

SELECTION OF SUITABLE GENOTYPES OF LENTIL (*Lens culinaris* MEDIK.) TOLERANT TO PHOSPHORUS DEFICIENCY

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Abstract. Lentil (*Lens culinaris* Medik.) is a popular Mediterranean legume crop grown for its nutritious seeds and to improve soil fertility. Lentil yield is a critical and difficult trait to improve in crop genetics because it is influenced by various factors that negatively affect seed yields and quality traits. The main constraint to legume production and symbiotic nitrogen fixation is the soil's insufficient phosphorus (P) availability. This P-deficiency also affects rhizobia multiplication in the rhizosphere, reducing the likelihood of infection and nodule growth. The primary goal of this study is to determine the degree of adaptation of lentils to P-deficiency to select the best genotypes grown, considering growth rate, grain yield, and efficiency in the use of rhizobial symbiosis. In this context, we are investigating the behavior of some lentil genotypes grown in the Khemis Miliana (Algeria) area over two growing seasons, 2018 and 2019. The results show that yield values vary between genotypes, ranging from 500 to 1200 kg·ha⁻¹; specifically, the genotypes 'Large blonde and 'Ibla 1 recorded the highest yield over two growing seasons. Furthermore, efficiency in the use of the rhizobial symbiosis of lentils was higher for 'Djendel during the 2018 season (1.57 g shoot DW·g⁻¹ nodule DW) than for 'Large blonde (0.2 g shoot DW·g⁻¹ nodule DW). The genotypes chosen for their P usage efficiency had higher yields and were the best at dealing with low P availability in the field.

Key words: legume; phosphorus; N₂ fixation; rhizobia; symbiosis.

INTRODUCTION

Legumes are among the most important crops due to their many agro-environmental benefits and high nutritional value. Legume seeds and powders contain significant amounts of protein, carbohydrates, vitamins, minerals, and dietary fiber [4, 39]. They significantly impact cropping systems, particularly as a source of symbiotic N₂ fixation, which may reduce the need for nitrogen fertilizer. They also aid in managing weeds and pests in crop rotations [18]. Phosphorus (P) may be a critical restriction for legumes in low-nutrient environments due to the high P requirements for the N₂ fixation process [45, 49]. Nodules are more sensitive to P-deficiency because of their high P requirements, a major constraint for legume growth, especially in acidic or calcareous soils [10, 19, 52]. P fertilizers must be used to increase crop production [17, 36]. However, P fertilization is frequently ineffective for crops; only a small portion of the supplied P is typically absorbed the same year, with the remainder deposited in the soil as organic, adsorbed, and mineral forms [15, 35, 56].

In response to P-deficiency, nodulated legumes employ two coping mechanisms [3]. The first approach involves plant-soil interactions, such as modification of soil exploration by roots, improved interactions with soil microorganisms such as arbuscular mycorrhizal fungi (AMF) and rhizosphere modification to increase P availability [21, 31, 54]. The second method entails efficient P partitioning and subsequent utilization within the plant, resulting in more biomass produced and N₂ fixed per unit of P taken up [31, 55]. This latter strategy has received little attention despite internal P requirements differing between legume species [10]. Indeed, choosing P deficiency tolerant symbioses may be based on the P use efficiency (PUE) for N₂ fixation, which appears to be unique to the interaction of the two

symbioses [50]. To increase crop productivity, it may be possible to identify physiological or biochemical techniques for screening tolerant cultivars by examining genotypic diversity in lentil responses to P deficiency. Therefore, this study aims to improve our understanding of the P function for symbiotic nitrogen fixation (SNF) to develop strategies to improve nodulated lentil resistance to low P-soils.

MATERIALS AND METHODS

Experimental site study area

The experiment was repeated throughout two seasons, 2018 and 2019. The experiment was carried out at the Demonstration Farm and Seed Production (FDPS), which belongs to the Technical Institute of Field Crops (ITGC) station of Khemis Miliana (Algeria) on a clayey-silt soil according to the USDA Duchaufour soil texture triangle [13] and a previous wheat crop. The experimental site is located at 277 m and has the coordinates 36°15'03''N 2°14'25''E. The Mediterranean climate of Khemis Miliana was characterized by a rainy and cold winter, a dry and hot summer, and a short spring (April-May) with varying thermal amplitudes between winter and summer and night and day. Rainfall in the region is erratic, with an annual mean of 565 mm in 2018 and 482.8 mm in 2019. Low rainfall occurred at the start of the two-year experiment, particularly in September. Soil's physicochemical characteristics were identified in 2018; it contained 47% clay, 45% loam, and 8% sand. The experimental soil was slightly alkaline (pH 7.4) [14], with 1.81 g·g⁻¹ CaCO₃ and 1.69% organic matter. Both N and P were insufficient in the soil (total N: 0.78gN·kg⁻¹[25], Olsen P: 9.52 mgP·kg⁻¹ [37]).

Cropping system and field plot design

Five lentil cultivars ('Djendel, 'Ibla 1, 'Large blonde, 'LVS, and 'Metropole) were used in the field experiment. They were grown over two seasons, 2018 and 2019, using a complete randomized block experimental design on an area of 122.4 m² and a density of 200 plants m⁻² for each genotype. The field trial was divided into three blocks, with each block subdivided into five subplots. Each subplot, measuring 5 m x 1.2 m with a surface area of 6 m², was used for one of the lentil genotypes.

Plant and soil sampling and measurements

Five topsoil samples (0-20 cm) collected by auger were subjected to soil physicochemical property measurements before the first sowing season. We collected rhizosphere and bulk soil samples 100 days after sowing (full flowering stage) to perform measurements. This sampling was done at a depth of 25 to 30 cm. The rhizosphere was sampled in the usual way by gently brushing <1–4 mm aggregates of soil adhering to lentil roots with a paintbrush [23, 29]. Each genotype received three replicates of rhizosphere and corresponding bulk soil samples. Plant samples were also taken at various stages of flowering. When flowering reached 50% of the elementary plot for each genotype, 9 plants were harvested, separated into shoots and roots, and dried for 48 h at 65°C before being weighed. Nodules were separated from the roots, dried, and weighed individually. Shoots and roots were ground and digested in nitric and perchloric acids in a microwave oven at 135°C and 1.5×10⁶ Pa. Correlation analysis was used to assess the efficiency of using the rhizobial symbiosis (EURS) for plant growth in order to test whether cropping treatment over two growing seasons would affect the relationship between nodulation and shoot growth [33].

Plant P uptake corresponded to the amount of nutrients taken up by plants during plant growth and was as follows: P concentrations in shoots and roots were measured using the malachite green method as performed for total soil P [8]. Before analysis, all soil and rhizosphere samples were dried, sieved (2 mm), and stored at 4°C for 72 h. P availability was assessed using the Olsen method, and pH was measured in soil suspension with deionized water (soil: water ratio=1:2.5; [47]. After soil digestion with perchloric and nitric acids, the total soil P content was measured spectrophotometrically at 630 nm using the malachite green method [51]. Kjeldahl's (1883) [26] and Walkley and Black's (1934) [53] methods were used to determine total N and organic matter contents, respectively.

$$P \text{ uptake (mg)} = [P \text{ concentration in shoot (mg}\cdot\text{g}^{-1}) \times \text{shoot DW (g)}] + [P \text{ concentration in root (mg}\cdot\text{g}^{-1}) \times \text{root DW (g)}] - \text{seed P content (mg)}.$$

The grain yield was determined at the plant maturity stage by harvesting all plants within 1 m² with three replicates in each plot.

Statistical analysis

A one-way ANOVA analysis was performed using genotype as a factor. We determined the efficiency in the use of rhizobia symbiosis (EURS) by the slope of the linear regression between shoot dry weight (SDW) and nodule dry weight (NDW) using analysis of variance (ANOVA) at a significance level of p=0.05. Tukey's test was used to determine if the difference between the mean values of each treatment was significant. Statistica 10 (v. 8.5) was used to perform all the statistics.

RESULTS

Plant growth and nodulation

Figs. (2, 3 and 4) show that three compartments of lentil biomass (shoots, roots and nodules) varied not significantly between genotypes. The largest shoot DW was found in genotypes 'Ibla 1 and 'Large blonde during the first growing season of 2018 with (7.33 g·plant⁻¹) and (7.14 g·plant⁻¹), respectively, followed by genotype 'Djendel (6.50 g·plant⁻¹). In the 2019 season, 'Metropole and 'Ibla 1 presented the highest shoot DW (7.20 g·plant⁻¹) and the lowest shoot DW was recorded for the 'LVS genotype during two growing seasons. Root DW was always substantially higher in the 'Large blonde and 'LVS genotypes, whatever the growing season and the lowest root DW was observed in the 'Ibla 1 genotype for the two seasons. The highest nodule dry DW was recorded by 'Ibla 1 and 'LVS for both seasons, and the lowest nodule DW was recorded for 'Large blonde for both seasons.

Estimated grain yield

The results in Fig. (1) show that yield values vary slightly between genotypes, ranging from 500 to 1200 kg·ha⁻¹. Specifically, the varieties 'Large blonde and 'Ibla 1 recorded the highest yield during two growing seasons: (1235 kg·ha⁻¹ and 1106 kg·ha⁻¹ in 2018) and: (1205 kg·ha⁻¹ and 940 kg·ha⁻¹ in 2019) respectively. The variety 'LVS produced the lowest yield during both seasons, with 544 kg·ha⁻¹ and 593 kg·ha⁻¹.

Efficiency in the use of the rhizobial symbiosis (EURS)

To determine whether N₂ fixation based on nodule biomass varied among lentil genotypes, shoot biomass was plotted as a function of nodule biomass for each plant. Shoot and nodule biomass were significantly correlated and varied among lentils (Fig. 5). The linear regression slope of the curves shown in (Fig. 5) represents the rhizobial symbiosis estimates efficiency. During two growing seasons, there was no significant correlation between NDW and SDW in 'Metropole. However, the remaining plant variables showed a significant correlation. The highest was for 'Djendel (1.57 g shoot dry weight g⁻¹ nodule dry weight) in 2018 with (R²=0.809***) and (1.07 g shoot dry weight g⁻¹ nodule dry weight) in 2019 with (R²=0.788***) followed by 'Large blonde (0.2 g shoot dry weight·g⁻¹

nodule dry weight) in 2018 and (0.87 g shoot dry weight·g⁻¹ nodule dry weight) in 2019 with (R²=0.756***) and (R²=0.749***) respectively. In 2018, the lowest value was for 'Metropole (0.03 g

shoot dry weight·g⁻¹ nodule dry weight) and in 2019, the lowest value was for (0.19 g shoot dry weight·g⁻¹ nodule dry weight).

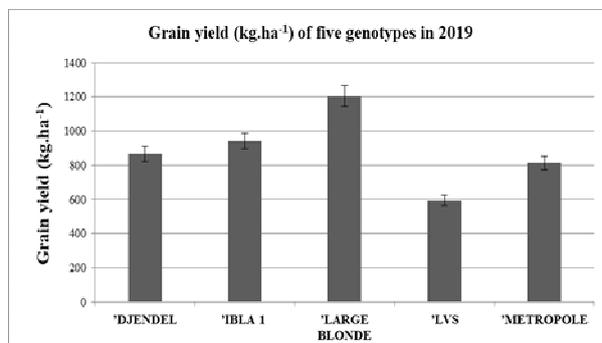
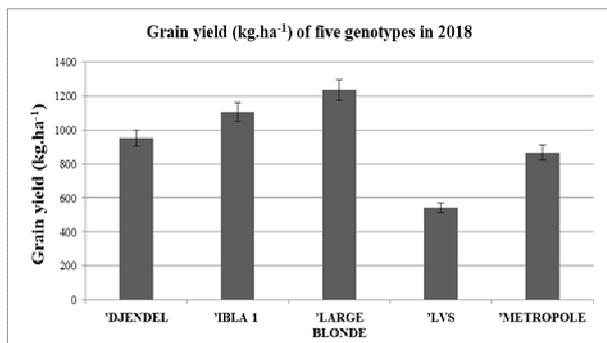


Figure 1. Grain yield (kg·ha⁻¹) of five genotypes of *lens culinaris* grown under P-deficient conditions during two growing seasons, 2018 and 2019. Data correspond to mean values ± standard error of three replicates harvested at the physiological maturity stage.

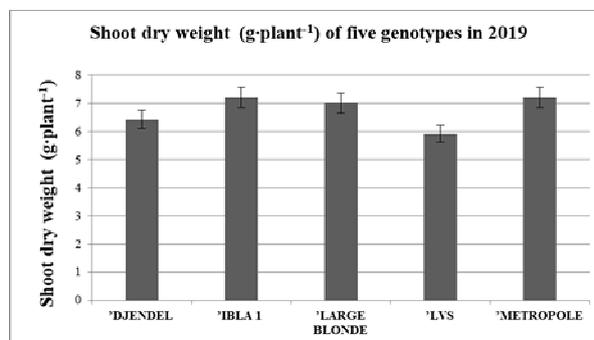
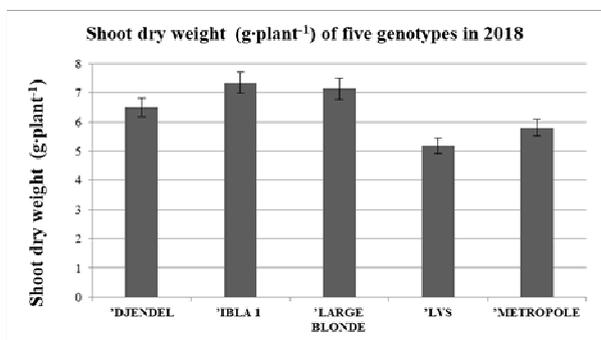


Figure 2. Shoot dry weight of five genotypes of *lens culinaris* grown under P-deficient conditions during two growing seasons, 2018 and 2019. Data correspond to mean values ± standard error as calculated with 9 plants.

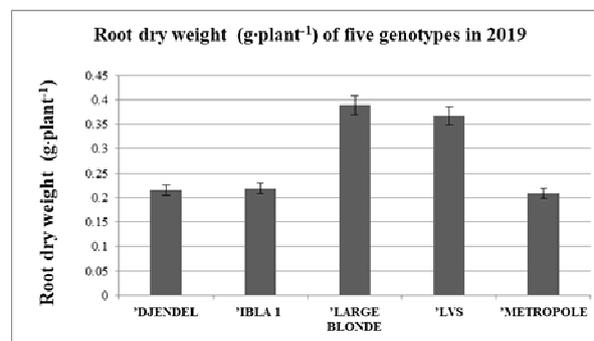
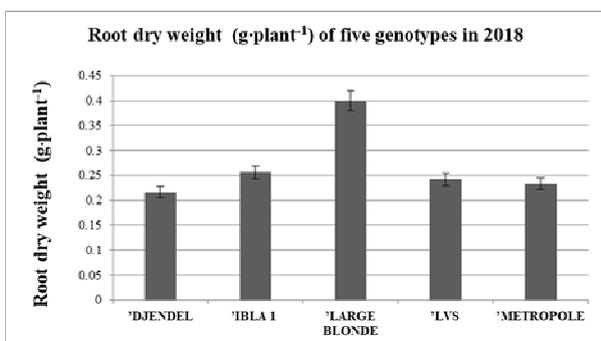


Figure 3. Root dry weight of five genotypes of *lens culinaris* grown under P-deficient conditions during two growing seasons, 2018 and 2019. Data correspond to mean values ± standard error as calculated with 9 plants.

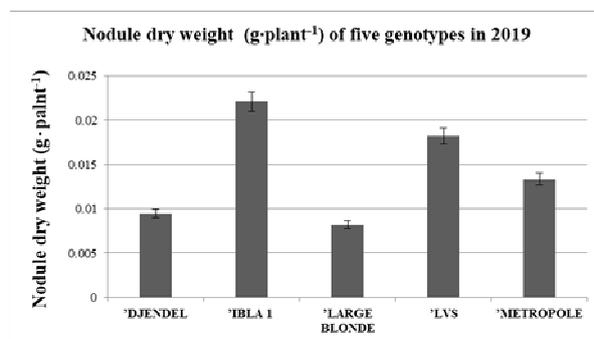
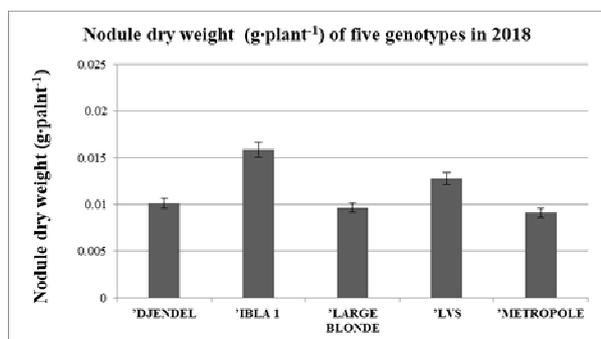


Figure 4. Nodule dry weight of five genotypes of *lens culinaris* grown under P-deficient conditions during two growing seasons, 2018 and 2019. Data correspond to mean values ± standard error as calculated with 9 plants.

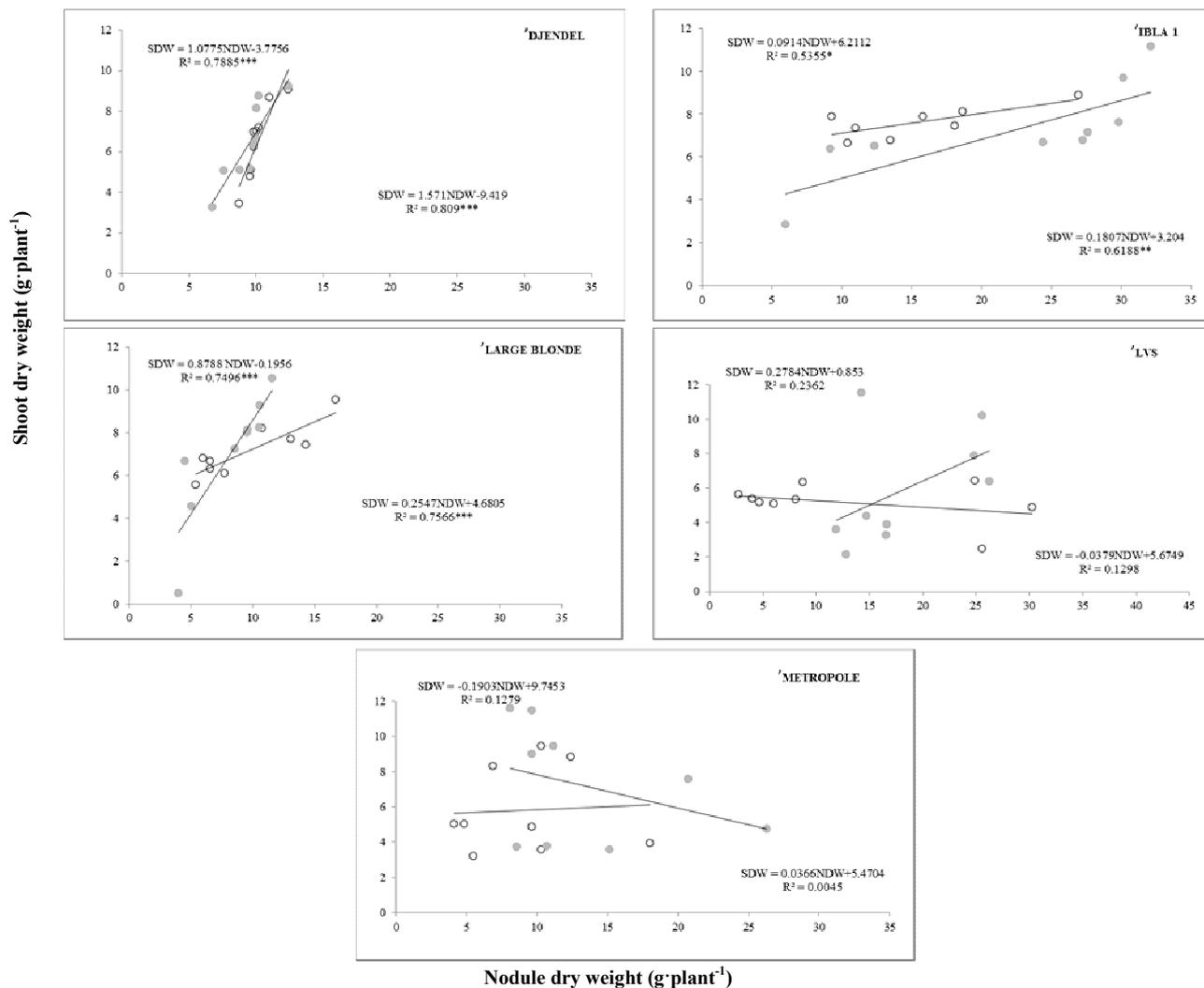


Figure 5. Linear relationships between nodule dry weight (NDW) and shoot dry weight (SDW) using 9 plants harvested 100 d after sowing in 2018 (open symbols) and 2019 (closed symbols). ** and *** denote $p < 0.01$ and $p < 0.001$, respectively.

Phosphorus concentration and uptake

The results summarized in Table (1) and Fig. (6) demonstrate that the levels of P concentrations in the shoot and root compartments of lentils exhibited significant variation ($p < 0.001$) across different genotypes. The shoots and roots of the 'Djendel genotype had the lowest phosphorus concentrations during both growing seasons, as indicated in Table 1. The plants of genotype 'Ibla 1 exhibited the highest

concentrations of P in both seasons, with 'Large blonde plants having slightly lower concentrations. During both growing seasons, the 'LVS variety had the lowest P uptake, with values of 27.05 and 26.77 for the first and second seasons, respectively. On the other hand, the 'Ibla1 variety had the highest P uptake during both seasons (Table 1). Some other genotypes showed moderate levels of P uptake.

Table 1. Phosphorus concentration in shoots and roots and P absorbed by lentil plants in two growth seasons, 2018 and 2019

Genotypes	Season	Shoot P concentration (mg)	Root P concentration (mg)	P uptake (mg·plant ⁻¹)
'DJENDEL	2018	4.52±0.005 ^a	2.29±0.005 ^a	29.90±5.03ns
	2019	4.45±0.005 ^a	2.19±0.005 ^a	29.42±4.93ns
'IBLA 1	2018	5.72±0.0115 ^c	3.52±0.017 ^d	42.59±3.84ns
	2019	5.83±0.014 ^c	3.45±0.005 ^c	43.74±3.67ns
'LARGE BLONDE	2018	5.21±0.115 ^d	3.11±0.034 ^c	38.45±3.02ns
	2019	5.11±0.23 ^b	3.26±0.01 ^d	37.76±2.42ns
'LVS	2018	5.07±0.011 ^c	3.04±0.005 ^c	27.05±1.53ns
	2019	4.95±0.005 ^b	2.95±0.011 ^b	26.77±1.89ns
'METROPOLE	2018	4.91±0.005 ^b	2.93±0.011 ^b	29.18±7.87ns
	2019	5.15±0.011 ^b	3.16±0.005 ^c	30.58±8.12ns

Values represent the mean of three repetitions±SE (standard error); different letters denote homogeneous groups at $p < 0.05$

Relationship between nodulation and phosphorus content

Given that the higher phosphorus (P) content in nodules suggests its significant role in nodule functioning compared to shoot organs, the nodule P content was graphed against nodule biomass (Fig. 5). A positive correlation was observed between these two parameters for all genotypes over two consecutive growing periods. In addition, the correlation was highly significant for 'Djendel ($R^2=0.96$, $P<0.001$) and 'Large blonde ($R^2=0.93$, $P<0.001$) in the 2018 seasons compared to other genotypes. In fact, with every 1 mg increase in nodule P content, the nodulation of genotypes 'LVS, 'Ibla 1, and 'Metropole increased by 339, 210, and 156 mg nodule dry weight per plant, respectively, in the first season. In the second season, the increase was 148, 247, and 230 mg nodule dry weight per plant, respectively. In contrast, in the 2018 season, the genotypes 'Large blonde and 'Djendel showed an increase of 132 mg and 27 mg in nodule dry weight per plant, respectively, for every 1 mg increase in nodule phosphorus content. In the second season of 2019, the increase was 31 mg and 58 mg in nodule dry

weight per plant, respectively, for the same increase in phosphorus content.

DISCUSSION

Algeria has a long-standing tradition of cultivating lentils. This plant is a traditional legume crop that has consistently been utilized in human diets. Furthermore, lentil exhibits a notable tolerance for low maintenance, making it suitable for cultivating impoverished soils enhancing their nutrient content and organic composition. Lentils and legumes are typically more ecologically sustainable as they necessitate less utilization of natural resources [46]. The results indicate that the biomass and growth of nodules for genotypes 'LVS and 'Metropole were comparatively lower than those of 'Ibla 1 and 'Large blonde (Figs. 2 and 4). This is because these genotypes are highly sensitive to low soil P availability, which aligns with a previous finding that a P-deficiency reduces the biomass of nodules and their nitrogenase activity [20, 38], consequently decreasing the total N_2 fixation [27].

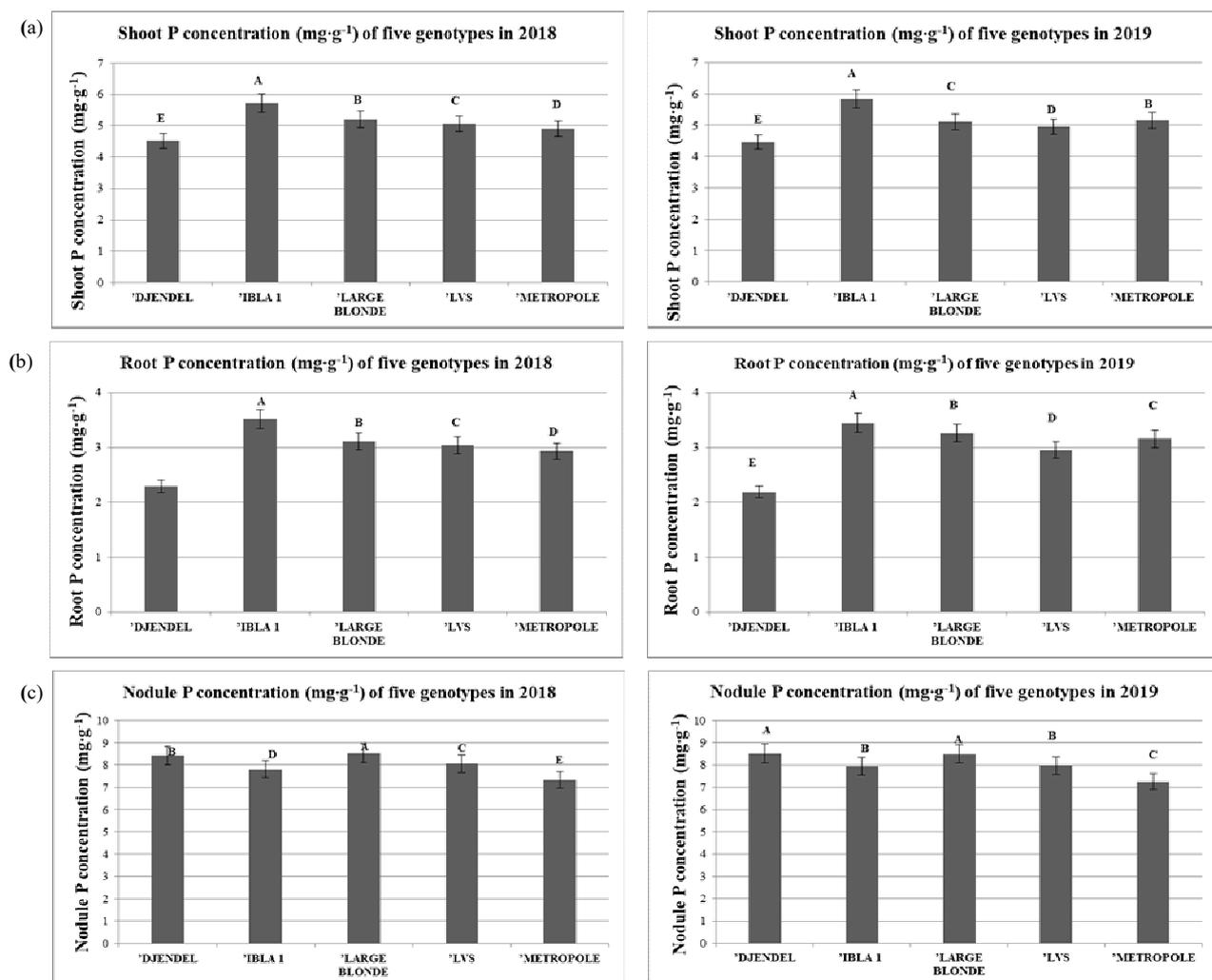


Figure 6. Shoot P concentration (a), Root P concentration (b) and nodule P concentration (c) of five genotypes of *lens culinaris* grown under P-deficient conditions during two growing seasons, 2018 and 2019. Data correspond to mean values \pm standard error as calculated with 3 plants sampled 100 \pm 3 d after sowing. Letters show significant differences between genotypes ($p<0.05$).

Our findings are also consistent with previous findings that P-deficiency may influence nodule development indirectly by limiting metabolite supply from the host plant in common bean [2, 12, 41], soybean [12, 41], and alfalfa [45]. Differences in the nodulation response to P deficiency appear to be related to legume species, genotype, rhizobial strain, and experimental conditions [10, 40]. Because nodules grow and fix N at the expense of roots [38, 44] and shoots during the vegetative stage, high nodule biomass was widely regarded as a trait for symbiotic efficiency. Legumes are important crops because they fix atmospheric nitrogen. This N₂ fixation process causes a number of rhizosphere-induced changes that contribute to soil P availability and interest in plant growth [1, 6, 22, 28].

The analysis of variance of the yield of five lentil accessions in this study revealed no significant differences between genotypes, demonstrating that the performance of all genotypes was not affected by different environmental conditions. The first season has slightly higher yields than the second season. During the same season, a slight difference in yield was

observed between genotypes, with 'Large blonde recording the highest yield and 'LVS recording the lowest for both seasons, which is explained by the difference in the amount of rain received during these seasons (565 mm in the first season and 482.8 mm in the second season). Water requirements for lentils in the semi-arid zone are estimated at 364-391 mm [24]. It is typically grown in areas with rainfall ranging from 300 to 450 mm. Racheed et al. (2010) [40] and Sadiq et al. (1998) [43] reported on the variable yield potential of different lentil genotypes. Lentil genotypes differ in root traits (root length and root-hair density), influencing plant nutrient uptake [16]. Genotypes with prolific root-hair formation are superior in nutrient uptake, resulting in greater growth and yield.

The correlation between lentil genotype nodulation and shoot biomass (Fig. 2) confirms similar earlier observations for common beans in farmers' fields in Lauragais [11], Montpellier [32] and Haouz, Morocco [6]. It suggests that nodules may be efficient enough to compensate for soil N deficiency by producing shoot biomass comparable to N-sufficient soil. Furthermore,

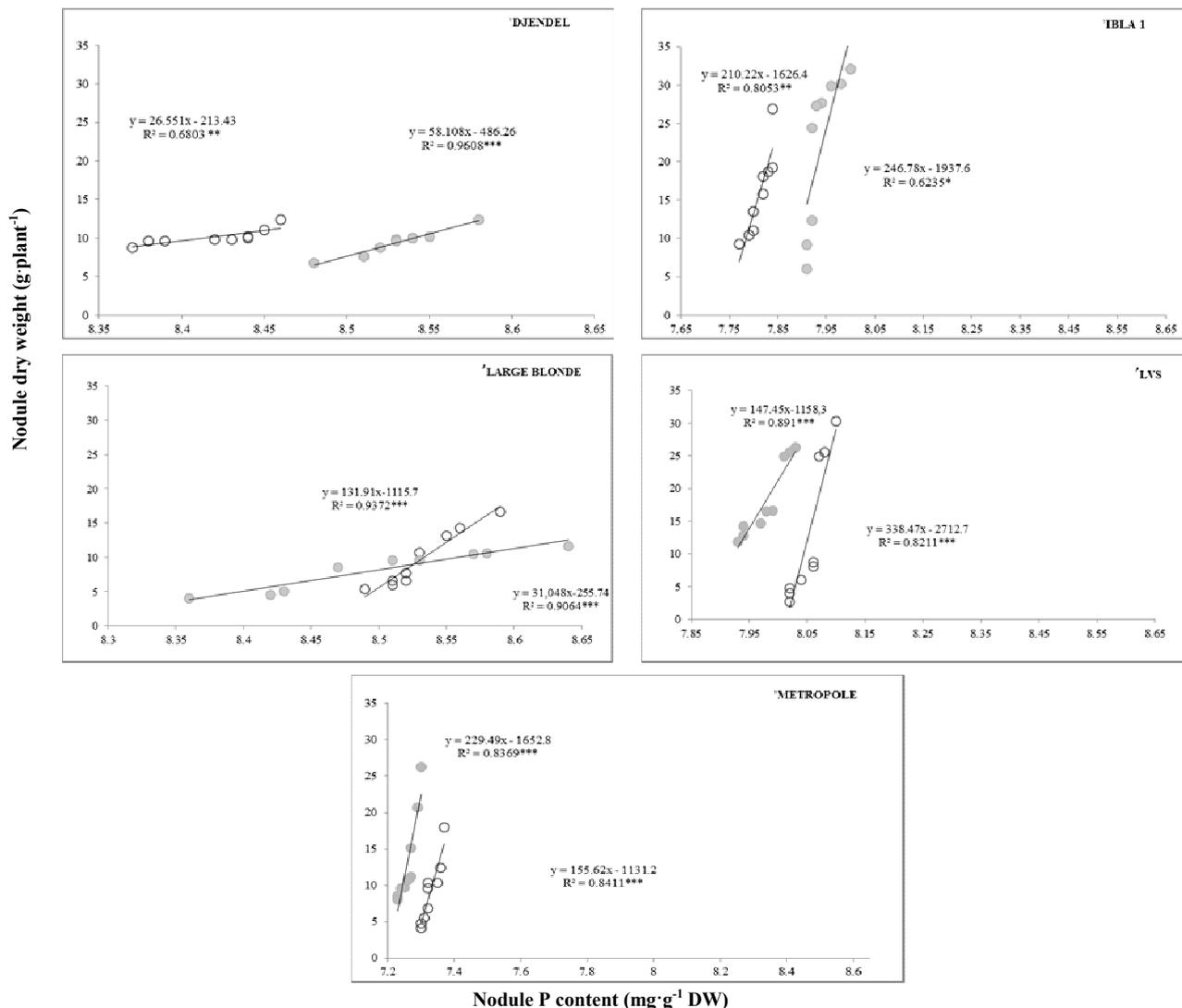


Figure 7. Relationship between nodule biomass and P content of five genotypes of lentil sowing in 2018 (open symbols) and 2019 (closed symbols), grown under low-soil P availability. Data are means of nine replicates per genotype, harvested at the flowering stage. *, **, *** indicate that the differences between means were significant at P<0.05; P<0.01 and P<=0.001, respectively.

the higher EUSR for 'Djendel and 'Large blonde compared to 'Metropole and 'LVS (Fig. 5) suggests that internal remobilization of acquired P via SNF ability may aid tolerant genotype in establishing higher shoot biomass under P deficient conditions. It distinguishes tolerant lines by combining a high individual nodule mass with a high EUSR. This finding agrees with the tolerance of nodulation to P-deficient conditions, which was attributed to lower P immobilization in nodules [45, 50] despite nodules having higher P concentrations than other parts, such as shoots.

The efficiency with which cropped legumes use rhizobial symbiosis is one of the most important biological indicators for monitoring environmental changes in the rhizosphere. Cropped legumes that rely on N₂ fixation generally help to increase P and N availability in the rhizosphere via rhizosphere acidification mechanisms [7, 9]. Indeed, the increased rhizobial symbiosis utilization efficiency in some genotypes under P-deficiency compared to others could be an adaptation involving mechanisms adjusting the plant's weight growth and nodular biomass [30]. The phosphorus concentrations in the shoot part differed significantly between genotypes ($p < 0.001$, Table 1, Fig. 6). During both seasons, the highest P content was recorded in 'Ibla 1, while the lowest P content was recorded in 'Djendel. The remaining genotypes had intermediate P levels in their shoots. The P content of lentil nodules varied significantly ($p < 0.001$, Table 1, Fig. 6) depending on the soil studied. The nodules of *Lens culinaris* plants accumulated more P than their corresponding shoots and roots. 'Large blonde had the highest concentration in the first season, followed by 'Djendel. In the second season, the opposite was observed with 'Djendel and 'Large blonde. In both seasons, 'Metropole had the lowest P content (Fig. 6C). This could be due to root nodules directly absorbing P from the soil solution. Our findings also show that P levels in shoots and nodules are higher in plants with high biomass [25]. This result confirms the beneficial effect of phosphorus on plant growth and nodulation. Similarly, Bargaz et al. (2012) [6] and Singh et al. (2016) [48] demonstrated that P accumulation was significantly and positively correlated with cultivated plant biomass.

The positive correlation between nodule biomass and P content reported (Fig. 4) suggests a tight regulation between N₂ fixation and nodule Pi requirement, most likely due to the high energy requirement of the SNF process. This statement appears to confirm the association between high P content in nodules and high N₂ fixation rates and nodule O₂ consumption [5, 34, 42]. Because high nodule P content induces an increase in nodule conductance to the O₂ diffusion, which is described as the main regulator for N₂ fixation [5, 34], high nodule P content may constitute an adaptive mechanism for P-deficiency tolerance. Thus, the rate of P allocation may be important in determining symbiotic efficiency and

the degree of legume adaptability under such adverse nutritional conditions. Furthermore, the results show significant genotype differences in nodular P demands and symbiotic N fixation (SNF) abilities. In response to P-deficiency, the 'Large blonde, 'Ibla 1, and 'Djendel genotypes were the most efficient in P use for SNF and recorded the highest yields compared to 'Metropole and 'LVS. The selection of P-efficient genotypes may help to improve the N₂-dependent growth of legumes in agro-ecosystems where P deficiency in the soil is a limiting factor of symbiotic N₂ fixation. As a result, improving legume P nutrition under P-deficient conditions requires using P-efficient genotypes and rhizobial strains with a high ability to grow and yield in P-deficient soil. Increased above-ground biomass and grain yield were associated with increased rhizobial symbiosis efficiency (indicating higher symbiosis efficiency), P availability, and soil resource use efficiency. More work is being done, and the results will be presented later.

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Conflict of interest. There is no actual or potential conflict of interest in relation to this article.

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