**Characterization and selection of some genotypes of lentil (***Lens culinaris***medik.) tolerant to phosphorus deficiency**

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Received \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_; Accepted \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_; Published \_\_\_\_\_\_\_\_\_\_

**Abstract**

Lentil are well recognized for their nutritional and health benefits as well as for their impact in the sustainability of agricultural systems, it is an excellent cultural precedent for other crops, capable of establishing a symbiotic relationship with rhizobia for nitrogen fixation. Low availability of phosphorus (P) in soil is a major constraint for symbiotic nitrogen fixation and productivity of legumes. This P-deficiency also affects multiplication of rhizobia in rhizosphere resulting in a reduction of the probability of infection and it also reduces growth of nodules. The main objective of this study is to see the degree of adaptation of lentil to P-deficiency in order to select the best genotypes grown taking into account growth rate, grain yield and the efficiency in use of rhizobial symbiosis. In this context, our work is focused on the study of behavior of some genotypes of lentil grown at Khemis Miliana area during two growing seasons 2018 and 2019. In terms of yield, the results illustrated show that the yield values are a little heterogeneous among genotypes, varies between 5 and 12 qha-1, specifically the genotypes Large blonde and Ibla1 recorded the highest yield during two growing seasons. In addition, efficiency in use of the rhizobial symbiosis of lentil was better for Djendel during 2018 season with (1.57gshoot DWg–1nodule DW) followed by Large blonde (0.2gshoot DWg–1nodule DW). It is concluded that the genotypes selected for their P use efficiency had a higher yield and could best adapt to low-P availability under field conditions.

**Keywords:** phosphorus, legumes, N2 fixation, rhizobia, symbiosis.

**Introduction**

Legumes are one of most important crops not only for their nutritional quality but also for their various agro-environmental benefits. Legume seeds and powders are important sources of protein, carbohydrates, vitamins, minerals and dietary fiber (Baljeet et al., 2014; Rachwa-Rosiak et al., 2015). They play a major environmental role in cropping systems, principally as a source of symbiotic N2 fixation that may save N fertilizer and also in weed and pest management in crop rotations (Graham, 2008). Phosphorus (P) may be a critical constraint for legumes under low nutrient environments because there is a substantial P requirement for the N2 fixation process (Schulze et al., 2006; Tsvetkova and Georgiev, 2007). This high P requirement of nodules increases the sensitivity of legumes to P-deficiency, a major limiting factor for legume production particularly in acidic or calcareous soils (Graham and Vance, 2003; Vance et al., 2003; Drevon et al., 2015). The application of P fertilizers is essential for improving crop yield (Gonzalez-Dugo et al., 2005; Li et al., 2007). However, P fertilization is generally little efficient for crops; i.e. only a few percent of the added P is usually taken up the same year, while the rest is stored in soil under organic, adsorbed and mineral forms (Li et al., 2008; Zhang et al., 2014; Gérard, 2016).

In response to P-deficiency, nodulated legumes implement two adaptive strategies (Araujo et al., 2008). The first strategy consists in developing a more efficient P acquisition system by increasing the root exchange surface (Hinsinger et al., 2018) or by secreting organic acids (Lazali and Bargaz, 2017) and phosphatases (Wang and Liu, 2018) whose role is to dissolve insoluble P from the soil. This strategy addresses the external P requirement of the plant, i.e. the minimum P concentration of the soil solution for optimal plant growth. The second strategy is to optimize the metabolic P use efficiency (PUE) for symbiotic N2 fixation (SNF) by producing more biomass or fixing more N2 per unit P consumed. It concerns the internal P requirement (Wang et al., 2018), i.e. the minimum P content of plant for optimal growth. Indeed, the selection of P deficiency tolerant symbioses could focus on the PUE for N2 fixation that seems specific to the interaction of the two symbioses (Wang et al., 2018).The exploration of genotypic variability in the responses of the lentil to P-deficiency may allow identification of physiological or biochemical tools for screening tolerant varieties and enable the productivity of this crop to be improved. Thus the aim of the present study was to improve our understanding of the role of P for SNF in developing strategies to improve the tolerance of nodulated lentil to low P-soils.

**Materials and methods**

**Experimental site study area**

The field experiment was conducted repeatedly over two seasons, 2017-18 and 2018-19. The experiment was conducted at the Demonstration Farm and Seed Production (FDPS) belonging to the station of the Technical Institute of Field Crops (ITGC) of Khemis Miliana. On a clayey-silt soil according to the USDA Duchaufour soil texture triangle (Duchaufour, 1970) and a previous wheat crop. The experimental site is located at the geographical coordinates 36°15'03''N 2°14'25''E at an altitude of 277 m. Khemis Miliana’s Mediterranean climate was characterized by a rather rainy and cold winter, dry and hot summer and a short spring (April-May) with different thermal amplitudes between winter and summer and between night and day. The region has irregular rainfall with an annual mean of 565 mm in 2018 and 482,8mm in 2019. The beginning of two-year experiment had low rainfall especially during September.

Physico-chemical properties of topsoil were determined in 2017. It was approximately 47% clay, 45% loam and 8% sand. The experimental soil was alkaline (pH 7); with1.81gkg−1CaCO3and low organic matter content (1.69 %). The soil was both N-deficient (Total N, 0.78 g N kg−1) and P-deficient (Olsen P, 9.52 mg Pkg−1).

**Cropping system and field plot design**

The field experiment was carried out using five lens cultivars (Djendel, Ibla1, Large blonde, LVS and Metropole). They were growing during two seasons 2018 and 2019 adopting a complete randomized block experimental design which covered an area of 122.4m2. With a density of 200 plants m-² for each genotype. The field trial was divided into three blocks, with each block further divided into five subplots. Each subplot was used for one of lentil genotypes and measuring 5 m x 1.2 m with a surface area of 6 m².

**Plant and soil sampling and measurements**

Before first sowing season, physico-chemical properties of soil were measured on five topsoil samples (0–20 cm) collected by auger. At the full flowering stage (100 days after sowing), we collected rhizosphere and bulk soil samples corresponding in order to perform measurements. This sampling was carried out from 25 to 30 cm depth. The rhizosphere was sampled in a standard manner; i.e. by brushing <1–4mm aggregates of soil adhering to lens roots gently using a paintbrush (Hinsinger, 2001; Latati et al., 2014). We bulked the three replicates of rhizosphere and corresponding bulk soil samples to each genotype.

Plants samples were also sampled at full flowering stage, when flowering reaches 50% of the elementary plot for each genotype, 9 plants were harvested and separated into shoots and roots, dried for 48 h at 65°C, and weighed. Nodules were separated from roots, dried and weighed separately. Shoot and roots were ground and digested in a microwave oven in nitric and perchloric acids at 135°C and 1.5 × 106 Pa. Biomasses data of shoots and nodules were used to assess the efficiency in use of the rhizobial symbiosis (EURS) for plant growth by correlation analysis in order to test whether cropping treatment over two growing season would affect the relationship between nodulation and shoot growth (Lazali et al., 2016).

Plant P uptake corresponded to the amount of nutrients taken up by plants during plant growth and were calculated as follows P concentrations in shoots and roots were measured using malachite green method as performed for total soil P. All soil and rhizosphere samples were dried, sieved (2 mm) and stored at 4°C for 72 h before analysis. We used Olsen method to assess P availability (Olsen et al. 1954) and pH was measured in soil suspension with deionized water (soil: water ratio = 1:2.5; Shen et al. 1996). The total soil P content was measured spectrophotometrically at 630 nm using malachite green method after soil digestion by perchloric and nitric acids (Valizadeh et al., 2003). Total N and organic matter contents were measured using the Kjeldahl (Kjeldahl, 1883) and Walkley–Black (Walkley and Black, 1934) methods, respectively.

Plant P uptake corresponded to the amount of nutrients taken up by plants during plant growth and were calculated as follows (Cavigelli and Thien, 2003).

P uptake (mg) = [P concentration in shoot (mg g-1) × shoot DW (g)] + [P concentration inroot (mgg-1) × root DW (g)]– seed P content(mg).

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

**Statistical analysis**

We performed a one-way ANOVA using genotype as factor. Plant biomass, P uptake, rhizosphere P availability, and grain yield were determined using analysis of variance (ANOVA) at a significance level of p = 0.05, and Tukey's test was used to determine if the difference between the mean values of each treatment was significant, we determined the efficiency in use of rhizobia symbiosis (EURS) by the slope of the linear regression between shoot dry weight (SDW) and nodule dry weight (NDW). All the statistics were performed with Statistica10 (V.8.5) software.

**Results**

**Plant growth and nodulation**

Figures (2, 3 and 4) show that three compartments of lentil biomass (shoots, roots and nodules) varied no significantly between genotypes. The largest shoot DW was found in genotypes Ibla1 and Large Blonde, during the first growing season 2018 with (7.33 g plant−1) and (7.14g plant-1) respectively followed by genotype Djendel (6,50 g plant-1). In 2019 season, Metropole and Ibla1 presented the highest shoot DW (7,20 g plant-1) and the lowest shoot DW was recorded for LVS genotype during two growing seasons

Root DW was always substantially higher in Large blonde and LVS genotypes whatever the growing season, and the lowest root DW were observed in Ibla1 genotype for the two seasons. Overall, the highest nodule dry DW was recorded by Ibla1 and LVS for both seasons and the lowest nodule DW was recorded for Large blonde for both seasons.

**Estimated grain yield**

The results illustrated in Fig. 1 show that the yield values are a little heterogeneous among genotypes, varies between 5 and 12 q.ha-1, specifically the varieties Large blonde and Ibla1 recorded the highest yield during two growing seasons: (12.35 q.ha-1 and 11,06 q.ha-1 in2017) and: (12,05q.ha-1 and 9,40q.ha-1in 2018) respectively. The variety LVS gave the lowest yield during both seasons with 5.44q.ha-1 and 5.93 q.ha-1.

**Efficiency in use of the rhizobial symbiosis (EURS)**

To assess whether the N2 fixation based on nodule biomass relative to the plant growth, varied among genotype of lentil, values of shoot biomass of each individual plant were plotted as a function of its nodule biomass. Biomass of shoots and nodules were significantly correlated and varied among lentil (Fig. 5). Efficiency in use of the rhizobial symbiosis estimates as the slope of the linear regression of the curves shown in (Fig. 5), No significant correlation was found in Metropole between NDW and SDW during two growing seasons. In contrast, these two plant variables were significantly correlated for rest. Was the highest for Djendel (1,57 g shoot dry weight g–1 nodule dry weight) during 2017 with (R²=0,809\*\*\*) and (1,07 g shoot dry weight g–1 nodule dry weight) during 2018 with (R²=0,788\*\*\*) followed by Large blonde (0,2 g shoot dry weight g–1 nodule dry weight)in 2017 and (0,87 g shoot dry weight g–1 nodule dry weight) in 2018 with (R²= 0,756\*\*\*) and (R²= 0,749\*\*\*) respectively, and the lowest for Metropole (0,03g shoot dry weight g–1 nodule dry weight) in 2017 and (0,19 g shoot dry weight g–1 nodule dry weight) in 2018.

**Phosphorus concentration and uptake**

Results showed in Table (1) and figure (6) indicate that P concentrations in shoot and root compartments of lentil varied significantly (p< 0.001) according to the different genotypes. The lowest P concentrations were found in shoots and roots of Djendel genotype during the two growing seasons (Table 1). Indeed, the highest P concentrations were found in plants of genotype Ibla1 in both seasons, followed by large blonde.

However, the lowest P uptake was found in LVS during two growing seasons with a value of 27.05 in the first season and 26.77 for the second season, and highest P uptake is noted in Ibla1 during two seasons (Table 1). Other genotypes exhibited intermediate values of P uptake.

**Relationship between nodulation and phosphorus content**

Since important role of P in nodule functioning is indicated by their higher P content compared with shoots organs, values of nodule P content were plotted as a function of nodule biomass (Fig. 5). A positive correlation was found between these two parameters for all genotypes during two growing seasons, but this correlation tended to be highly significant in Djendel (R2 = 0.96, P < 0.001) and Large blonde (R2 = 0.93, P < 0.001) in 2018 seasons compared to other genotypes. Indeed, for each increase of 1 mg nodule P content nodulation of genotypes LVS, Ibla1 and Metropole increased in first season by 339, 210, and 156 mg nodule DW per plant, respectively, and in second season by 148, 247 and 230 mg nodule DW per plant, respectively. By contrast, for each increase of 1 mg nodule P content nodulation of genotypes Large blonde and Djendel increased by 132 and 27 mg nodule DW per plant, respectively during the season 2017 and 31 and 58 mg nodule DW per plant, respectively in second season 2018.

**Discussion**

Lentil cultivation is traditional in Algeria. This plant is a classic grain legume that has always been used in human nutrition. Additionally to relatively low maintenance requirement, lentil is well adapted to poor soils which enriches with nutrients and organic matter. In general, lentil and legumes are more environmentally sustainable because they require fewer natural resources to grow (Sellami et al., 2021). In this study, nodule biomass and growth for genotypes LVS and Metropole were lower than those of Ibla1 and Large blonde (Fig. 2.4). This is due to the sensitivity of these genotypes to low soil-P availability, which is consistent with a previous conclusion that P-deficiency decreases the biomass of nodules as well as their nitrogenase activity (Qiao et al., 2007; Hernandez et al., 2007) and thereby the total N2 fixation (Kouas et al., 2005). Our results are also in line with previous findings that P-deficiency may indirectly affect nodule development by restricting metabolite supply from the host plant in common bean (Alkama et al., 2012), soybean (Ribet and Drevon, 1995; Drevon and Hartwig, 1997), and alfalfa (Schulze and Drevon, 2005). Differences in the response of nodulation to P-deficiency appear to be related to legume species, genotype, rhizobial strain, and experimental conditions (Rodiño et al., 2011; Drevon et al., 2015). In this study, the analysis of variance of the yield of 5 lentil accessions did not reveal any significant difference between these genotypes, proving that the performance of all genotypes was not affected by different environmental conditions~~.~~ The slightly higher yields in the first season (9.41qha-1) compared to the second (8.83qha-1) is explained by the difference in the quantity of rain received during these seasons (565 mm in the first season and 482,8 mm in the second season). In the semi-arid zone, water requirements of lentil are estimated at 364–391 mm (INRA, 2015). It is generally cultivated in areas with rainfall between 300 and 450 mm.

The significant correlation between nodulation and shoot biomass indicates that nodules were efficient (Fig. 5). This statement confirms previous observations in farmers’ fields of Lauragais in southern France (Drevon et al., 2011), Setif in Algeria (Lazali et al., 2016) and Mauguio Southeast of Montpellier, France (Lazali et al., 2017). High nodule biomass was generally considered as a trait for symbiosis efficiency because nodules grow and fix N at the expense of roots (Schulze et al., 1999; Qiao et al., 2007) and also at the expense of shoots during the vegetative stage (Qiao et al., 2007). Legumes are important crops as they are atmospheric nitrogen fixers. This process of N2 fixation induce a number of rhizosphere-induced changes that contributes to P-availability in the soil and interest in plant growth (Hinsinger et al., 2003; Alkama et al., 2009, 2012; Bargaz et al., 2012; Latati et al., 2016). Indeed, the higher efficiency of nodules of genotypes Djendel and Large blonde than Metropole and LVS (Fig. 5) suggests that internal remobilization of acquired P through SNF ability may help the lentil genotypes to establish higher shoot biomass under P-deficient conditions. This is in agreement with the tolerance of nodulation to P-deficient conditions that was attributed to a lower immobilization of P in nodule (Vadez et al., 1999; Schulze and Drevon, 2005).The efficiency in use of the rhizobial symbiosis is one of the most important biological indicators for monitoring environmental changes in the rhizosphere of cropped legumes, which rely on N2 fixation generally help to increase P and N availability in the rhizosphere through rhizosphere acidification mechanisms (Betencourt et al., 2012; Brooker et al., 2014). Indeed, the increase in rhizobial symbiosis utilization efficiency under P deficiency in some genotypes compared to others could be a kind of adaptation involving mechanisms adjusting the weight growth of the plant and its nodular biomass (Lazali, 2019).

Phosphorus concentrations in the shoot part showed significant variations between genotypes (p<0.001, Table 1, Fig. 6) of soil studied. This parameter varied from 4.45 to 5.83 mg of Pg-1DW. The highest content of P concentrations was recorded in Ibla 1 with a mean value of 9.65 mg Pg-1 DW while the lowest P content was recorded in Djendel with mean value of 5 mg Pg-1DW during both seasons. The rest of the genotypes showed intermediate shoots P levels. Nodules P content of lentil plants showed significant variations (p <0.001, Table1, Fig. 6) of soil studied. In general, the nodules of *Lens culinaris* plants accumulated more P compared to their corresponding shoots and roots parts. In the first season, Large blonde recorded the highest concentration with 8.53 mg Pg-1 DW followed by Djendel with 8.42mgPg-1 the opposite was observed in the second season, with Djendel recording 8.53 mg Pg-1 and Large blonde recording 8.50 mg Pg-1. Metropole showed the lowest P content in both seasons with mean value of 7.33 mg P.g-1 DW and 7.26 mg P.g-1 DW respectively, (Fig.6C). This could be due to the fact that root nodules could uptake P directly from soil solution. Our results have also shown that P contents in shoots and nodules are generally higher for plants with high biomass (Khadraji, 2020). This further proves the positive effect of phosphorus on the growth and nodulation of plants. Similarly, Bargaz et al. (2012) and Singh et al. (2016) showed that the accumulation of P was significantly and positively correlated with the biomass of cultivated plants.

In our study, the positive correlation between nodule biomass and P content (Fig. 7) suggests tight regulation between N2 fixation and the nodule P requirement. The high level of P in nodules may constitute an adaptive mechanism for P deficiency since high nodule P content induces an increase in nodule conductance to the O2 diffusion which is described as the main regulator for N2 fixation (Jebara et al., 2005; Bargaz et al., 2013; Lazali and Drevon, 2014). Thus, P allocation rate may play an important role in the determination of the symbiotic efficiency as well as the degree of legume adaptability under low-P conditions.

**Conclusion**

Our results showed significant differences between genotypes for yield, 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 contribute to the improvement of the N2– dependent growth of legumes in agro-ecosystems where P is deficient in the soil, which is a limiting factor of symbiotic N2 fixation. The use of P-efficient genotypes and rhizobial strains with a great ability to grow and yield in P-deficient soil is, therefore, an important goal for improving legume P nutrition under P-deficient conditions. Although the increased above-ground biomass and grain yield were associated with the stimulation of efficiency use of the rhizobial symbiosis (indicating higher symbiosis efficiency), P availability, and soil resource use efficiency.

**Acknowledgments**

The authors are grateful to the technical institute of field crop (ITGC) for the support we received from its experimental station (Khemis Miliana) and for giving us the opportunity to use the five lentil genotypes during this research, as well as hosting our experiment to achieve this study. We also acknowledge the master students of the University of Djilali Bounaama of Khemis Miliana who helped us, without which this study could not have been completed.

**Author Contributions**

Wassila BOUGHANEM: Experimental work (sowing in the field), explained the results, Statistical analysis, Writing of the original project, Revision of the writing and editing. Ibrahim BOUSSALHIH: Supervision, Revision and editing of the text. Mohamed LAZALI: Supervision, Revision and editing of the text.

**Conflict of Interest**

All authors declare no conflict of interest

**Data Availability**

Data presented in this study will be available on a fair request to the corresponding author

**Ethics Approval**

Not applicable to this paper

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**Table 1:** Phosphorus concentration in shoots and roots and P absorbed by lentil plants in two growth seasons 2017-2018 and 2018-2019.

|  |
| --- |
| Phosphorus concentration and uptake  |
| Genotypes | **Season** | **Shoot P concentration (mg)** | **Root P concentration (mg)** | **P uptake (mgplant-1)** |
| DJENDEL | 2018 | 4,52±0,005a | 2,29±0,005a | 29,90±5,03ns |
| 2019 | 4,45±0,005a | 2,19±0,005a | 29,42±4,93ns |
| IBLA 1 | 2018 | 5,72±0,0115e | 3,52±0,017d | 42,59±3,84ns |
| 2019 | 5,83±0,014c | 3,45±0,005e | 43,74±3,67ns |
| LARGE BLONDE | 2018 | 5,21±0,115d | 3,11±0,034c | 38,45±3,02ns |
| 2019 | 5,11±0,23b | 3,26±0,01d | 37,76±2,42ns |
| LVS | 2018 | 5,07±0,011c | 3,04±0,005c | 27,05±1,53ns |
| 2019 | 4,95±0,005b | 2,95±0,011b | 26,77±1,89ns |
| METROPOLE | 2018 | 4,91±0,005b | 2,93±0,011b | 29,18±7,87ns |
| 2019 | 5,15±0,011b | 3,16±0,005c | 30,58±8,12ns |
| Values represent the mean of three repetitions±SE (standard error), different letters denots homogeneous groups at p<0.05 |

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**Fig. 1**Grain yield (q.ha-1) of five genotypes of *lens culinaris* grown under P-deficient conditions during two growing seasons 2018 and 2019. Data are corresponding to mean values ± standard error of three replicates harvested at physiological maturity stage.

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

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

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

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**Shoot dry weight (g.plant-1)**



**Nodule dry weight (g.plant-1)**

**.palnt-1)**

**Fig. 5** Linear relationships between nodule dry weight (NDW) and shoot dry weight (SDW) found using 9 plants harvested 100 d after sowing in 2017-2018 (open symbols) and 2018-2019 (closed symbols). \*\* and \*\*\* denote p< 0.01 and p< 0.001, respectively.



**(**a)



**(**b)



**(**c)

**Fig. 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 are corresponding to mean values ± standard error as calculated with 03plants sampled 100±3 d after sowing. Letters show significant differences between genotypes (p< 0.05).

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**Nodule dry weight (g.plant-1)**

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**Nodule P content (mg.g-1 DW)**

**Fig. 7** Relationship between nodule biomass and P content of five genotypes of lentil sowing in 2017-2018 (open symbols) and 2018-2019 (closed symbols), grown under low-soil P availability. Data are means of nine replicates per genotype, harvested at flowering stage. \*, \*\*, \*\*\* indicate that the differences between means were significant at P < 0.05; P < 0.01 and P < 0.001, respectively.