

IMPACT OF NATIVE SOIL MICROBIOTA ON NODULATION AND SEED YIELD IN WHITE LUPIN (*Lupinus albus* L.) CULTIVARS

Lizica SZILAGYI, Nicolae FARCAȘ

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd,
District 1, 011464, Bucharest, Romania

Corresponding author email: lizicasz@yahoo.com

Abstract

During the 2025 growing season, a field experiment was conducted at the University of Agronomic Sciences and Veterinary Medicine of Bucharest to evaluate the effect of native soil microbiota on biological nitrogen fixation and seed yield in four white lupin (*Lupinus albus* L.) cultivars. The results showed that, even without artificial inoculation, plants developed an average of 10.59 nodules per root. The cultivars Medi and Mariuca exhibited the highest nodulation (11.33 and 11.15 nodules, respectively) and achieved the highest seed yields: 1,440 kg ha⁻¹ and 1,363 kg ha⁻¹. These values represent an increase of 13.11% and 7.06%, respectively, over the trial mean (1,273 kg ha⁻¹). A strong positive correlation ($r = 0.962$; $R^2 = 0.926$) between nodule count and seed yield suggests that nodulation serves as a key indicator of nitrogen fixation potential and overall productivity. These findings indicate that native soil microbiota significantly support lupin development, with important implications for cultivar selection and sustainable crop management focused on reducing synthetic nitrogen inputs.

Key words: *Lupinus albus* L., native soil microbiota, nodulation, biological nitrogen fixation, seed yield, correlation.

INTRODUCTION

White lupin (*Lupinus albus* L.) is native to the Mediterranean basin and the coastal regions of the Black Sea, where it has been cultivated for millennia for its high-protein seeds and significant ecological benefits (Gladstones et al., 1998; Hondelmann, 1984). Although it historically played a vital role in traditional agriculture across Southern Europe and Western Asia, it remains globally underrecognized compared to other major legumes (Kurlovich, 2002). Taxonomically, *L. albus* belongs to the *Fabaceae* family. It is an annual, dicotyledonous species, typically reaching heights of 0.5 to 1.5 meters. While primarily self-pollinating, low levels of cross-pollination can occur depending on environmental factors and pollinator activity (Wolko et al., 2011). Cytogenetic studies identify white lupin as a diploid species with a chromosome number of $2n = 50$ (Naganowska et al., 2003). Its seeds possess a remarkable nutritional profile, comparable to soybean in protein content, essential amino acids, and dietary fiber (Lucas et al., 2015), making it a valuable resource for both human consumption and livestock feed.

Beyond its nutritional value, *L. albus* is distinguished by its highly efficient symbiosis with soil bacteria, specifically *Bradyrhizobium* strains, which enables biological nitrogen fixation (BNF). This process enhances soil fertility and reduces the ecological footprint of agriculture by decreasing dependency on synthetic nitrogen fertilizers (Sulas et al., 2016; Vance, 2001). As the global agricultural sector faces energy crises and an urgent need for sustainable protein, white lupin has emerged as a crop of considerable scientific interest. In rainfed Mediterranean environments, it can exhibit a remarkable nitrogen-fixing capacity, with peak values exceeding 300 kg N ha⁻¹ (Sulas et al., 2016), underscoring its potential under favorable conditions.

The efficiency of biological nitrogen fixation in white lupin is regulated by complex molecular interactions between the host plant and symbiotic bacteria, leading to the formation of specialized "lupinoid" nodules. Unlike peas or beans, which typically associate with *Rhizobium leguminosarum*, *Lupinus* species are primarily nodulated by slow-growing *Bradyrhizobium* bacteria, particularly *Bradyrhizobium lupini* (Clúa et al., 2018). Within these nodules,

bacteria differentiate into bacteroids that convert atmospheric nitrogen into a bioavailable form in exchange for plant carbohydrates. This symbiosis allows the plant to meet its nitrogen requirements independently, supporting vigorous growth and high seed yields even in nitrogen-deficient soils (Huyghe, 1997). Recent research indicates that the success of this symbiosis is influenced by intricate interactions between host genotypes and native soil microbiota (Tounsi-Hammami et al., 2021). The diversity of indigenous microbial populations allows different cultivars to adapt to specific pedoclimatic conditions, directly impacting nitrogenase activity and productivity. Despite this potential, white lupin remains a neglected crop in many regions, including Romania, where it is considered a minor species. To promote wider adoption, it is essential to understand how native soil microbiota facilitate effective nodulation without artificial inoculation. This study aims to evaluate the impact of native soil microbiota on nodulation patterns and seed yield in four white lupin (*Lupinus albus* L.) cultivars, providing a scientific basis for integrating this species into sustainable crop rotation systems in temperate climates.

MATERIALS AND METHODS

Experimental Site and Setup

The field trial was conducted at the experimental site of the University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania, during the 2025 growing season. The experiment was carried out on reddish preluvosol soil, characterized by a slightly alkaline pH of 7.79, moderate humus content of 2.95%, moderate total nitrogen level of 0.166%, high mobile phosphorus ($P_{AL} = 92$ mg/kg), and very high mobile potassium ($K_{AL} = 329$ mg/kg). Soil analysis was performed in 2023 at the National Institute of Research and Development for Pedology, Agrochemistry, and Environmental Protection in Bucharest (Szilagyi & Gidea, 2025). The trial was arranged in a Randomized Complete Block Design (RCBD) with three replications. Each experimental plot covered an area of 12 m² and comprised six rows, each 6 m in length. Sowing was performed

on April 2, 2025, at a depth of 3-4 cm, maintaining an inter-row spacing of 0.4 m.

Cultural Practices

Conventional tillage was applied. To evaluate the natural symbiotic potential of the soil, no nitrogen fertilization or artificial inoculation with *Bradyrhizobium* sp (*Lupinus*) was used. During the growing season, weed control was maintained through two manual hoeings. Additionally, two irrigation events of 30 mm each were performed: the first immediately following seedling emergence (BBCH 09-10) and the second prior to the flowering stage (BBCH 59-61). Phenological stages were identified according to the extended BBCH scale (Meier, 2001). All other agrotechnical measures were performed according to local standard practices for white lupin (*Lupinus albus*) cultivation. Manual harvesting was conducted between July 10 and 13, 2025, when the plants ranged between harvest maturity and overripeness (BBCH 89-92).

Plant Material

The biological material consisted of four white lupin (*Lupinus albus* L.) cultivars with distinct phenological, morphological, and chemical profiles (Table 1). Mihai, Mariuca, and Ruxandra varieties were developed at the Iasi University of Life Science (IULS), while 'Medi' originated from the Agricultural Research and Development Station (ARDS) Livada. According to the Official Catalogue of Varieties of Agricultural Plant Species in Romania, the genotypes were classified by seed alkaloid content: 'sweet' (Mariuca, lacking bitter substances) and 'bitter' (Mihai, Ruxandra, and Medi, which contain bitter substances in the seed). The selection included both determinate (Dt) and indeterminate (Ndt) growth habits.

Table 1. Characteristics of the white lupin cultivars used in the study

Cultivar	Origin	Maturity	Growth habit*	Alkaloid Type
Mihai	IULS	Mid-early	Ndt	Bitter
Mariuca	IULS	Mid-early	Dt	Sweet
Ruxandra	IULS	Mid-late	Dt	Bitter
Medi	ARDS Livada	Mid-early	Ndt	Bitter

*Dt – determinate upright, Ndt – indeterminate upright

Weather Conditions

In 2025, according to data from the National Meteorological Administration (ANM) and Meteoblue, Bucharest recorded a positive deviation in the average annual temperature compared to the 1991-2020 multi-annual average, ranging between +0.8°C and +1.2°C. This temperature increase had a detrimental impact on agricultural crops, being particularly severe from June to August, when maximum values exceeding 35°C induced significant heat and water stress, negatively affecting yields. The precipitation regime in 2025 was characterized by rainfall slightly below the climatological average, with an uneven distribution cumulating approximately 520-540 mm/year (Figure 1). The spring months and early summer were marked by prolonged periods of drought, followed by short episodes of torrential rain, often accompanied by convective phenomena such as hail and frequent electrical discharges. This alternation between water deficit and pluvial excess further amplified the physiological stress on the agricultural crops.

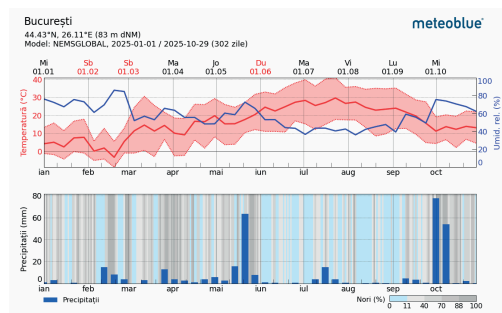


Figure 1. Climate conditions (average temperature and precipitation) in Bucharest - January-October 2025 (Source: <https://www.meteoblue>)

Data Collection

Nodulation: to quantify the number of nodules, a sample of six plants per replicate was randomly collected for each lupin cultivar during the flowering phenophase, which is recognized as the stage of maximum symbiotic nitrogen-fixing activity (Zahran, 1999).

These authors emphasize that nitrogen fixation efficiency is driven by the metabolic sink strength of the flowers and developing pods, reaching its absolute maximum at full bloom.

Seed yield: the total seed yield from each plot was bulked and weighed, with the results

extrapolated to calculate seed yield in kilograms per hectare (kg ha^{-1}).

Statistical Analysis

Data were subjected to analysis of variance (ANOVA). Mean values were compared using Fisher's Least Significant Difference (LSD) test at a significance level of $p < 0.05$. Additionally, Pearson's correlation coefficient (r) was calculated to assess the relationship between nodule count and seed yield.

RESULTS AND DISCUSSIONS

Symbiotic Performance (Nodulation)

The assessment of nodule formation in the absence of artificial inoculation serves as a critical indicator of the soil's indigenous rhizobial potential. Our results revealed that the experimental site hosts a viable and functionally active population of native *Bradyrhizobium* strains capable of establishing symbiosis with white lupin (*Lupinus albus* L.). Across all tested cultivars, plants developed a trial mean of 10.59 nodules per root system (Table 2).

A key result of this research is the substantial nodule development observed even without artificial inoculation.

This spontaneous nodulation can be attributed to the established rhizobial presence within the experimental site. Although the preceding crop in 2024 was winter barley (*Hordeum vulgare* L.), the field's extensive history, marked by the frequent cultivation of legumes like beans, chickpeas, lentils, peas, white lupin and blue lupin, has likely maintained a persistent microbial reservoir. The survival of these indigenous populations highlights the environmental persistence of native *Bradyrhizobium*, which continued to colonize the lupin roots effectively without the need for new inoculants. Statistically the cultivars Medi and Mariuca exhibited the highest numerical values, with 11.33 and 11.15 nodules per plant, respectively (Table 2). These figures align with findings by Tounsi-Hammami et al. (2021), who observed that in non-inoculated Mediterranean soils, *Lupinus albus* maintains a baseline nodulation similar to our results. However, as noted by those authors, while baseline nodulation exists, it often falls below the threshold required for maximum biomass

production compared to inoculated treatments. The Analysis of Variance (ANOVA) for nodule count (Table 3) yielded a p-value of 0.6326, indicating that the differences between cultivars were not statistically significant at the (5%) threshold. The calculated LSD (0.05) of 3.20 was not exceeded by any pair of cultivars, suggesting a relatively uniform compatibility across the tested cultivars with the indigenous soil microbiota.

Table 2. Descriptive statistics, variability, and significance indicators for the number of nodules per plant in white lupin cultivars

Cultivar	Mean (nodules/plant)	SD*	Observations
Medi	11.33	3.29	Highest variability
Mihai	9.86	0.11	Most consistent results across replications.
Ruxandra	10.01	0.55	Homogeneous results
Mariuca	11.15	0.64	High and stable performance
Trial Mean	10.59		
LSD ($p < 0.05$) - 3.20; CV (%) -16.03			

*SD – standard deviation

Despite the lack of significance, the Coefficient of Variation (CV = 16.03%) and the Standard Deviation (SD) analysis provide deeper insights into cultivar-specific stability. The higher variability observed in Medi (SD = 3.29) implies that while this cultivar possesses a high symbiotic potential, its interaction with native bacteria is more sensitive to micro-environmental fluctuations or soil heterogeneity. In contrast, Mihai demonstrated exceptional stability (SD = 0.11), though at a lower numerical frequency.

Table 3. ANOVA analysis for nodules number to white lupin

Source of Variation	df	Sum of Squares (SS)	Mean Square (MS)	F-value	P-value ns*
Between cultivars	3	5.1912	1.7304	0.6005	0.6326
Residual Error	8	23.0521	2.8815		
Total	11	28.2433			

*(ns) – not significant

These results suggest that in soils with established native populations, cultivar selection may influence the stability of the symbiotic interaction as much as its magnitude.

Seed Yield Performance

The results revealed highly significant differences among the white lupin cultivars studied, with seed yields ranging from 1,080 to 1,440 kg ha⁻¹ (Table 4). The experiment showed a high level of accuracy, evidenced by the low coefficient of variation (CV = 2.86%) and relatively small standard deviations (less than 40 kg ha⁻¹), indicating a strong consistency and uniformity among the replicates.

The cultivar Medi demonstrated the highest adaptive capacity and production potential, recording a very highly significant positive difference of +167 kg ha⁻¹ (113.11%) over the trial mean of 1,273 kg ha⁻¹, establishing this genotype as a reference for high productivity in the analyzed environment. Similarly, Mariuca showed a distinctly significant yield increase of +90 kg ha⁻¹ (107.06%), confirming its status as a high-performing cultivar (Table 4).

In contrast, Ruxandra yielded 1,209 kg ha⁻¹ (94.97% relative yield). Although it produced 64 kg ha⁻¹ less than the control, this difference did not exceed the critical LSD 5% threshold (68.58 kg ha⁻¹), indicating that its performance was statistically comparable to the trial average. The lowest yield was recorded for Mihai (1,080 kg ha⁻¹), which exhibited a very highly significant deficit, producing 15.17% less than the mean, suggesting limited genetic adaptation to the experimental conditions or lower resource-use efficiency for this specific cultivar.

Table 4. Seed yield performance and statistical significance of white lupin cultivars compared to the trial mean (control)

Cultivar	Mean yield (kg ha ⁻¹)	Relative yield (%)	Difference ± Control (kg ha ⁻¹)	SD	Sig.
Medi	1,440	113.11	+167	±39.05	***
Mihai	1,080	84.83	-193	±30.41	000
Ruxandra	1,209	94.97	-64	±37.32	ns
Mariuca	1,363	107.06	+90	±38.43	**
Trial Mean	1,273	100			

Note: LSD 5% = 68.58 kg ha⁻¹; LSD 1% = 99.76 kg ha⁻¹; LSD 0.1% = 149.88 kg ha⁻¹

*** = $p < 0.001$; ** = $p < 0.01$; ns = not significant; 000 = significantly lower at $p < 0.001$.

Table 5. ANOVA analysis for seed yield to white lupin cultivars

Source of Variation	df	Sum of Squares (SS)	Mean Square (MS)	F-value	P-value ***
Between cultivars	3	231,342.0	77,114.0	58.14	<0.0001
Residual Error	8	10,610.7	1,326.3		
Total	11	241,952.7			

***– highly significant at $p < 0.001$

The analysis of variance (ANOVA) confirmed the highly significant effect of the cultivar on seed yield ($p < 0.001$) (Table 5).

These results align with findings by López-Bellido et al. (2000), who noted that white lupin yields are highly dependent on genotype-environment interactions, with modern cultivars showing superior harvest indices even under varying climatic conditions.

Correlation Between Nodulation and Seed Yield

The strong positive correlation ($r = 0.962$) suggests that for these specific cultivars (Medi, Mihai, Ruxandra, Mariuca), nitrogen fixation is the primary limiting factor for productivity (Figure 2), a relationship consistent with the findings of Sulas et al. (2016).

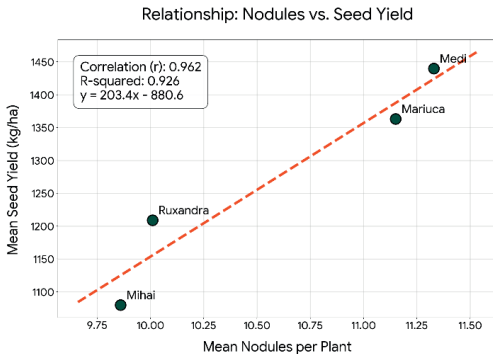


Figure 2. Correlation between nodulation and seed yield in white lupin cultivars

The data indicates that cultivars capable of supporting higher nodule populations, such as Medi (11.33 nodules/plant), are significantly more effective in achieving superior seed yields ($1,440 \text{ kg ha}^{-1}$). Medi and Mariuca demonstrated superior symbiotic efficiency. Their ability to maintain higher nodule counts suggests either an enhanced genetic compatibility with

Bradyrhizobium bacteria or a more vigorous root system capable of supporting more infection sites, aligning with the breeding priorities for *Lupinus* species identified by Wolko et al. (2011). Conversely, Mihai was the least productive ($1,080 \text{ kg ha}^{-1}$), directly corresponding to its lowest nodule count (9.86). This suggests a potential genetic barrier or a reduced physiological demand for nitrogen that limits its symbiotic potential relative to the top-performing cultivars. With a coefficient of determination R^2 of 0.926, nearly all observed yield variation is dictated by nodulation intensity. This implies that while environmental factors (e.g., soil moisture, temperature) affected the trial uniformly, genotype-specific affinity for local microbiota remained the principal driver of the results, supporting the conclusions of Annicchiarico et al. (2010) regarding the genotypic basis of adaptation as the primary determinant of white lupin productivity.

CONCLUSIONS

This study highlights the crucial role of native soil microbiota, sustained by a long-term persistent microbial reservoir from previous leguminous crops, which provides a solid foundation for white lupin productivity even without artificial inoculation. The strong positive correlation between nodulation and seed yield identifies biological nitrogen fixation as the primary determinant of yield potential in these conditions.

The superior performance of cultivars Medi and Mariuca highlights the importance of genotype-specific affinity for indigenous *Bradyrhizobium* populations. Furthermore, the discrepancy between the high correlation observed and the non-significant variance in nodule quantity (ANOVA) suggests that nodule effectiveness and nitrogen translocation efficiency are more critical to final output than the absolute number of nodules. Therefore, breeding and cultivar selection for sustainable management should prioritize symbiotic functionality as a key indicator of adaptive capacity and high production potential in white lupin.

These findings provide a vital baseline for future research, which should explore whether artificial inoculation can further bridge the yield

gap or enhance the performance of these cultivars.

ACKNOWLEDGEMENTS

This work was supported by project ADER 115/18.07.2023, funded by the Ministry of Agriculture and Rural Development of Romania. We would also like to express our gratitude to Professor Dănuț Petru Simioniuc from the Iasi University of Life Sciences (IULS) for kindly providing the seeds of the Măriuca, Mihai, and Ruxandra cultivars.

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