to high NaCl concentrations. It is well documented that symbiotic nitrogen fixation can be affected by several types of environmental stress, such as acidity, alkalinity, drought, salinity and others, which reduce the effectiveness of legume-rhizobia symbiosis (Zahrán *et al.* 1999; Giller *et al.* 2001). Hence, selecting efficient strains that tolerate these abiotic stresses could improve the resistance of white lupin plants to these factors and will give rise to a more sustainable agriculture (Glick *et al.* 2007).

2. Plant nodulation assay

The symbiotic effectiveness of new native strains was assessed using a plant nodulation test (Table 3). Fourteen strains were able to nodulate white lupin with a number of nodules/plant ranging from 2.66 to 14, while the rest failed to induce root nodules on their host plants. On the other hand, the positive and negative control plants did not form any nodules, confirming aseptic experimental conditions. In agreement with our results, several studies demonstrated that legume root nodules can be cohabited by several non-nodulating bacteria called endophytes (De Lajudie *et al.* 1999; Mhamdi *et al.* 2005; Peix *et al.* 2015). Some of them can promote plant growth through direct and indirect mechanisms (Glick, 2012). Recently, Ferchichi *et al.* (2019) reported that root nodules of spontaneous *L. angustifolius* and *L. luteus* plants collected from five locations in northern Tunisia harbor several endophytic bacteria exhibiting several plant growth promotion traits.

Table 2. Phenotypic characteristics of the new strains isolated from *Lupinus albus* L.

Strains	Origin	Identification	Growth	NaCl inhibiting concentrations (mM)	PEG6000 inhibiting concentrations (%)	Low pH tolerated values	High pH tolerated values
LAa8	BorjMassaoudi	<i>Agrobacterium</i> sp.	FG	1000	30	4	11
LAa9	BorjMassaoudi	<i>Agrobacterium</i> sp.	FG	1000	25	4	11
LAa14	BorjMassaoudi	<i>Agrobacterium</i> sp.	FG	1000	30	4	11
LAa15	BorjMassaoudi	<i>Agrobacterium</i> sp.	FG	1000	30	4	11
LAa16	BorjMassaoudi	<i>Agrobacterium</i> sp.	FG	1000	25	4	11
LAa19	BorjMassaoudi	<i>Rhizobium</i> sp.	FG	1000	25	4	11
LAa21	BorjMassaoudi	<i>Agrobacterium</i> sp.	FG	1000	30	4	11
LAa24	BorjMassaoudi	<i>Rhizobium</i> sp.	FG	1000	30	5	11
LAa35	SidiThabet	<i>Rhizobium</i> sp.	FG	1000	25	4	11
LAa37	SidiThabet	<i>Rhizobium</i> sp.	FG	1000	25	5	11
LAa50	Sejnen	<i>Agrobacterium</i> sp.	FG	900	25	4	11
LAa51	Sejnen	<i>Agrobacterium</i> sp.	FG	900	25	4	11
LAa53	Sejnen	<i>Agrobacterium</i> sp.	FG	900	25	5	9
LAa54	Sejnen	<i>Agrobacterium</i> sp.	FG	800	20	4	11
LAa55	Sejnen	<i>Agrobacterium</i> sp.	FG	800	25	4	11
LAB8	Madian	<i>Rhizobium</i> sp.	FG	1500	30	5	11
LAB9	Madian	<i>Agrobacterium</i> sp.	FG	1000	30	5	11
LAB30	Jrissa	<i>Agrobacterium</i> sp.	FG	1500	25	5	11
LAB32	Jrissa	<i>Agrobacterium</i> sp.	FG	1500	30	5	11
LAB33	Madian	<i>Rhizobium</i> sp.	FG	1000	20	5	9

FG: fast-growing strain

On the other hand, shoot dry weight and nitrogen content, as an indirect measurement of nitrogen fixation benefit, varied considerably among infective strains. The shoot dry weight of white lupin of inoculated plants varied from 0.53 g/plant for isolate LAa19 to 1.63 g/plant for isolate LAb8, while the nitrogen content ranged from 11.9 to 26.63% g/plant. Compared to the uninoculated control plants (T0), all strains showed significant increases in shoot dry weight and nitrogen content, indicating that inoculated plants benefited more from symbiosis with the native rhizobial strains, except strain LAa19. However, only strains LAb8 and LAb33 accumulated a shoot dry weight and a nitrogen content that were statistically similar to those recorded in fertilized uninoculated plants (TN), and this indicated their efficiency.

However, strain LAb8 has been shown to have more adaptability and a greater ability to tolerate adverse stressful conditions than strain LAb33. Strain LAb8 was therefore selected as an appropriate candidate for further studies with a view to develop efficient inoculants to promote white lupin production in Tunisian soils.

Table 3. Results of plant nodulation and efficiency tests on *L. albus* inoculated with the new native strains

Strains	Nodule number (nodules plants ⁻¹)	Shoot dry weight (g plant ⁻¹)	Nitrogen content (%)
LAa8	2.66f	0.92d	17.50d
LAa9	9.00cd	0.97d	19.50c
LAa14	0.00	ND	ND
LAa15	14.00a	1.59a	21.80b
LAa16	0.00	ND	ND
LAa19	3.33f	0.53f	11.90f
LAa21	7.00d	1.66a	21.53b
LAa24	8.33d	0.77e	15.50e
LAa35	7.00d	0.61e	14.50e
LAa37	10.33c	1.45b	19.33c
LAa50	13.00a	1.0d	19.50c
LAa51	8.66d	0.87d	20.80b
LAa53	0.00	ND	ND
LAa54	0.00	ND	ND
LAa55	5.00e	1.17c	21.50b
LAB8	9.66c	1.63a	26.63a
LAB9	0.00	ND	ND
LAB30	7.66d	1.33c	17.50d
LAB32	0.00	ND	ND
LAB33	12.00b	1.63a	26.26a
TN	0.00	1.60a	26.89a
T0	0.00	0.52f	12.33f

T0: uninoculated unfertilized negative control; TN: positive control (fertilization equivalent to 90 units of nitrogen applied per ha); ND: not determined. Different letters indicate significant differences according to the LSD-test ($p \leq 0.05$).

3. Pot experiment

3.1. Nodulation

The nodulation test highlighted the potential of strain LAb8 inoculation to improve the growth and nitrogen content of white lupin when cultivated in sterilized carrier sand. This strain was therefore adopted for testing, under non-sterile conditions, with a liquid and a solid inoculum.

Nodulation was examined at flowering stage and results are presented in Figure 1. Significant differences in the number and dry weight of nodules per plant were observed between treatments. Twenty nodules per plant were recorded in the unfertilized control plants with a respective dry weight of 15.7 mg per plant. This indicated the presence of native compatible rhizobia able to nodulate white lupin plants in the collected soils with no history of growing lupins or rhizobial inoculation. However, the application of chemical nitrogen fertilizer led to a reduction in the nodulation number (9.25 nodules per plant) and in the dry weight (7 mg per plant). It was previously reported that high rates of chemical nitrogen fertilization inhibited the nodule formation and the nitrogen fixation of several legumes under both controlled and field conditions (Giller *et al.* 2001; Ferchichi *et al.* 2019). In the same context, Zhang *et al.* (2000) reported that mineral nitrogen application reduced the isoflavonoids concentration in the soybean root system and limited root colonization. Moreover, previous studies showed that applying chemical fertilizers at high rates may negatively affect bacterial populations in the root environment (Thilagar *et al.* 2016).

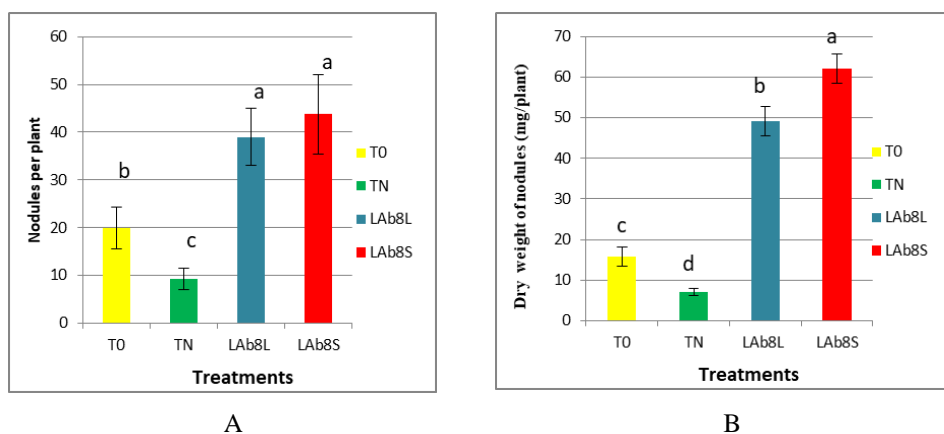


Figure 1: Effect of selected strain LAb8 on the number (A) and dry weight (B) of nodules per white lupin plant cultivated in non-sterilized soil.

T0: uninoculated unfertilized negative control; TN: positive control (fertilization equivalent to 90 units of nitrogen applied per ha); L: liquid inoculant, S: solid inoculant; Different letters indicate significant differences according to the LSD-test ($p \leq 0.05$).

However, in soils with low nitrogen fertility a moderate amount of nitrogen, called starter nitrogen, would appear to improve legume responses to rhizobial inoculants (Kouki *et al.* 2016; Mathenge *et al.* 2019). Several authors demonstrated the need for starter N, especially in low-fertility soil. In contrast, Mendes *et al.* (2003) reported that applying starter N had an insignificant effect on soybean nodulation.

Confirming the results obtained from the nodulation assay, a significant positive effect on nodulation was observed when inoculating white lupin plants with the selected strain LAb8, whether as solid or liquid inoculant. The increased number (95-118%) and dry weight of nodules (213-296%) over the unfertilized control plants brought about by rhizobial inoculation indicated the efficiency of the selected strains. Our results were in line with previous studies reporting that inoculation with efficient rhizobial strains can significantly increase nodulation (Hungria and Mendes, 2015; Tounsi-Hammami *et al.* 2016).

Interestingly, it seems that LAb8 conserved in a compost carrier was more efficient than liquid inoculant, since it displayed the highest number and dry weight of nodules per plant. It is important to note that the compost used in this study as a carrier to formulate the solid inoculant was able to support and maintain a similar or larger population of rhizobia than peat during a long storage period (Tounsi-Hammami *et al.* 2015). Similarly to our results, Albareda *et al.* (2008) reported that a solid formulation led to a larger number of nodules than the corresponding liquid inoculant.

3.2. Growth parameters

In terms of growth parameters, the leaf chlorophyll content (SPAD), plant height (PH), and shoot and root dry weight were analyzed and the data are shown in Table 4. The lowest values of all the measured growth parameters were recorded in the unfertilized control plants indicating the non-efficiency of the indigenous stains present in the tested soil. This result highlighted the need to inoculate with efficient compatible rhizobial strains (Date, 2001). Compared to the negative control plants, the white lupin plants benefited significantly from rhizobial inoculation, regardless of the type of the applied inoculants (solid or liquid formulations). For instance, inoculation with LAb8 applied as a liquid inoculant induced significant increases in SPAD, PH, SDW and RDW by 9%, 18%, 38% and 51%, respectively, compared to the negative control plants, while use of the same strain conserved in a compost carrier showed better increases in SPAD (11%), PH (16%), SDW (44%) and RDW (61%). Interestingly, rhizobial inoculation led to growth parameters similar to those obtained by applying 90 units of nitrogen ha⁻¹.

Our findings supported those obtained by Ouma *et al.* (2016), who reported that inoculation with efficient rhizobial strains significantly increased plant height, and shoot and root dry weights compared to uninoculated common bean and soybean plants. In contrast, Samudin *et al.* (2018) reported that rhizobial inoculation significantly increased the root dry weight of soybean, but did not affect plant height and shoot dry weight. Additionally, Herliana *et al.* (2019) reported that rhizobial inoculation increased the leaf area, which improved photosynthetic activities.

Table 4. Effect of selected strain LAb8 on the growth parameters of white lupin cultivated in non-sterilized soil

Treatments	SPAD	Plant height (cm/plant)	Shoot dry weight (g/plant)	Root dry weight (g/plant)
LAB8L	49.75a	17.375a	0.8525a	0.23ab
LAB8S	50.925a	17.125a	0.8875a	0.245a
T0	44.65b	14.75b	0.6175b	0.1525c
TN	53.225a	17.075a	0.8225a	0.1825bc
LSD	6.897	1.520	0.126	0.054

LAB8L; LAB8S

T0: uninoculated unfertilized negative control; TN: positive control (fertilization equivalent to 90 units of nitrogen applied per ha); L: liquid inoculant, S: solid inoculant Different letters indicate significant differences according to the LSD-test ($p \leq 0.05$).

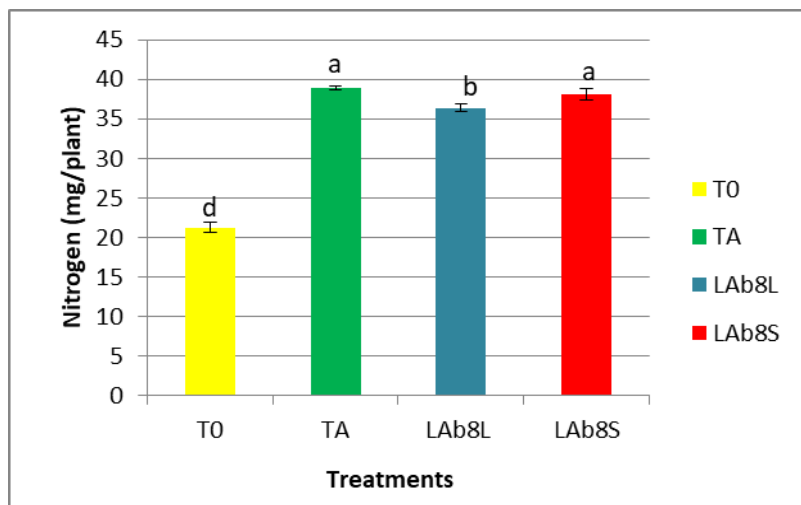


Figure 2. Effect of selected strain LAb8 on the nitrogen content of white lupin cultivated in non-sterilized soil. T0: uninoculated unfertilized negative control; TN: positive control (fertilization equivalent to 90 units of nitrogen applied per ha); L: liquid inoculant, S: solid inoculant; Different letters indicate significant differences according to the LSD-test ($p \leq 0.05$).

For shoot nitrogen accumulation, the highest value was recorded in the nitrogen control plants. Interestingly, the selected strain applied as a liquid or solid formulation exhibited significant increases in shoot nitrogen accumulation ranging from 71% to 79% compared to the negative control plants. It is important to note that only the solid inoculants resulted in greater accumulation of nitrogen in plant shoots similar to that recorded in the fertilized control plants, indicating the efficiency of the selected strains when conserved in compost as a carrier material. Our findings were in line with those obtained by Albareda *et al.* (2008) and Rice *et al.* (2000) who reported that solid formulations were more efficient than liquid ones, while Singleton *et al.* (2002) and Tittabutr *et al.* (2007) showed similar efficiency for liquid and solid inoculants.

It has been reported that increasing the rhizobial density in the rhizosphere results in maximum increases in nodulation, as well as growth parameters (Kumar *et al.* 2002). It could be imagined that the compost used as the carrier material to support LAb8 was able to maintain a larger number of rhizobia than the liquid inoculant.

CONCLUSION

The main purpose of this study was to select efficient white lupin nodulating rhizobia in Tunisia. Phenotypic characterization of the native strains identified a Tunisian strain with high tolerance of several types of abiotic stress, such as drought, alkalinity, acidity and salinity. A nodulation test revealed the potential of the selected native strain LAb8 as a useful, efficient inoculant. Additionally, in a pot experiment, the selected strain showed great potential for promoting the introduction of white lupin as a crop plant in Tunisian soils,

especially when conserved in compost as a carrier material. This study should be complemented with multi-field trials to elucidate the effectiveness of the selected strain.

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