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Relationship between phosphorus deficiency tolerance and root/rhizosphere management in *Vicia faba* and *Vicia sativa*

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1 ABSTRACT

The present study aims to assess the response of *Vicia faba* (Broad bean) and *Vicia sativa* (Common vetch) to phosphorus (P) deficiency as well as the implication of root/rhizosphere processes on P deficiency tolerance. Seedlings of *Vicia sativa* and two Tunisian *Vicia faba* L. cultivars (Locale and Saber 2) were grown hydroponically under three P treatments: control (C); P deficiency (D) and induced P deficiency by bicarbonate (ID). Both cultivars showed higher tolerance to P deficiency compared to *V. sativa*. An intra-specific variability was also revealed between *V. faba* cultivars. The tolerant one (Locale) was characterized by an efficient root/rhizosphere processes, revealed in a stimulation of root biomass, phosphatase activity, rhizosphere acidification capacity and exudation of phenols and soluble sugars. As protein rich forage legume plant, the identification of *V. faba* cultivar tolerant to P deficiency should be an important result for forage production in calcareous soils.

2 INTRODUCTION

Phosphorus (P) is involved in several key plant functions, including plant energy transfer, photosynthesis and nutrient transport within the plant. Although abundant in soils, its bioavailability is restricted. As a consequence, P deficiency is affecting plant growth across the world, particularly in developing countries where the access to P fertilizers is onerous (Balemi and Negisho, 2012). To solve this nutritional problem, it is recommended to incorporate P-efficient plants that are able to mobilize P from soil, into cropping systems. Nutrient-efficient plants are plants with the ability to produce higher yields per nutrient applied or absorbed more than other plants grown in similar agro-ecological conditions (Fageria *et al.,* 2008). Selection of P-efficient plants with greater performance under phosphorus (P) deficiency can be described as an approach to improve production sustainability in P-deficient soils. Numerous studies investigated P deficiency responses in crop species (Kostadinova *et al.,* 2016). A previous study dealing with rice showed a significant difference in response to P deficiency stress among genotypes (Kakade *et al.,* 2017). Additionally, Leiser *et al.* (2015) indicated a large genetic variation among sorghum genotypes under low P disponibility. In response to low P bioavailability, leguminous crops can acquire P from soil by improving their P-use efficiency through a variety of adaptive strategies such as root architecture modifications, rhizosphere acidification or the release of phosphatases and phenolic compounds (Niu *et al.,* 2013; Rose *et al.,* 2013). Namayanja *et al.* (2014) showed genotypic differences in tolerance to phosphorus deficiency among common bean genotypes. Pang *et al.* (2010) reported a genotypic variation in herbaceous perennial legumes response to phosphorus supply. Zhou *et al.* (2016) assessed the genetic variation on P use efficiency among soybean genotypes in field and

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in hydroponic media. Their obtained results confirm the existence of a genetic variability concerning P use efficiency. In Tunisia, the Vicia genus has a considerable economic importance; it is characterized by a very wide diversity enumerated 16 annual spontaneous and sub-spontaneous species (El Bok *et al.*, 2014). Among them, Faba bean (*Vicia faba* L.) is the most cultivated leguminous species in Tunisia (Chaieb *et al.*, 2011) which covers more than 68% (70,000 ha) of the total area annually devoted to grain legumes crops (Rebaa *et al.*, 2017). Nevertheless, Kharrat and Ouchari (2011) showed that the average productivity of *V. faba* in Tunisia is 1.03 tons/ha, nearly 40% below the world average (1.7 tons/ha). The Vicia genus includes more than a dozen forage plants among them the most important is *Vicia sativa* (Common vetch). This specie is an important fodder or green manure. In recent years, the interest in this crop both in the Mediterranean region and in other areas with a Mediterranean climate is growing (Van De Wouw *et al.*, 2003). All over the world, including Tunisia, the calcareous soils are predominant and lead to a low P availability in soils, which leads to significant yield loss of *Vicia faba* and *Vicia sativa* production. The main objective of the current research study was to assess P deficiency responses and the implication of root/rhizosphere processes on phosphorus (P) deficiency tolerance in *Vicia faba* and *Vicia sativa*.

3 MATERIALS AND METHODS

3.1 Plant material and culture conditions: *Vicia sativa L.* (Common vetch, commercial variety) and two Tunisian *Vicia faba* L. var. minor cultivars (Locale and Saber 2) were used in this experiment. After imbibition with distilled H_2O, seeds were germinated for 6 days at 19°C in Petri dishes. Six-day-old seedlings were cultivated for 7 days in a half strength nutrient solution properly aerated for 7 days Vadez's *et al.* (1996). After that, a similar sized seedlings was selected which are cultured as groups of 8 plants in 5 l of full strength (Vadez *et al.*, 1996) modified nutrient solution, containing macronutrients: $MgSO_4$ (1 mM), KNO_3 (2 mM), K_2SO_4 (0.7 mM), $CaCl_2$ (1.65 mM) and micronutrients as a mixture of salts: $MnSO_4$ (6.6 µM), $CuSO_4$ (1.56 µM), $ZnSO_4$ (1.55 µM), $(Na)_2\ M_OO_4$ (0.12 µM), $COSO_4$(0.12 µM) and H_3BO_3 (4 µM). EDTA-K–Fe complex was added to the medium. Three treatments were established as follows: C = control (+ 360 µM KH_2PO_4), D = P deficiency treatment (+ 10 µM KH_2PO_4), and ID = induced P deficiency (+ 360 µM KH_2PO_4 + 0.5 g l^{-1} $CaCO_3$ + 10 mM $NaHCO_3$). The choice of P concentrations was based on a preliminary experiment showing that *V. sativa* and *V. faba* minor reached their optimal growth at 360 µM P. The pH was adjusted to 6.0 with HCl (0.1 N) for both (C) and (D) conditions and reached 8.2 for bicarbonate treatment (ID). The experiments were carried out in a growth chamber, under controlled conditions. The treatment lasts 27 days. The nutrient solution was continuously aerated and renewed every 5 days.

3.2 Pigment determination : Eight plants per treatment were used to determine leaf chlorophyll, anthocyanins and carotenoids concentrations ($mg.g^{-1}FW$) according to Lochtenthaler and Welburn (1983). Total anthocyanins concentrations were estimated according to Gould *et al.* (2000).

3.3 Chlorophyll fluorescence parameters: Values for maximum fluorescence (Fm) and initial fluorescence (F0) from the fluorescence induction curve were measured with a portable chlorophyll fluorometer (OS1-FL).

3.4 Evaluation of iron reducing power (FRAP) and lipid peroxidation (MDA): The reducing power was determined through the transformation of Fe^{3+} to Fe^{2+} induced by plant extracts (Ferreira *et al.,* 2007). Sample solutions at different concentrations were mixed with 2.5 ml of 0.2M phosphate buffer (pH 6.6) and 2.5 ml of potassium ferricyanide (1%, w/v). After incubation at 50°C for 20 min, 2.5 ml of (10%) trichloroacetate (TCA) was added and the mixture was centrifuged for 10 min at 1000 g. Then, 2.5 ml of the supernatant was mixed with the same volume of distilled water and 0.5 ml of ferric chloride (0.1%, w/v), and the absorbance was read at 700 nm against ascorbic acid as

authentic standard. Higher absorbance indicates greater reducing power. EC50 value (g ml^{-1}) is the effective concentration of the extract at which the absorbance was 0.5 and it was obtained from linear regression analysis. Lipid peroxidation (MDA) was determined using Cakmak and Horst (1991) method.

3.5 Enzyme assays: Extracts were prepared by homogenizing 200 mg of roots and leaves in a mortar with 10% (v/v) polyvinyl-polypyrrolidone and 1 ml of 50 mM phosphate buffer (pH=7,8) containing 0,1% (v/v) triton x-100, 1 mM PMSF as an inhibitor of protease. Then, the homogenate was centrifuged at 12 000 g for 30 min at 4°C. The supernatant was used to study superoxide dismutase (SOD, EC 1.15.1.1) and glutathione peroxidase (GPOX, EC 1.11.1.9) activities (Mhadhbi *et al.*, 2005). Protein content of each sample was measured based on the method described by Bradford (1976) using bovine serum albumin as standard.

3.6 Determination of phosphorous concentrations and acid phosphatase activity: Phosphorus concentration was assayed using the vanado-molybdate method (Fleury and Leclerc, 1943). For acid phosphatase (EC 3.1.3.2) activity determination, 200 mg of roots at 4°C was mixed in 100 mM Na-acetate buffer (pH 5.0), containing 6 mM β-mercaptoethanol, 0.1 mM phenylmethylsulphonylfluoride (PMSF) and 10% (w/w) polyvinylpyrrolidone (PVP). After centrifugation (12 000g for 30 min at 4°C) the supernatants were collected. The assay mixture contained 100 mM Na-acetate buffer (pH 4.8), 5 mM *p*-nitrophenylphosphate and the enzyme. The mixture was incubated at 30°C for 30 min. The reaction was finished by adding 0.1 M NaOH. Acid phosphatase activity was measured spectrophotometrically at 410 nm by monitoring the *p*-nitrophenol released (Talbi Zribi *et al.*, 2015). The activity of acid phosphatase excreted into solution was assayed after 24 h of root exudation, using p-nitrophenyl phosphate (pNPP) as a substrate (Talbi Zribi *et al.*, 2015).

3.7 Analysis of polyphenols, soluble sugars and nutrient solution acidification: Root extracts were obtained by magnetic stirring process during 30 min mixing 1 g of dry root powder with 10 ml pure methanol for 30 min (M'sehli *et al.*, 2008). Phenolic compounds was determined by using Folin–Ciocalteu reagent according to (M'sehli *et al.*, 2008). Total flavonoids were measured using a colorimetric assay according to Dewanto *et al.* (2002). Soluble sugars were quantified with the anthrone reagent according to Sairam *et al.* (2002). The nutrient solution acidification was determined by the follow-up of the medium pH during the treatment using a Radiometer PHM 84 pH meter. The capacity to extrude protons by roots was also determined by incubating eight plants per treatment for three hours in a KCl (10 mM)/-$CaCl_2$ (1 mM) solution initially adjusted to pH 6.2, then proton extrusion fluxes were measured by a return titration using a NaOH solution (1 mM).

3.8 Data and statistical analysis: The relative growth rates (RGR), Phosphorus acquisition efficiency (PAE) and Phosphorus use-efficiency (PUE) were calculated. The root surface area was determined on freshly harvested roots using the Image J software. Relative root elongation (RRE) was calculated following Watanabe and Okada (2005) method. Principal component analysis (PCA) of physiological and biochemical parameters of control and P-deficient plants was carried out. ANOVA two-way analysis was performed for the whole data using the STATI-CF statistical software. Means were compared using the Newman Keuls test at $p<0.05$ when significant differences were found.

4 RESULTS

4.1 Plant Growth and phosphorus status: The plant biomass was significantly reduced by P deficiency (D) and (ID) in both species. This depressive effect was less pronounced in *V. faba* cv Locale) (Table 1). Relative growth rate (RGR) decreased significantly with P deficiency only in *V. faba* cv Saber 2 and *V. sativa* (Table 1). Concerning root response to P deficiency, it was noticed that this nutritional stress led to an enhancement of the root RGR in *V. sativa* and in

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the two cultivars of *V. faba* (Table 1). The marked increase was observed in Locale (50% and 57% increase for D and ID treatments, respectively, as compared to the control (C)). In the same way, bicarbonate supply resulted in a higher root/shoot DW ratio values in Locale and Saber 2 cultivars reaching 1.5- and 1.3-fold increase, respectively (Table 1). Values related to root area were particularly increased in *V. faba* cv Locale submitted to P deficiency treatments (D and ID). Relative root elongation (RRE) results revealed that P deficiency treatment has no significant effect on principal root elongation in the both studied species (Table 1).

Table 1: Total dry weight, whole plant relative growth rates (RGR), root relative growth rates (RGR), root/shoot DW, root surface area (cm²/Plant) and relative root elongation (RRE) of *V. sativa* and *V. faba* cultivars grown on a control nutrient solution (C), under direct P deficiency (D) or under induced P deficiency (ID).

	V.faba cv Locale			**V.faba cv Saber 2**			**V.sativa**		
	C	D	ID	C	D	ID	C	D	ID
Total dry weight (mg)	1438±43a	1205±55b	1317±6ab	1216±32b	920±25d	1040±64c	937±7cd	546±52e	613±41e
Whole plant RGR (day-1)	0.102a	0.107a	0.107a	0.103a	0.091b	0.095b	0.087b	0.078d	0.081c
Root RGR (day-1)	0.038c	0.057a	0.06a	0.036c	0.042b	0.047b	0.038c	0.044b	0.05b
Root/shoot DW	0.4c	0.5b	0.6a	0.32e	0.37d	0.42c	0.3e	0.3e	0.3e
Root surface area	137±2.9c	167±4.7b	182±6.9a	132±1.6c	139±2c	171±1.9b	125±1.2d	130±1.8d	141±6.06c
RRE (%)	85±3a	83±2a	8 4±4a	84±3a	83±6a	84±2a	79±2a	77±7a	80±4a

Values followed by different letters are significantly different at $p<0.05$ according to Newman-Keuls test.

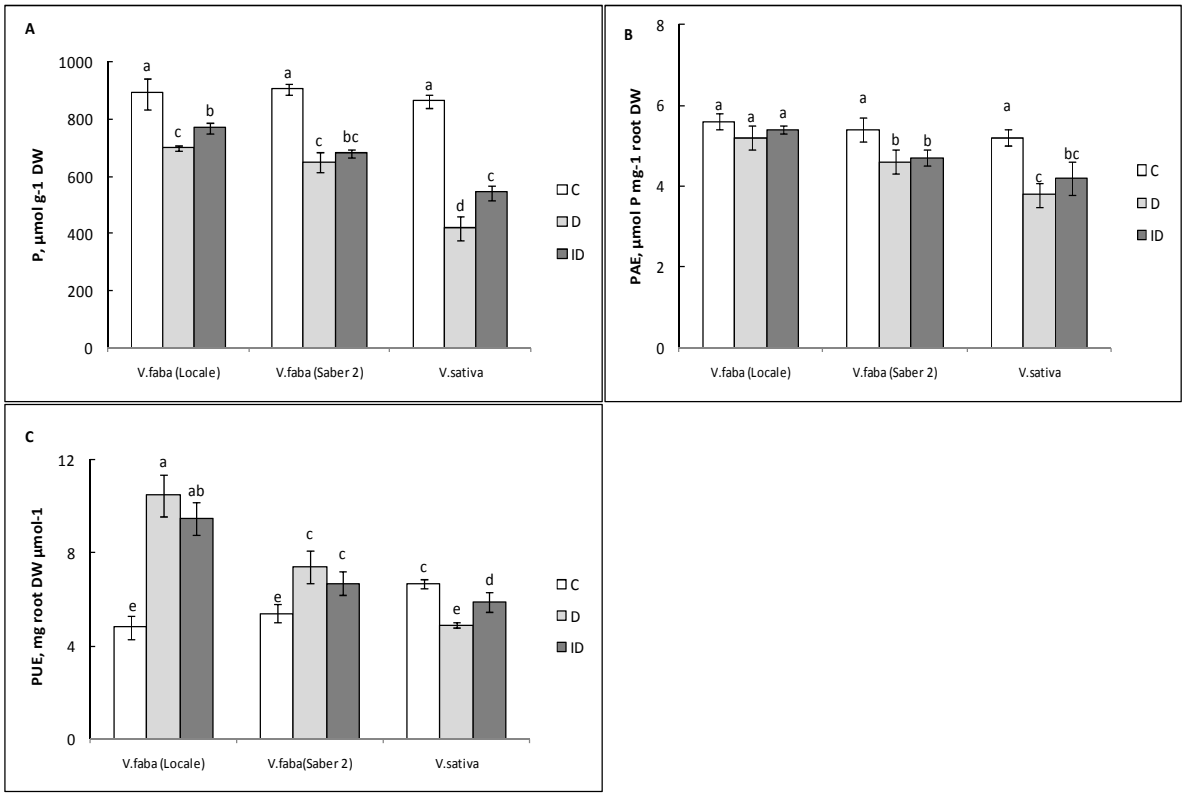

Figure 1: Whole plant P concentrations, P absorption (PAE) and use (PUE) of *V. sativa* and *V. faba* cultivars grown on a control nutrient solution(C), under direct P deficiency (D) or under induced P deficiency (ID). Values followed by different letters are significantly different at $p<0.05$ according to Newman-Keuls test.

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A significant decrease of phosphorus content was found in P deficient plants in both species, especially under (D) treatment. The greater decrease was recorded in *V. sativa* where the reduction rate reached 51% (Figure 1 A). The reduction of P concentrations in plants was accompanied with a significant decrease in phosphorus acquisition (PAE) only in *V. sativa* and in Saber 2, cultivar (Figure 1 B) against a significant increase in phosphorus use efficiency (PUE) mainly in Locale plants (Figure 1 C).

4.2 Pigment concentration and fluorescence parameters: Total Chl concentration was not modified by P deficiency treatment (D); however, it was decreased by the addition of bicarbonate in the nutrient solution (ID) in both species. The effect of bicarbonate was less pronounced in *V. faba* than in *V. sativa* (Table 2). A stimulation of carotenoids and anthocyanins concentrations by P deficiency was observed in *V. faba* cultivars. The highest values were recorded in cv Locale plants cultivated under ID conditions (Table 2). The addition of bicarbonate in the nutrient solution affected significantly Fv/Fm and Fv/F0 ratios. The highest decrease was observed in *V. sativa* (31 and 43% for Fv/Fm and Fv/F0, respectively).

4.3 Leaf membrane damages and antioxidant capacity: P deficiency induced an important increase of MDA content in leaves of both species. The increase was more marked in *V. sativa* (+ 62%) compared to *V. faba* (+ 26% and + 45% in cv Locale and cv Saber 2, respectively) under ID treatment (Table 2). P deprivation increases iron reducing power capacity (FRAP) in *V. faba* cultivars (+ 41% and + 29% in Locale and Saber 2, respectively) while no significant variation was noticed for *V. sativa* (Table 2). SOD and GPOX activities were higher in leaves of P-deficient plants. These activities were remarkably stimulated in *V. faba* than in *V. sativa* (Table 2).

Table 2: Chlorophyll (mg.g^{-1} FW), carotenoids (mg.g^{-1} FW), anthocyanins (µg.g^{-1} FW), chlorophyll fluorescence parameters, MDA concentration (µmol.g^{-1} FW), FRAP activity (µmol.g^{-1} FW), SOD (U SOD µg^{-1}.Prot.) and GPOX (µM H_2O_2 min^{-1}.mg^{-1}.Prot.) activities of *V. sativa* and *V. faba* cultivars grown on a control nutrient solution (C), under direct P deficiency (D) or under induced P deficiency (ID).

	***V.faba* cv Locale**			***V.faba* cv Saber 2**			***V.sativa***		
	C	**D**	**ID**	**C**	**D**	**ID**	**C**	**D**	**ID**
chlorophyll	0.8^{a}	0.78^{a}	0.73^{b}	0.8^{a}	0.81^{a}	0.71^{b}	0.81^{a}	0.79^{a}	0.59^{c}
carotenoids	2.5±0.1^{c}	3.8±0.2^{a}	3.9±0.1^{a}	2.3±0.3^{c}	3.2±0.1^{b}	3.6±0.2^{a}	2.7±0.1^{b}	2.9±0.1^{b}	2.9±0.2^{b}
anthocyanins	0.19±0.03^{c}	0.23±0.02^{b}	0.31±0.04^{a}	0.19±0.03^{c}	0.24±0.02^{b}	0.29±0.05^{a}	0.18±0.01^{c}	0.2±0.02^{c}	0.2±0.01^{c}
Fv/Fm	0.80±0.04^{a}	0.78±0.03^{a}	0.67±0.01^{b}	0.80±0.06^{a}	0.78±0.03^{a}	0.65±0.02^{b}	0.7±0.04^{a}	0.7±0.03^{a}	0.5±0.05^{c}
Fv/Fo	3.45±0.2^{c}	3.25±0.08^{c}	3.13±0.07^{c}	4.06±0.1^{a}	4.19±0.05^{b}	3.23±0.09^{c}	3.9±0.3^{a}	3.78±0.1^{d}	2.25±0.09^{e}
MDA	2.89±0.3^{d}	3.57±0.2^{c}	3.67±0.1^{c}	2.8±0.2^{d}	3.85±0.4^{bc}	4.06±0.2^{b}	2.7±0.1^{d}	4.2±0.3^{b}	4.46±0.2^{a}
FRAP activity	34.4±0.9^{c}	46.5±1.2^{a}	48.6±0.7^{a}	36.76±1.1^{c}	43.78±1.5^{b}	47.67±1.7^{a}	37.5±0.8^{c}	38.4±1.2^{c}	39.76±0.9^{c}
SOD activity	15.84±0.7^{c}	20.14±1^{b}	23.29±0.8^{a}	14.28±0.5^{d}	17.91±0.4^{c}	20.18±1.7^{b}	10.5±0.6^{e}	12.5±0.2^{d}	13.02±0.5^{d}
GPOX activity	0.45±0.01^{c}	0.57±0.03^{a}	0.55±0.03^{a}	0.49±0.02^{b}	0.54±0.04^{a}	0.53±0.02^{a}	0.4±0.01^{c}	0.46±0.02^{b}	0.45±0.05^{b}

Values followed by different letters are significantly different at $p<0.05$ according to Newman-Keuls test.

4.4 Root acidification capacity and acid phosphatase activities: Under P-deficiency conditions, plants exhibited an acidification of the medium. The decrease of pH values was more pronounced in Locale reaching the 4.05 pH unit (Table 3) through a higher levels of roots proton release (1,86 µmol h^{-1} g^{-1} Root FW) under D treatment (Table 3). Acid phosphatases activities in roots (Figure 2 A) or released into solution (Figure 2 B) were stimulated by D treatment in both species mainly in *V. faba* cv Locale. However, the induced P deficiency (ID) leads to a decrease of these activities in both species (Figure 2 A and B).

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Table 3: pH values of the culture media (pHi= pH initial; pHf= pH final) and proton extrusion by roots (PRR, µmol $h^{-1}g^{-1}$ Root FW) during the 4 weeks treatment of *V. sativa* and *V. faba* cultivars grown on a control nutrient solution (C), under direct P deficiency (D) or under induced P deficiency (ID).

Treatment		1 week		2 week		3 week		4 week	
	pH_i	pH_f	PRR	pH_f	PRR	pH_f	PRR	pH_f	PRR
V.faba cv Locale									
C	6	6.16 ± 0.1 b	0.7 ± 0.1 d	6.93 ± 0.1 b	0.9 ± 0.04 b	7.11 ± 0.2 b	0.8 ± 0.02 d	7.21 ± 0.5 b	0.7 ± 0.04 e
D	6	4.63 ± 0.08 c	1.78 ± 0.08 a	5.51 ± 0.3 c	1.4 ± 0.02 a	6.8 ± 0.5 b	0.97 ± 0.04 a	4.05 ± 0.3 d	1.86 ± 0.06 a
ID	8	8.09 ± 0.2 a	1.43 ± 0.02 b	7.96 ± 0.3 a	1.35 ± 0.02 a	8. 25 ± 0.1 a	0. 9 ± 0.01 b	7.96 ± 0.2 a	1.52 ± 0.07 b
V.faba cv Saber 2									
C	6	6.23 ± 0.3 b	0.69 ± 0.09 d	7.01 ± 0.2 b	0.8 ± 0.03 c	6.97 ± 0.4 b	0.7 ± 0.05 d	6.25 ± 0.4 bc	0.65 ± 0.01 e
D	6	4.86 ± 0.4 c	1.11 ± 0.04 c	5.9 ± 0.5 b	0.9 ± 0.01 b	7.25 ± 0.1 b	0.8 ± 0.03 c	5.53 ± 0.1 c	1.27 ± 0.03 c
ID	8	8.25 ± 0.5 a	0.91 ± 0.07 cd	7.88 ± 0.2 a	0.85 ± 0.03 b	8.07 ± 0.3 a	0.8 ± 0.01 b	8.06 ± 0.3 a	1.07 ± 0.05 d
V.sativa									
C	6	6.2 ± 0.5 b	0.71 ± 0.03 d	7.13 ± 0.1 b	0.67 ± 0.03 d	7.3 ± 0.4 b	0.73 ± 0.03 d	7.21 ± 0.6 b	0.75 ± 0.02 e
D	6	5.8 ± 0.4 b	0.97 ± 0.04 cd	7.11 ± 0.1 b	0.85 ± 0.04 c	7.14 ± 0.2 b	0.81 ± 0.04 c	5.9 ± 0.2 c	1.03 ± 0.03 d
ID	8	8.1 ± 0.1 a	0.99 ± 0.01 cd	7.8 ± 0.5 a	0.78 ± 0.01 c	8.2 ± 0.5 a	0.79 ± 0.02 c	8 ± 0.3 a	1.07 ± 0.04 d

Values followed by different letters are significantly different at $p<0.05$ according to Newman-Keuls test.

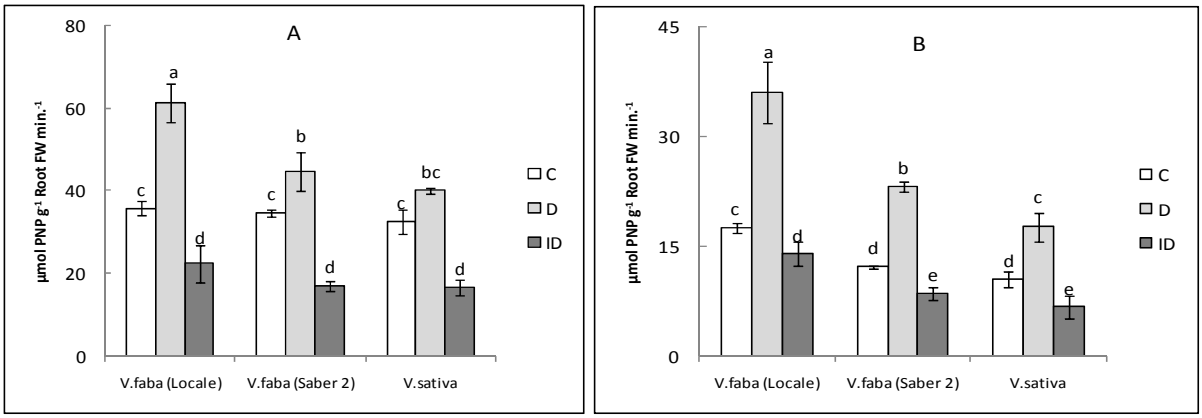
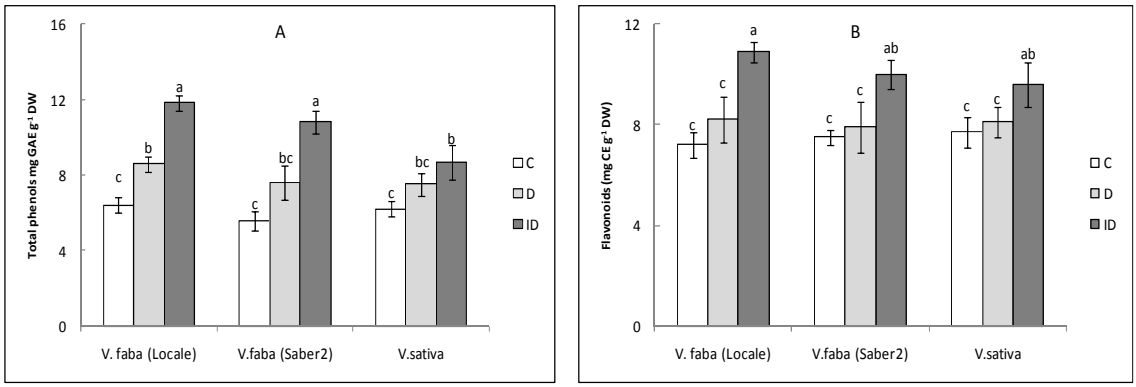


Figure 2: Acid phosphatase activity in roots (A) and as released into solution (B) of *V. sativa* and *V. faba* cultivars grown on a control nutrient solution(C), under direct P deficiency (D) or under induced P deficiency (ID). Values followed by different letters are significantly different at $p<0.05$ according to Newman-Keuls test.



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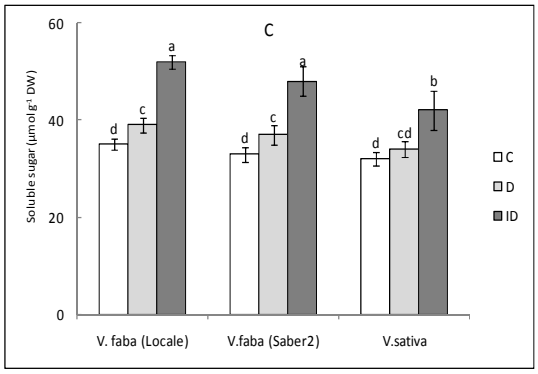
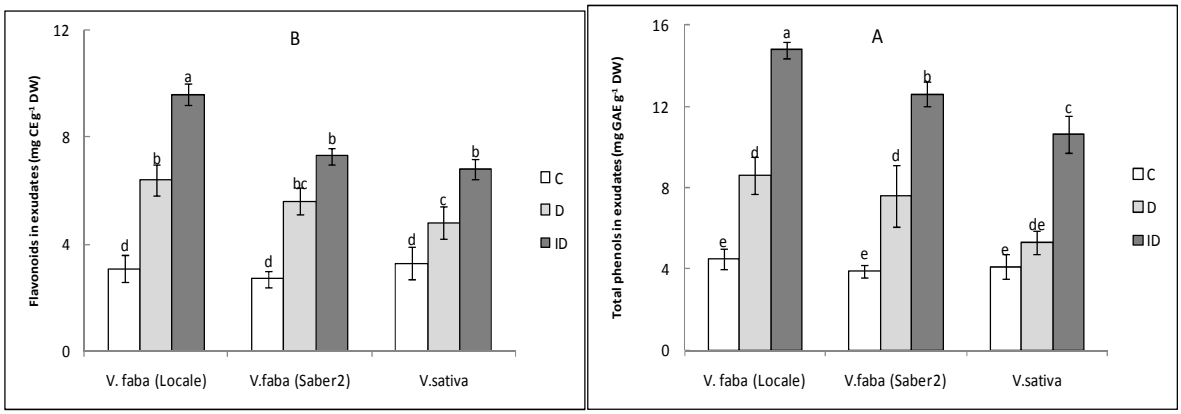


Figure 3: Root phenols (A), flavonoids (B) and soluble sugars (C) contents of *V. sativa* and *V. faba* cultivars grown on a control nutrient solution(C), under direct P deficiency (D) or under induced P deficiency (ID). Values followed by different letters are significantly different at $p<0.05$ according to Newman-Keuls test.



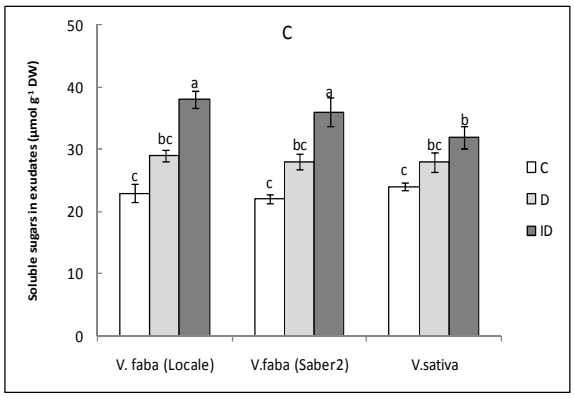


Figure 4: Amounts of phenols (A), flavonoids (B) and soluble sugars (C) in root exudates of *V. sativa* and *V. faba* cultivars grown on a control nutrient solution(C), under direct P deficiency (D) or under induced P deficiency (ID). Values followed by different letters are significantly different at $p<0.05$ according to Newman-Keuls test.

4.5 Soluble sugar and phenol concentrations in roots and exudates: Larger concentrations of phenols (polyphenols and flavonoids) and soluble sugars were observed in both P-deficient roots (Figure 3) and in root exudates (Figure 4), especially in the presence of

bicarbonate in the nutrient solution. It should be noticed that the observed increase was greater for Locale cultivar (Figure 3 and 4).

4.6 PCA analysis: The PCA of the physiological and biochemical parameters in the studied plants showed clearly a separation between control (T1) and P-deficient plants (T2 and T3). The first two PCs explained 79 % of the total variance. The first component (PC1 55.4 % of the variance) is slightly negatively determined by P content, PAE and root phosphatases. In addition, the PC1 has strong positive loading on the root surface area, FRAP capacity and root RGR. The second component (PC2 23.7 % of the variance) was mainly explained by soluble sugars and secondary metabolites in roots and exudates (Figure 5). The PCA plots revealed four clusters.

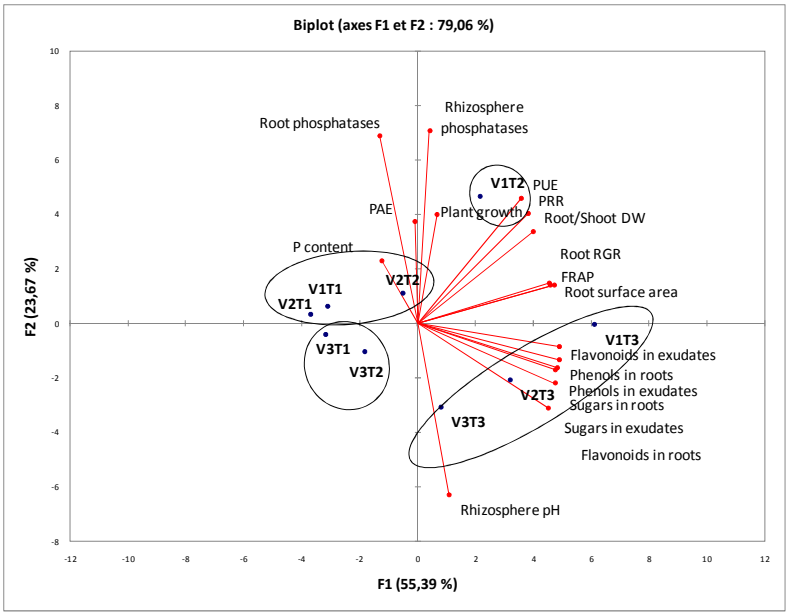


Figure 5: *Scatter plot* of the PCA of the biochemical and physiological parameters in *V. sativa* (V3), *V. faba* cv Locale (V1) and *V. faba* cv Saber 2 (V2) plants grown during 27 days on a control nutrient solution(T1), under direct P deficiency (T2) or under induced P deficiency (T3). The percentage of variation is presented on each corresponding axis. The circles encompass four groups based on treatment, species and cultivars.

5 DISCUSSION

5.1 P deficiency responses in *Vicia faba* and *Vicia sativa*: Phosphorus deficiency decreased plant growth in the two studied species. However, the observed reduction was less important in *V. faba* compared to *V. sativa*, suggesting a higher tolerance of *V. faba* to this nutritional stress (Table 1). Significant differences among cultivars were found in *V. faba* specie. Data obtained demonstrated that, compared to cv Saber 2, Locale cultivar showed a better growth under P deficiency conditions (D and ID treatments). Leaf P starvation often inhibits photosynthesis and leaf chlorophyll content (Zribi *et al.*, 2012). These results contrasted with this study findings where D treatment has no significant effect on leaf total chlorophyll content. Nevertheless, this study is in accord with other researches suggesting that photosynthesis can be sustained under P deficiency (Wissuwa, 2005). Interestingly, a decrease in the concentrations of total chlorophyll, Fv/Fm and Fv/F_0 was detected only in plants cultivated in the presence of bicarbonate in the nutrient solution. This fact is not the specific effect of P deficiency, but was probably caused by a decrease in leaf Fe content. In this

context, several studies have shown that the bicarbonate causes the precipitation of some nutrients like P, Fe and Zn in plants, which affects photosynthesis (Roosta *et al.*, 2015).
Relatively to other abiotic stress (salinity, drought), few investigations studied the effect of P deficiency on ROS production and on antioxidative defense system. The current research showed that the lack of P in the medium (D) or the addition of the bicarbonate (ID) induced a considerable increase of lipid peroxidation. Yet, interspecific variability was observed. Leaf MDA content was significantly higher in the P-sensitive specie *V. sativa* than in the tolerant one *V. faba*, which in turn shows an intraspecific variability: MDA concentration in Locale cultivar deficient plants was lower than in cv Saber 2. Several research showed that an efficient antioxidant system (enzymatic and non-enzymatic) was associated with the tolerance to multiple abiotic stress (M'sehli *et al.*, 2014; Liu *et al.*, 2015). Moreover, in the current study, P deficiency caused a significant increase of SOD and GPOX activities in P-deficient plants in both species mainly in *V. faba* cv Locale. Besides, P deficient plants of *V. faba* cultivars exhibited an increase of iron reducing capacity (FRAP) which supplies information about total non-enzymatic antioxidant activity; the higher values of this capacity detected in the tolerant cultivar (Locale) suggests that it has a better protection against oxidative damage caused by P starvation. The accumulation of carotenoid and anthocyanin pigments in P deficient plants of both *V. faba* cultivars (especially in Locale) indicate their restricted oxidative injury as compared to *V. sativa* plants. Taken together, all data showed the presence of an interspecies variability for P deficiency in Vicia genus. In fact, *V. faba* was more tolerant to P deficiency compared to the *V. sativa* one. In addition, the two cultivars of *V. faba* (Locale and Saber 2) differ in their tolerance to P starvation. The obtained results indicated that the P-efficient cultivar (Locale) was characterized by an efficient enzymatic and non-enzymatic antioxidant defense system.

5.2 Implication of root/rhizosphere traits for improving P tolerance in *Vicia faba* and *Vicia sativa:* Under P deficiency conditions, plants enhance their capacity to acquire P from soil (Shen *et al.*, 2013). The results of this study showed a negative effect of P deficiency on plant P nutrition. However, this depressive effect was less pronounced in *V. faba* cultivars. This can be explained by its higher stimulation of P acquisition efficiency (PAE) and its P use efficiency (PUE). Especially, results showed a better P acquisition and P-use capacity in the P-tolerant cultivar Locale. This behavior is described for wheat tolerant genotypes by Yaseen and Malhi (2011) and Zhou *et al.* (2016). Plants submitted to P deficiency conditions showed an increase in root-to-shoot dry weight ratio and root RGR (Hammond and White, 2007). In this study, the P deficiency induced a significant increase of those parameters and was strongly stimulated in the tolerant cultivar (Locale) under both P deficiency treatments (D and ID) as compared to control. This study results are in accord with those obtained by Zribi *et al.* (2014) in barley. In fact, those authors reported that the P-efficient wild barley (*Hordium maritimum*) maintained a higher root RGR and root/shoot DW under P deficient conditions as compared to P-inefficient cultivated barley (*Hordium Vulgare*). In an analogous way, these findings showed that in both species, P deficiency increased root surface area; the P-efficient cultivar (Locale) was characterized by a high root surface area. Balzergue *et al.* (2017) showed that low P biodisponibility affect primary root growth in many plant species. In this study case, relative root elongation (RRE) results revealed that P deficiency treatment has no significant effect on principal root elongation in the both studied species. According to Shah *et al.* (2015), the response of root growth and architecture to low P bioavailability varies among species and is strongly controlled by plant regulators and inherent genetic factors. The modification of the root architecture observed during P stress is a consequence of change in root sugar concentration (Niu *et al.*, 2013). In this experiment, it was found that for both species, P deficiency increased soluble sugars in P-deficient

roots and this observed increase was more pronounced in the tolerant cultivar (Locale).

When studying the root acidification capacity, *V. faba* and *V. sativa* plants grown in the presence of 10 µM P ((D) treatment) shows an ability to enhance proton release, leading to acidification of the nutrient solution. Li *et al.* (2007) demonstrated that decreasing the pH soil resulted in an increased solubilization of soil P. Significant intra and inter-differences were recorded in the rhizosphere acidification; results showed that the P tolerant cultivar (Locale) acidified its rhizosphere intensively, with pH declining 2 units in the nutrient solution. Several works suggests that extracellular APases are involved in hydrolysis of various organic phosphate monoesters in the soil, whereas intracellular enzymes are important for the remobilization of Pi from rich P components inside the plant cell (Zribi *et al.*, 2014). Enhancement of acid phosphatase activity with phosphate starvation has been demonstrated for several species such as rice (Rose *et al.*, 2013) and barley (Zribi *et al.*, 2014). This study results revealed that in both Vicia species, P shortage significantly increased the intracellular (in roots) and extracellular (released in the rhizosphere) acid phosphatase activities, with a higher extent in the tolerant cultivar (Locale). However, under ID treatment acid phosphatase activity was not stimulated in both species, which suggested that the addition of bicarbonate in the nutrient solution has an inhibitor effect on the activity of those enzymes since acid phosphatases (APases) hydrolyze different forms of organic P at low pH. Abiotic stress induced biosynthesis and accumulation of phenolic compounds in plants (Dixon and Paiva, 1995). In the current study, plants cultured on phosphate-deficient media showed an increase in their root phenolics concentration and the higher values were detected in *Vicia faba* specie under induced P deficiency. Similar results were observed for bean plants (Juszczuk *et al.*, 2004). Phenolics are also the major components of root exudates that solubilize the different nutrients from unavailable sources to facilitate their uptake by plants (Massalha *et al.*, 2017). The study findings showed that the amount of exuded phenolics from phosphate-deficient roots was higher than that from the roots of control plants for both species (Figure 5), mainly in the tolerant cultivar (Locale). This result is consistent with that of (Neumann, 2000) who reported that in white lupin, phosphorus deficiency increased secretion of phenolic compounds in the rhizosphere. Several reports have highlighted the importance of sugars (as secondary messengers) in plant tolerance to adverse environmental conditions (Ko and Helariutta, 2017, Zandalinas *et al.*, 2017). The afore-mentioned studies are in accordance with the study findings. In fact, compared to the control plants, increased concentrations of soluble total sugars were found in roots and in exudates collected from P deficient plants. In order to reduce the dimensionality of the data and visualize samples grouping, an unsupervised multivariate data analysis method PCA was performed on the biochemical and physiological data generated from control (T1) and P-deficient plants (T2 and T3). This approach allows transforming the set of measured parameters into fewer variables that determine the changes in plant physiological and biochemical state. The first and second principal component explained 55.4% and 23.7%, respectively, of the total variance with a cumulative eigenvalue of 79.1% (Figure 5). The results of the PCA for the three group samples indicated that an obvious separation between the control (T1) and treated samples (T3) was detected, while no clear difference was observed between the T1 and T2 groups (Figure 5). In order to understand of which physiological and biochemical mechanisms were affected by phosphorus stress, a graphical approach-so-called biplot or dual graph, which describes the coordinates of points, reflecting the state of the investigated samples and simultaneously it shows vectors presenting observed variables was reported. These vectors give us information about the relative "contribution" of each variable to the formation of the principal components (Comp1 and Comp2). The direction and magnitude of the vector are indicators of this. The cluster composed of plants cultivated in the presence of bicarbonate in the nutrient solution

(T3) is located in the negative region of Comp 1 and Comp 2. This means that the induced P deficiency in *V. sativa* (V3), *V. faba* cv Locale (V1) and *V. faba* cv Saber 2 (V2) can be easily determined by measuring the physiological and biochemical traits. The formation of the first component is due to the changes in morpho-agronomical and physiological parameters (plant growth, Root/Shoot DW, Root surface area,), P content and phosphatases activity. The parameters reflecting the primary (sugars), secondary (flavonoids and polyphenols) metabolism, and rhizosphere pH contributed to decrease in Comp 2.

6 CONCLUSION

The results of the present study showed that *Vicia faba* was more tolerant to P deficiency than *Vicia sativa*. In addition, the obtained findings demonstrated that the two cultivars of *V. faba* (Locale and Saber 2) differ in P starvation tolerance. In fact, the efficient cultivar (Locale) is characterized by a greater ability to maintain an efficient root/rhizosphere management defined by a stimulation of root biomass, phosphatase activity, rhizosphere acidification capacity and exudation of phenols and soluble sugars, which provides higher P acquisition. Increased P-deficiency tolerance and its use efficiency may occur through formed mycorrhizal symbioses (Zhang *et al.,* 2017). Thus, further research should focus on the effect of mycorrhizae for improving legumes performance during growth in low-phosphate environments.

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