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Biochemical and antioxidant proprieties associated with the adaptation of faba bean (*Vicia faba* L.)–rhizobia symbiosis to phosphorus deficit

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Abstract

Faba bean is an important legume species in Morocco due to its ability to fix N_2 in association with indigenous rhizobia. So, exploring this symbiosis to cope with abitic stresses such as phosphorus deficieny is of great importance. This study aims to assess the P deficit (50 µmol plant^{-1} week^{-1}) effects on growth, biochemical and antioxidant responses in six faba bean-rhizobia symbioses involving three Mediterranean varieties, *Alfia* (*AL*), *Reina Mora* (*RM*) and *Luz do Otoño* (*LO*), and two rhizobial strains *RhF16T* and *RhF5T* isolated from Haouz region of Morocco. The results showed that P deficit decreased growth and nodulation, increased acid phosphatase (APase), phytase, peroxidase (PO), polyphenoloxidase (PPO) activities and decreased malonyldialdehyde (MDA) content in the nodules. *AL-RhF16T* presented the lowest reductions in growth (11.3%), nodulation (34.4%), the highest APase, phytase, PO and PPO activities and the lowest MDA content under P deficit. In general, high enzymatic activities in the nodules may improve faba bean tolerance to P deficit, especially in the presence of rhizobial strains with high phosphate solubilization capacity. Based on our results, *AL-RhF16T* symbiotic combination was qualified as more tolerant to P deficit, while *LO-RhF5T* was qualified as less tolerant.

1. Introduction

Legumes are one of the most important families of Angiosperm. They owe much of their importance to their ability to fix atmospheric nitrogen in symbioses with soil bacteria known as "rhizobia" housed in root structures called nodules.. Besides, they have been important source of proteins, starch, oil, minerals, vitamins and health protecting compounds for direct human consumption [1]. Faba bean (*Vicia faba* L.) is currently the third most important winter food legume [2]. Despite its low productivity, it occupies the greatest area planted with legume crops in many countries [3]. Its low yield may be attributed to several factors including low and unpredictable rainfalls, acid soil conditions, salinity, low soil nutrient and low yielding cultivars [4]. Indeed, the soil P deficiency is one of the most significant abiotic factors limiting crop productivity [5]. It has been reported that only 0.1% of phosphorus exists in a soluble form available for plants absorption[6]. Thus, P deficiency is considered one of the most important factors limiting agricultural production in many countries around the world, especially in the Mediterranean area [7, 8].

Phosphorus is the second major macronutrient required for growth and plant development [9]. Its major role is the energy storage and release during cellular metabolism [10]. In addition, in legumes, symbiotic nitrogen fixation requires additional demands of P with up to 20% of total plant P being allocated to nodules [11] and any phosphorus deficiency may influence the activity of rhizobia and the efficiency of its symbiosis [12]. Indeed, several authors have shown that the nodules contain a high content of phosphorus compared to aerial parts [13], while other investigations revealed that P availability influences nodular and roots biomasses [14].

Efficient acquisition and utilization of phosphorus requires ubiquitous class of enzymes known as phosphatases. These enzymes are relatively non-specific. They play an important role in the production, transport and remobilization of available phosphorus (P_i)[15]. In fact, they catalyze the hydrolysis of phosphate esters and anhydrides of organic compounds releasing Pi near the roots. Studies revealed that most of acid

phosphatases are released from bacterial origin [16]. In the same sense, Raboy [17] mentioned that the phytic acid, hydrolyzed by phytase enzyme (a family of highly specific phosphatase) constitutes a major form of phosphorus reserve. Moreover, Kouas, Alkama [18] and Mandri, Drevon [19] noted a significant increase in phytase and acid phosphatase activity in bean's nodules. Yet, Araújo, Plassard [20] suggest that these enzymatic activities are an adaptive mechanism for more tolerant N_2 fixing legumes to P deficit. Indeed, the presence of tolerant rhizobia strains has been reported to enhance the tolerance of legumes to abiotic stresses such as salinity and drought, especially when associated with more tolerant genotypes of these species [21]. Furthermore, the phosphorus deficiency is evidently associated with the increase of oxidative stress due to the accumulation of ROS (reactive oxygen species), particularly O^{2-} and H_2O_2 in chloroplasts, mitochondria, and peroxisomes [22]. As a result, plants have developed several defense strategies for ROS detoxification in their tissues under stress [23]. The induction of antioxidant enzymatic activities and antioxidant compounds are general adaptation strategy, which plants use to overcome oxidative stresses [23]. Juszczuk, Malusà [24] noted a decrease in lipid peroxidation with an increase of catalase and peroxidase activities in bean's roots subjected to P deficiency. Mouradi, Bouizgaren [25] and Mouradi, Bouizgaren [26] reported that tolerant alfalfa genotypes presented high GPO and CAT levels under water deficit. Moreover, several works have reported that the ROS may inactivate nitrogenase activity in nodules [27].

Therefore, the aim of this study was to evaluate the effect of P deficiency on faba bean-rhizobia symbiosis. Six symbiotic combinations involving three Mediterranean faba bean genotypes and two rhizobial strains isolated from Moroccan soil of Haouz region with P sub-deficiency were tested. The analysis was focused on several agro-physiological and biochemical parameters related to P deficiency tolerance. The plant growth, nodulation, acid phosphatase and phytase activities were assessed. The lipid peroxidation and the role of antioxidant enzymes in P deficiency tolerance were also studied.

2. Material and Methods

2.1. Plant material and experimental conditions

Three faba bean (*Vicia faba* L.) genotypes *Alfia* (*AL*), *Luz do Otoño* (*LO*) and *Reina Mora* (*RM*) were used in the present study. These varieties were selected for their frequent use in local fields. The experiments were conducted in greenhouse conditions at the Faculty of Sciences and Techniques, Marrakesh, Morocco. Seeds were surface sterilized with 5% sodium hypochlorite for 5 min, rinsed several times with sterile deionized water and germinated at 25°C and complete darkness for five days on sterile sand. After germination, young seedlings with the same size and shape were transferred to pots of 12 cm tall and 16 cm diameter. Each pot was filled with sterile sand and peat with the proportion of 5:1 respectively. The plants were then separately inoculated with 10 mL of *RhF16T* and *RhF5T* rhizobia suspensions. Each inoculum was prepared by growing the bacteria in liquid yeast extract mannitol (YEM) medium at 28°C for 72h and contained approximately 10^9 CFU mL^{-1}(CFU=Colony-forming unit). These strains were isolated from nodules of faba bean grown in Haouz region of Morocco. The strains were previously subjected to infectivity test and evaluated for their inorganic phosphate solubilization ability. For stress application, plants were watered with two phosphorus nutrient solutions, 50 and 250 µM of P $plant^{-1}$ $week^{-1}$ as deficient and sufficient supplies provided as KH_2PO_4 respectively. The composition of the nutrient solution was as follows: $MgSO_4$ (100 µM), K_2SO_4 (750 µM), $CaCl_2$ (1650 µM), sequestrene (16 µM), $MnSO_4$ (6 µM), H_3BO_3 (4 µM), $ZnSO_4$ (1 µM), $NaMoO_4$ (0.1 µM) and $CuSO_4$ (1 µM). Five pots containing three plants each were used per symbiotic combination per treatment. The experiment was replicated three times. At flowering stage, plants were harvested, and several physiological and biochemical analysis were evaluated.

2.2. Acid phosphatase and phytase activities

Samples of fresh nodules (50 mg) were ground in mortar using 500 µL of sodium acetate extraction buffer (0.1 M, pH 5.5), 2.5% polyvinylpyrrolidone (PVP) and 5 µL of β mercaptoethanol under 0ºC. The homogenates were centrifuged at 12,000×g for 30 min at 4 ºC. The supernatants were collected and used for the determination of acid phosphatase and phytase activities [19, 20]. For acid phosphatase activity, 100 µL of enzymatic extract were mixed with 200 µL of p-NPP (p-nitrophenyl phosphate) and incubated for 30 min at 37ºC. The reaction was stopped by adding 1 mL of NaOH 1N. The activity was measured by a spectrophotometer at 410 nm and expressed as µmol of pNP (p-nitrophenyl) per min per g of fresh nodules. The phytase activity was determined by mixing 200 µL of 10 mM phytic acid (Sodium hexaphosphate inositol with 9 moles of H_2O per mole-Sigma P 8810) with 100 µL of nodule enzymatic extract. The reaction was maintained at 37ºC and stopped after 90 min by adding 1 mL of 10% TCA (Trichloroacetic acid). For each sample, a control was prepared by adding immediately 1 mL of 10% TCA to the reaction medium containing phytic acid. The reaction media were centrifuged at 12,000×g during 5 min. The concentration of Pi of the extract was calorimetrically determined as

described by Huang and Zhang [28]andMandri, Drevon [19]. The phytase activity was defined as the difference between the Pi in the extract and its corresponding blank and expressed in µmol of Pi min^{-1} g^{-1} FM.

2.3. Guaiacol peroxidase (GPO) and polyphenol oxidase (PPO) activities

Fresh nodules (100 mg) were crushed in 1 mL of phosphate buffer (20 mM, pH 7) using a mortar at 0°C. The homogenate was centrifuged at 15,000×g for 20 min at 4°C. The supernatant was used for the determination of the GPO (EC 1.11.1.7) and PPO (EC 1.14.18.1) activities according to the technique described by Hori, Wada [29]. For GPO activity, the reaction medium contained 2 mL of 0.1M phosphate buffer (pH 7) 5% of PVP; 200 µL of 0.3% H_2O_2, 300 µL of 20 mM guaiacol, 1 mL of distilled water and 100 µL of enzymatic extract. After 3 min, the GPO activity was determined at 470 nm against a blank, where the enzymatic extract was replaced by distilled water. The activity of GPO was presented as µmol of gaïacol^{-1} min^{-1}mg g^{-1} FM. For the PPO activity, the reaction medium contained 500 µL of 1.6% catechol prepared in 0.1M phosphate buffer (pH 6) and 200 µL of 0.1M phosphate buffer (pH 7). After 3 min of incubation in ambient temperature (25°C), the enzyme activity was measured at 410 nm using a spectrophotometer. The PPO activity was presented as µmol of catechol^{-1} min^{-1} g^{-1} FM.

2.4. Malonyldialdehyde (MDA) content

The malonyldialdehyde (MDA) content was determined as described by Zhang and Kirkham [30]. Fresh nodules (100 mg) were crushed in 1.5 mL of 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was then centrifuged at 10,000×g for 10 min. The supernatant obtained (1 mL) was added with 1 mL of 20% (w/v) TCA containing 0.5% (w/v) of thiobarbituric acid (TBA). The mixture was then boiled for 30 min. The reaction was stopped by a bath of ice and followed by centrifugation at 10,000×g for 10 min. The absorbance of the supernatant was determined at 532 nm. The non-specific absorbance was measured at 600 nm. The MDA contents were calculated using the molecular extinction coefficient of the complex MDA-TBA: 155 mM^{-1} cm^{-1}. The results were expressed as mg g^{-1} FM.

2.5.Statistical analysis

Statistical analysis was performed using SPSS (21.0) software. It concerned the two-way analysis of variance (ANOVA II). The experimental layout was a completely randomized design. Means comparison was performed using Student–Newman–Keuls (SNK) test. Six plants per symbiotic combinations per treatment were used as repetition and grouped as three replicates. The means and calculated standard errors were reported.

3. Results

3.1.Dry biomass

The P deficiency (50 µM) significantly ($P< 0.001$) decreased dry biomass in all of the studied faba bean-rhizobia symbioses, with no significant ($P>0.05$) variation between them according to SNK test (Table 1). The combinations *AL-RhF16T* and *RM-RhF16T* presented the least TDW reductions of 11.4% and 23.3% respectively under stress. For the SDW, the reductions were 11.3% and 17.9% respectively under the same conditions of stress. The most severe TDW and SDW reductions of 74.9% and 66.3% were presented by *AL-RhF5T* respectively under stress. Like shoots, the latest presented the highest RDW reduction of 85.7%, while *AL-RhF16T* presented the lowest reduction of 50.2% under stress (Table 2). According to our results, P deficiency significantly ($P<0.001$) decreased the nodulation in all of the tested symbiotic combinations with significant ($P<0.001$) variation between them (Table 2). *AL-RhF16T* presented the less important reduction of 34.4% followed by *RM-RhF16T* with 34.6% under P deficit. Meanwhile, *AL-RhF5T* and *LO-RhF16T* presented the highest reductions of 74.3% and 60.6% respectively.

3.2.APase and phytase activities

As response to P deficiency, the nodules APase activity was significantly increased ($P<0.001$) in all of the tested combinations with significant ($P<0.05$) variation between them according to SNK method (Figure 1). The highest activity was recorded in *AL-RhF16T* and *RM-RhF16T* reaching 220.6±4.2 and 218.5±8.7 µmol pNP min^{-1} g^{-1} FM respectively under stress in comparison with their respective controls. Meanwhile, LO-RhF5T presented the lowest APase activity of 84±7.2 µmol pNP min^{-1} g^{-1} FM among all of the tested symbioses. The phytase activity was also significantly ($P<0.001$) increased in almost all of the tested symbiotic combinations (Figure2). The behavior of each combination was significantly ($P<0.05$) different according to SNK test. The highest increases were presented by *AL-RhF16T* and *RM-RhF16T* with phytase activities of 24.2±0.9 and 13.8±0.61 µmol P_i min^{-1} g^{-1} FM respectively under stress conditions. *LO-RhF5T* presented the lowest activity of 1.65±0.14 µmol P_i min^{-1} g^{-1} FM under stress.

Table 1: Effect of P deficit (50 µM plant^{-1} week^{-1}) on the total (TDW) and shoot dry weight (SDW) in the six faba bean-rhizobia symbioses involving (*Alfia*, *Luz do Otoño* and *Reina Mora*) and (*RhF16T* and *RhF5T*) rhizobia strains. Values are means of three replicates. Two ways ANOVA results are also presented in the table.

Symbiotic combinations	TDW (mg plant^{-1})			SDW (mg plant^{-1})		
	Treatment (µM P plant^{-1})		Reduction (%)	Treatment (µM P plant^{-1})		Reduction (%)
	+P (250)	-P (50)		+P (250)	-P (50)	
AL-RhF16T	4.21d	3.73e	*11.4*	2.65bc	2.35cd	*11.3*
AL-RhF5T	6.11b	1.53g	*74.9*	2.65bc	0.90g	*66.3*
LO-RhF16T	4.94c	2.59f	*47.5*	3.43a	1.73e	*49.6*
LO-RhF5T	4.96c	1.61g	*67.5*	2.67bc	1.28f	*52*
RM-RhF16T	5.86b	4.49e	*23.3*	3.23a	2.65bc	*17.9*
RM-RhF5T	6.67a	3.49c	*47.6*	2.78b	2.21d	*20.5*
	df	F	Significance	df	F	Significance
Symbiosis	2	1.7	*NS*	2	2.8	*NS*
Stress	1	754.3	***	1	362.4	***
Interaction	2	25.2	***	2	24.6	***

Notes: *: Significance at 0.05 probability level; **: Significance at 0.01 probability level; ***: Significance at 0.001 probability level; NS: Not significant at 0.05. Letters are significance at 0.05 probability using post hoc Tukey's tests. df: degree of freedom; F: Fisher value; -: No reduction.

Table 2: Effect of P deficit (50 µM plant^{-1} week^{-1}) on the root (RDW) and nodules dry weights (NDW) in the six faba bean-rhizobia symbioses involving (*Alfia*, *Luz do Otoño* and *Reina Mora*) and (*RhF16T* and *RhF5T*) rhizobia strains. Values are means of three replicates.Two ways ANOVA results are also presented in the table.

Symbiotic combinations	RDW (mg plant^{-1})			NDW (mg plant^{-1})		
	Treatment (µM P plant^{-1})		Reduction (%)	Treatment (µM P plant^{-1})		Reduction (%)
	+P (250)	-P (50)		+P (250)	-P (50)	
AL-RhF16T	1.41cd	1.28cd	*50.2*	0.151b	0.099c	*34.4*
AL-RhF5T	2.21b	0.31f	*85.7*	0.082c	0.021d	*74.3*
LO-RhF16T	1.28cd	0.77e	*39.5*	0.231a	0.091c	*60.6*
LO-RhF5T	3.40a	0.60ef	*82.3*	0.067c	0.036d	*46.2*
RM-RhF16T	2.48b	1.74c	*29.8*	0.150b	0.098c	*34.6*
RM-RhF5T	3.75a	1.20d	*68*	0.140b	0.087c	*37.8*
	df	F	Significance	df	F	Significance
Symbiosis	2	2.8	*NS*	2	25.7	***
Stress	1	388.6	***	1	129.6	***
Interaction	2	6.7	**	2	4.23	*

Notes: *: Significance at 0.05 probability level; **: Significance at 0.01 probability level; ***: Significance at 0.001 probability level; NS: Not significant at 0.05. Letters are significance at 0.05 probability using post hoc Tukey's tests. df: degree of freedom; F: Fisher value; -: No reduction.

3.3.Guaiacol peroxidase (GPO) and polyphenol oxidase (PPO) activities

Figure 3 indicates that the nodules GPO activity significantly (P<0.001) increased as response to P deficit. According to SNK test, the tested symbiotic combinations presented significant (P<0.05) variation in their behavior. Indeed, the highest GPO activity was mentioned by *AL-Rh16T* and *LO-Rh16T*, which reached 46.3±2.3 and 38.9±0.4 µmol guaiacol. min^{-1} g^{-1} FM in comparison with their respective controls.

Meanwhile, *RM-RhF5T* presented the lowest GPO activity of 24.9±1.67 µmol gaïacol.min^{-1} g^{-1} FM under P deficit. The PPO activity was also significantly (P<0.001) increased under P deficit in all of the studied combination with significant (P<0.05) variation between them (Figure 4). Important increases have been noted in *AL-RhF16T* and *RM-RhF16T*, which reached 6.5±0.27 µmol catechol min^{-1} g^{-1} FM under P deficit. Meanwhile, no significant variation has been noted for *AL-RhF5T* under stress in comparison with the respective control.

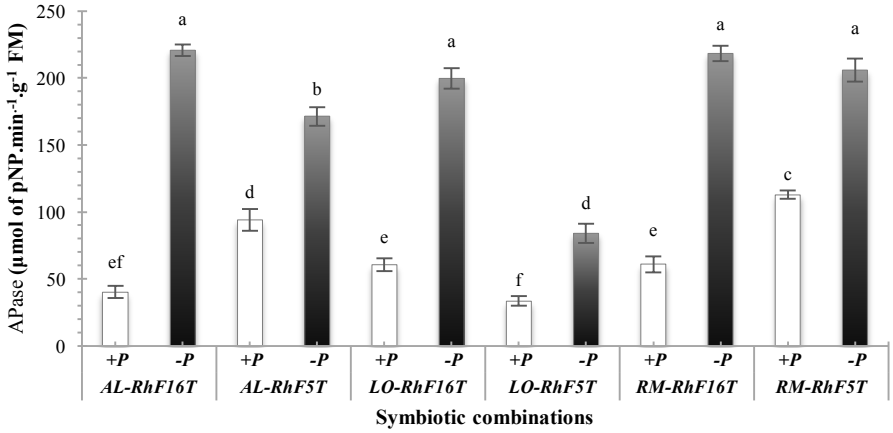

Figure 1: Effect of P deficit (50 µM plant^{-1} week^{-1}) on the nodules acid phosphatase (APase) activity in three faba bean genotypes (*AL*, *LO* and *RM*) inoculated with rhizobial strains *RhF16T* and *RhL5T*. Bars showing means ± standard errors and letters showing differences at 0.05 probability according to Tukey's test.

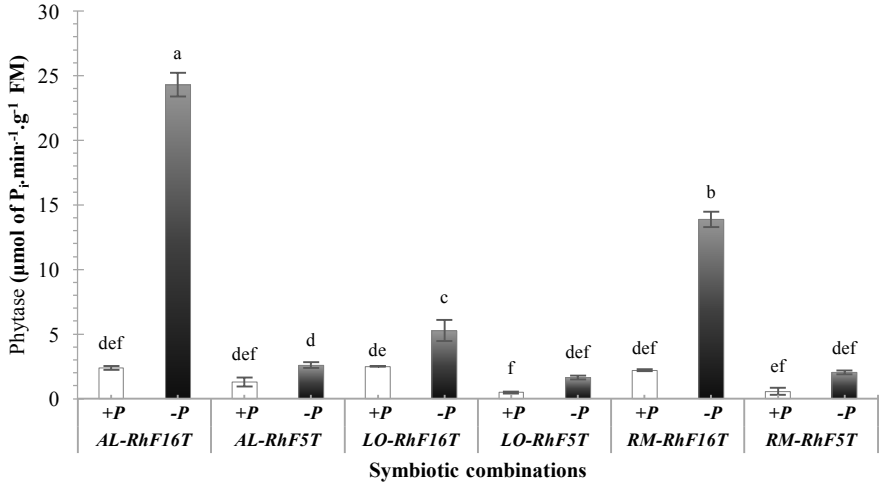

Figure 2: Effect of P deficit (50 µM plant^{-1} week^{-1}) on the nodules Phytase activity in three faba bean genotypes (*AL*, *LO* and *RM*) inoculated with rhizobial strains *RhF16T* and *RhL5T*. Bars showing means ± standard errors and letters showing differences at 0.05 probability according to Tukey's test.

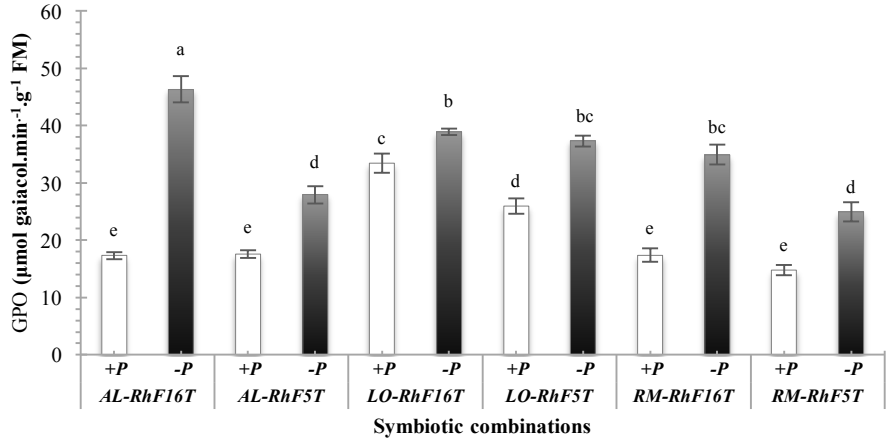

Figure 3: Effect of P deficit (50 µM plant^{-1} week^{-1}) on the nodules peroxidase (GPO) activity in three faba bean genotypes (*AL, LO* and *RM*) inoculated with rhizobial strains *RhF16T* and *RhL5T*. Bars showing means ± standard errors and letters showing differences at 0.05 probability according to Tukey's test .

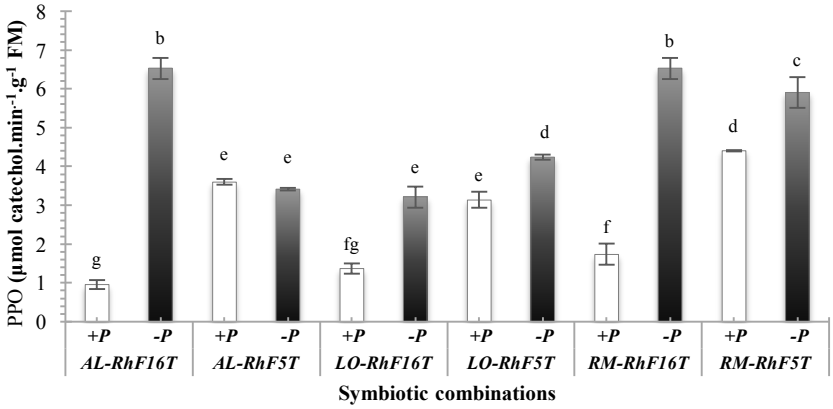

Figure 4: Effect of P deficit (50 µM plant^{-1} week^{-1}) on the nodules polyphenoloxidase (PPO) activity in three faba bean genotypes (*AL, LO* and *RM*) inoculated with rhizobial strains *RhF16T* and *RhL5T*. Bars showing means ± standard errors and letters showing differences at 0.05 probability according to Tukey's test.

3.4.Malonyldialdehyde (MDA) content

As reported in Figure 5, P deficiency significantly (P<0.001) increased MDA contents in all of the tested symbiotic combinations with significant variation in their behaviors in comparison with their respective controls. The lowest increases were presented by *RM-RhF16T* and *AL-RhF16T*. These two combinations presented the lowest MDA content of 0.57±0.02 mg g^{-1} FM under P deficit. Whereas *LO-RhF5T* presented the highest value of 1.34±0.03 mg g^{-1} FM under the same conditions.

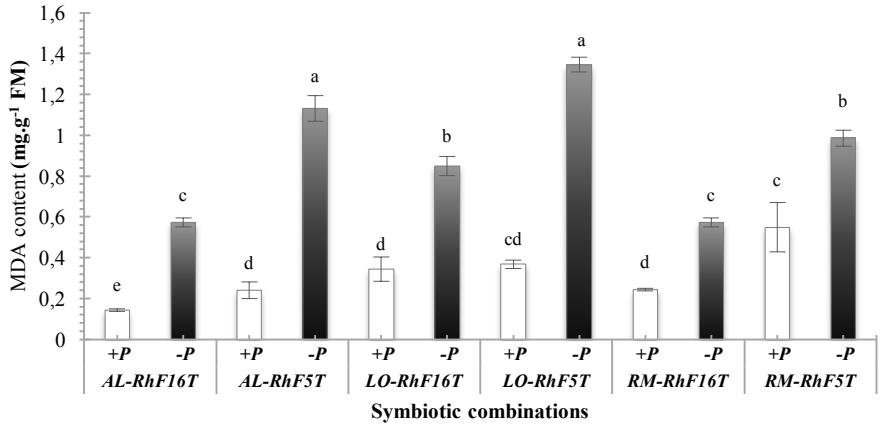

Figure 5: Effect of P deficit on the nodules malonyldialdehyde (MDA) content in three faba bean genotypes (*AL, LO* and *RM*) inoculated with rhizobial strains *RhF16T* and *RhL5T*. Bars showing means ± standard errors and letters showing differences at 0.05 probability according to Tukey's test.

4. Discussion

Phosphorus deficiency is one of the major problems for plants in the Mediterranean region. Many soils of Morocco suffer from P deficit circumstances that directly influence crop productivity. In fact, the aluminum and iron are major metals to rapidly chelate P and make it non-available for plants [31, 32]. Understanding the legumes N_2-fixing responses to P deficit is of great importance in improving faba been cultivation in these regions. In the present study, we evaluated the effects of P deficit (50 µmol plant^{-1}) on symbiotic interactions associating three faba bean varieties and two rhizobial strains isolated from Haouz region of Morocco. The results indicated that P deficit significantly (P<0.001) decreased growth traits in the tested symbioses. The decrease in the biomass could be due to low P acquisition, which is the major element for structural molecules, energy transformation and the regulation of enzyme activities. The low availability of this element imposes severe limitation of photosynthesis and therefore plants growth [33]. Significant variation between the studied symbioses has been noted according to SNK test. This led to the conclusion that the positive effects of the rhizobial strains are evident for the whole plant tolerance under this abiotic circumstance. Indeed, combinations involving *RhF16T* strains showed the lowest biomass reductions under P deficit (Table 1). It has been reported that rhizobial symbioses may contribute to the enhancement of legumes tolerance to several abiotic stresses [21,

34-37]. Neila, Adnane [38] reported that the inoculation with highly solubilizing strains enhances N_2-fixing in common been under P deficit. Legumes require phosphorus nutrition also for reassuring effective N_2-fixing, especially under difficult environmental conditions. P is fundamental element for nodules growth, formation and function [39]. In this study, rhizobial symbiosis with *RhF16T* enhances growth under stress. This may be related to their effective N_2 fixing ability manifested by the activation of several nodular biochemical traits. P deficit negatively ($P<0.001$) affected the nodulation. *AL-RhF16T* showed the lowest reduction. It has been proved that the shoot growth and N_2 fixation are mainly determined by the efficiency of absorption and use of P [40]. Similarly, Ribet and Drevon [41] showed that P deficit has a direct effect on the N_2 biological fixation of owing to the high ATP needs for nitrogenase activity in the nodules. Likewise, Divito and Sadras [42] also reported that nodules are highly sensitive to nutrient deficiency in comparison to shoots, with the hypothesis that plants grown under P-deficiency tend to reduce their nodule number rather than nodule mass, in this manner they facilitate oxygen diffusion into the nodule to assure effective N_2-fixing. In this study, the P deficit-responses of the studied symbioses were significantly different. This is in accordance with the results reported by Mandri, Drevon [19] for common bean and may be due to the specific and complex interaction between each rhizobial strain and the considered faba been genotype. It has been suggested that combining tolerant rhizobial strains with more tolerant plant genotypes may improve plants growth and persistence under stress [21, 35, 43, 44]. Under low P conditions, significant stimulation of APase activity has been noted in the nodules of faba bean. Mandri, Drevon [19] showed that nodules APase and phytase activities were significantly increased in the majority of the tested common bean-rhizobia combinations under P deficiency. Our result also agrees with those published by García, Olivera [45] for bean. In this way, *AL-RhF16T* presented the highest APase and phytase activity in comparison to the other combinations. Generally, the plants inoculated by the *RhF16T* showed high APase and phytase activity. This strain presented an important ability for tricalcium phosphate (TCP) solubilization when evaluated in liquid and solid NBRIP (national botanical research institute phosphate) media (data not shown). In fact, the APase and phytase may improve the availability of P required for the nodules function [19, 20]. In our study, strong positive correlation has been noted between the phytase activity and growth (r = 0.148**). This could be one of the main adaptation strategies developed by plants to maintain adequate level of P under stress. Several studies reported that the increase of phosphatase is highly correlated to the expression of phosphatase genes in response to stress [23]. In the present study, significant increases of the GPO and the PPO activities under P deficit have been noted in all of the tested combinations (Figure 3 and 4). These results agree with those reported by Bargaz, Faghire [23]. Several reports highlighted that the increase of antioxidant defense enzyme activities against the reactive oxygen species (ROS) in the nodules improve the plant stress tolerance [46]. Our results indicated that *AL-RhF16T* presented the highest GPO and PPO activities and the lowest MDA contents in comparison to other combinations. The increase in MDA content reflects the degree of cell membrane damage in the nodules under P-deficit and consequently proves the high occurrence of the oxidative stress in these organs. It was reported that the high membrane permeability is generally accompanied with enhanced lipid peroxidation as an evidence of oxidative damage [13, 47]. As a result, the induction of antioxidant enzyme activities is a general adaptation strategy, by which plants overcome the oxidative stresses [23, 26, 48].

Conclusion

In summary, faba bean varieties inoculated by *RhF16T* strains showed high APase, phytase, GPO, PPO activities and low MDA content in their nodules under P deficit especially *AL-RhF16T*. Therefore, maintaining better growth and nodulation compared to the other combinations may led to the conclusion that the symbioses relating *RhF16T*rhizobial strain and faba bean varieties showed more tolerance levels than the others. APase and phytase activities as well as the antioxidant enzymes revealed the responses of the more tolerant variety (*AL*) and the less tolerant one (*LO*). Therefore, according to the studied traits, P deficit tolerance may strongly related to the both partners the rhizobial strains and faba been genotype.

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