

Effect of phosphate deficiency on growth and phosphorus content of three Voandzou (*Vigna subterranea* (L.) Verdc.) varieties.

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Abstract: The aim of this study was to investigate the effect of phosphate (P_i) deficiency on growth and phosphorus content of three varieties (V1: white, V2: red and V3: burgundy/purple) of Bambara groundnut (*Vigna subterranea* (L.) Verdc.). For this purpose, plants were grown for 30 days in a greenhouse on river sand, watered with Hoagland solutions of two different concentrations of P_i (0 and 1000 $\mu\text{M } P_i$). The results obtained showed that from 1000 to 0 $\mu\text{M } P_i$, *V. subterranea* shoot fresh biomass reduced by 13.48%, 9.46% and 14.57% for varieties V1, V2 and V3 respectively. Its total fresh biomass also reduced by 8.29% for V1, 3.32% for V2 and 6.94% for V3. But P_i deficiency (0 $\mu\text{M } P_i$) led to an increase in root fresh biomass (V2: 8.82% and V3: 7.90%) and root/shoot ratio (V1: 15.17%, V2: 21.57% and V3: 25%). These results show a preferential allocation of biomass to the roots in P_i deficient plants. P_i deficiency had no significant effect on the number of emerged leaves and the plant water content of *V. subterranea*. However, it increased the specific leaf weight (0.027 for 0 $\mu\text{M } P_i$ and 0.023 g.cm^{-2} for 1000 $\mu\text{M } P_i$ in V1). The total leaves and roots phosphorus content of P_i deficient plants (0 $\mu\text{M } P_i$) significantly decreased compared to non-deficient plants (1000 $\mu\text{M } P_i$). The P_i deficient plants showed better efficiency in phosphorus assimilation. V1 had the best vegetative growth, V2 showed highest phosphorus use efficiency and V3 contained more phosphorus in its organs.

Keywords: *Vigna subterranea*, phosphorus, P_i deficiency, plant biomass, Hoagland solution

I. Introduction

Seeds legumes (pulses), especially *Vigna subterranea* (L.) Verdc. also known as Voandzou or Bambara pea have an important socio-economic role in tropical Africa, where they constitute a tradition in the culinary habits of the inhabitants [7][21] [22]. In Cameroon, it is called "Matob" in Bassa'a language, "Motoh" in Fe'efe'e language, "ngalaa-ji" in Fulfulde and "Motobo" in Bafia language. Bambara groundnut is highly caloric (387 kcal/100g), rich in vitamins and minerals and very balanced in protein elements. It contains 63% of carbohydrates and 18% of oil and fatty acids [22]. The leaves of Bambara pea, rich in phosphorus, are used to feed livestock [7][43]. This pea is incorporated into crop rotation systems with cereal and is also used in fallow to replenish soil fertility [1][2]. A drop in production of pulses has been observed in Cameroon since the 1980s despite the high demand for consumption. The environmental stresses like nutritional deficiencies including the phosphate deficiency are among the main causes of the drastic decline in yields and are important limiting factors for the growth, production and survival of cultures [30].

Phosphorus (P) is a limiting factor for crop yields on over 30% of arable land on the planet [19][38]. It is the most limiting element for mineral nutrition of plants because of its low availability in the soil [19]. At present, P is mostly obtained from rock phosphate extracts. At the current rate of extraction, world reserves of commercial phosphate (P_i) will be exhausted by 2050 [11]. Improving the efficiency of acquisition and use of phosphorus by plants is therefore essential for economic, humanitarian and environmental reasons [38].

In addition, phosphorus concentrations of tropical soils are generally less than half that of temperate soils [32][33]. To increase production, farmers amend the soil with chemical phosphate fertilizers. But these are expensive and harmful to the environment if leached [11][27]. In order to reduce the use of phosphate fertilizers, research of efficient biological/organic fertilizers (Plant Growth Promoting Rhizobacteria, arbuscular mycorrhizal fungi) under limited phosphorus conditions is envisaged. However, a prerequisite to this research is the mastery of the phosphate nutrition of plants [18][48]. Some agronomic studies have been done on the

response of *Vigna subterranea* [26][36] to the P_i deficit. But, to the best of our knowledge, no study has been done on understanding the morpho-physiological and biochemical response mechanisms of this Bambara pea to P_i deficiency. The aim of this work was therefore to study the effect of P_i deficiency on growth and phosphorus content of *V. subterranea* (L.).

II. Materials And Method

2.1 Study site

The experiment was carried out in a greenhouse at the University of Yaoundé I. The phosphorus content in roots and stems of *Vigna subterranea* plants was analysed at the International Institute of Tropical Agriculture (IITA) at Nkolbisson in Yaounde, Cameroon.

2.2 Plant Material

Seeds of three varieties of voandzou (Bambara pea) were used for this study. Variety V1 corresponding to white seeds, variety V2 to red seeds and variety V3 to burgundy/purple seeds (Fig. 1).



Figure 1. Seeds of three varieties of *Vigna subterranea* (L.) Verdc. A: V1 white seeds, B: V2 red seeds and C: V3 burgundy seeds.

2.3 Methodology

2.3.1 Nutritive solution

The Hoagland's solutions (Hoagland and Arnon, 1950) of different phosphate concentrations (0 and 1000 $\mu\text{M } P_i$) were prepared from macronutrients (KH_2PO_4 , KNO_3 , $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, NH_4NO_3 , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and Fe EDDHA) and micronutrients (H_3BO_3 , $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$). These solutions were then adjusted to pH 6.

2.3.2 Culture substrate

The culture substrate used was river sand. It was previously sieved to obtain particle sizes ranging from 0 to 2 mm. Then, this sand was washed, autoclaved (120 °C for 4 h) and dried in an oven until a constant weight [35].

2.3.3 Obtaining seedlings

The sorted seeds were disinfected in ethanol (70%) for 1 min and then in sodium hypochlorite for 10 min [34]. They were then rinsed three times with distilled water and then spread over a seedbed between two filter papers soaked with distilled water to promote good germination. The seedbed was washed with distilled water using a squeeze bottle and kept in the dark at room temperature in the laboratory until germination.

2.3.4 Transplanting tube

A plastic bag was placed in each cylindrical tube (20 × 10 cm). These tubes were then filled with 1.5 kg of river sand with 10% of moisture; 1350 g of dry sand and 150 g of the corresponding Hoagland solution. A hole was made in each tube to allow planting of germinated seeds. Small holes were also made at the base of the bags to allow the substrate to lose the excess solution and to prevent asphyxia of seedlings. Homogeneous seedlings (sprouts) with radicles were selected for further experimentation. These seedlings were delicately removed from the seedbed using pliers. Then, they were planted in the previously prepared tubes, one seedling per tube. All of this was placed in the greenhouse (12 hour photoperiod, 25 ± 3 °C, 60% of moisture and 2400-3500 lux) in randomized complete blocks. The experimental design consisted of three (3) blocks, three (3) varieties (V1, V2 and V3) and two (2) treatments (0 $\mu\text{M } P_i$ and 1000 $\mu\text{M } P_i$). In each block, there were five (5) seedlings per P_i concentration (treatment) and variety, 30 seedlings / block, and 90 seedlings in total.

2.3.5 Application of treatments

From the day of transplanting, the *Vigna subterranea* seedlings were maintained for 10 days at 10% of moisture, with the corresponding Hoagland solution. They were then sprayed with 150 ml of Hoagland solution from days 10, 15, 20 and 25. The harvesting was carried out on the 30th day of growth and various parameters were measured.

2.3.6 Measurement parameters

The number of leaves was obtained by counting the newly formed and completely open leaves each week. In order to facilitate detection of new leaves, the last leaf issued was marked by a sign on the petiole. At harvest, plants were carefully collected and the roots were rinsed carefully to remove sand and residues on their surface. The root portion was then separated from the stem. Fresh biomass of each part was weighed with an analytical balance (Scaltec SPB55). Each portion of 72 of the 90 harvested plants was dried in an oven (P-Selecta) at 65°C for 96 hours, and weighed with the balance to obtain the dry biomass. The plant water content (WC) was evaluated by the following formula:

$$WC (\%) = \frac{(FW - DW)}{DW} * 100, \text{ where FW: Fresh weight of plant and DW: Dry weight of plant.}$$

The specific leaf weight (SLW) was calculated from the formula [4]:

$$SLW (g.cm^{-2}) = LFW/LA, \text{ where LFW: Leaf fresh weight and LA: Leaf area.}$$

The phosphorus content in roots and stems of *Vigna subterranea* plants was analysed at the International Institute of Tropical Agriculture (IITA) of Nkolbisson, Yaounde in Cameroon. Phosphorus was extracted after plant material calcination (450°C for 16 h) in a muffle furnace. It was then analysed by colorimetric method [6][25]. The amount of phosphorus contained in each part of the plant material was expressed as a percentage of dry matter. The phosphorus (P) use efficiency (PUE) is the amount of dry biomass (g) produced by a given plant for each milligram of P contained in the plant [13]. This was calculated using the following formula [5][42]:

$$PUE = \frac{\text{Plant DW}}{\text{Plant P content}}, \text{ where DW: Dry weight of plant.}$$

2.3.7 Data analysis

An analysis of variance (ANOVA) with three classification criteria (AV3) was made on the data with Minitab Statistical Software Release 16. The Newman-Keuls test at the 5% threshold allowed comparing the averages in order to classify the treatments and identify those that are different from each other. The relationships between the measured parameters were identified by the Pearson correlation test. Differences were declared significant at $p < 0.05$. The results, presented in the form of curves, tables and histograms were produced using Microsoft Excel 2010 software.

III. Results

3.1. Number of leaves

Figure 2 shows the evolution of the number of leaves of *Vigna subterranea* versus time (weeks) after 30 days of growth. It is observed that phosphate starvation (0 $\mu\text{M P}_i$) does not significantly affect ($p > 0.05$) the number of leaves produced by *V. subterranea* over time (Fig. 2). The variety does not influence the leaves emission in *V. subterranea*.

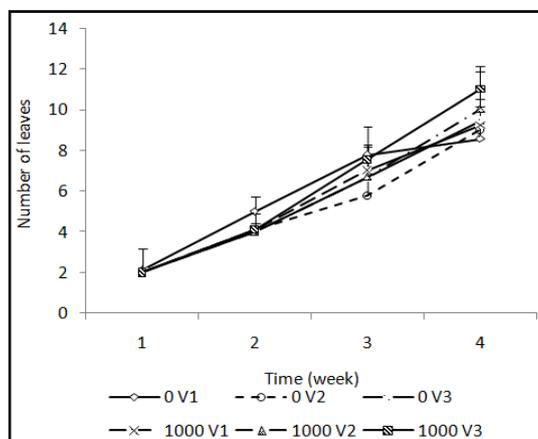


Figure 2. Evolution of the number of *Vigna subterranea* (L.) leaves versus phosphate concentrations after 30 days of growth. V1: white variety, V2: red variety, V3: burgundy variety; 0 and 1000: phosphate (P_i) concentrations in μM .

3.2 Shoot and root fresh weights

Shoot fresh biomass of *Vigna subterranea* increased significantly ($p < 0.01$) with increase of the P_i concentration in the Hoagland watering nutrient solution (Figure 3A). Thus, an increase in shoot fresh biomass by 15.58%, 10.45% and 16.39%, was respectively recorded for white (V1), red (V2) and burgundy (V3) varieties of *V. subterranea*. The variety significantly influenced ($p < 0.01$) production of shoot fresh biomass of *V. subterranea* plants. The white variety (V1: 8.24 ± 1.96^a g) significantly ($p < 0.01$) produced more shoot fresh biomass than the red variety (V2: 7.05 ± 1.32^{ab} g) and the burgundy variety (V3: 6.62 ± 1.49^b g).

The decrease of P_i concentration in the Hoagland solution generated an increase in the root fresh biomass production of *V. subterranea* (Fig. 3B). But statistically, the P_i deficit had no effect on root production by *V. subterranea*. An important varietal variation was observed. The white variety (V1: 5.75 ± 1.33^a g) significantly produced ($p < 0.001$) more fresh root biomass than the red (V2: 3.91 ± 0.69^b g) and the burgundy (V3: 3.81 ± 0.69^b g) varieties.

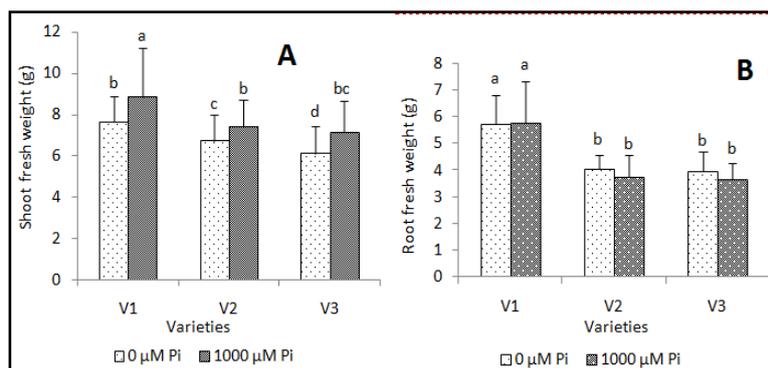


Figure 3. Effect of phosphate concentrations on production of shoot fresh biomass (A) and root fresh biomass (B) of *Vigna subterranea* (L.) after 30 days of growth. V1: white variety, V2: red variety, V3: burgundy variety. For each parameter, the average with the same letter are not significantly different at $p < 0.05$ with Newman-Keuls test.

3.3 Total plant fresh weight and roots/shoot ratio

A very highly significant increase ($p < 0.001$) of the plant total fresh biomass of *V. subterranea* was observed with increase of P_i concentration in the nutritive solution (Fig. 4A) after 30 days of growth. Thus, in the white variety (V1), a significant increase of the total fresh biomass of 9.04% was observed from 0 to 1000 $\mu\text{M } P_i$. Moreover, a very highly significant ($p < 0.001$) varietal effect was also observed. The averages ordering test of Newman-Keuls classified varieties into two groups, the white variety (V1: 13.99 ± 3.10^a g), and the red (V2: 10.96 ± 1.81^b g) and the burgundy (V3: 10.43 ± 2.02^b g) varieties.

A very highly significant ($p < 0.001$) reduction of the root / shoot ratio was observed with the increase of the phosphate (P_i) concentration in the Hoagland solution (Fig. 4B). Thus, from 0 to 1000 $\mu\text{M } P_i$, a very highly significant decrease in the root / shoot ratio of 13.16%, 17.74% and 20.00% was respectively observed for the white (V1), the red (V2) and the burgundy (V3) varieties. As shown in Figure 4B, the averages ordering test of Newman-Keuls indicated that the white variety (V1: 0.71 ± 0.11^a) had a significantly ($p < 0.001$) higher roots / shoot ratio than those of the red variety (V2: 0.56 ± 0.10^b) and the burgundy variety (V3: 0.59 ± 0.09^b).

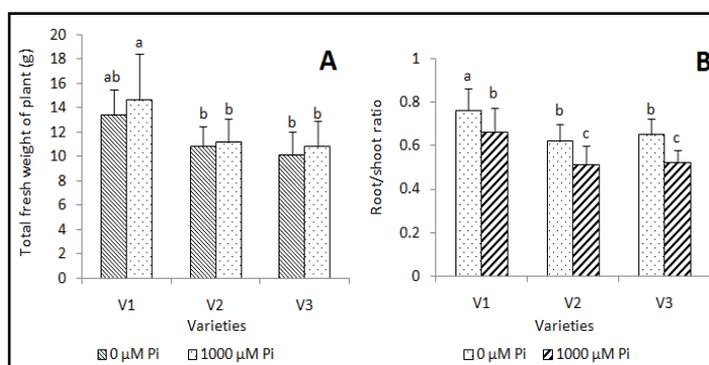


Figure 4. Effect of phosphate concentration on total fresh biomass of *Vigna subterranea* (L.) plants (A) and root/shoot ratio (B) after 30 days of growth. V1: white variety, V2: red variety, V3: burgundy variety. For each parameter, the averages with the same letter are not significantly different at $p < 0.05$ with Newman-Keuls test.

3.4 Plant water content and specific leaf weight

The phosphate starvation ($0 \mu\text{M P}_i$) did not significantly change ($p > 0.05$) the plant water content of *Vigna subterranea* (Table 1). The three varieties of *V. subterranea* statistically had the same water content. On the other hand, the decrease of the P_i concentration in the nutrient solution led to a very highly significant ($p < 0.001$) increase of the specific leaf weight of *V. subterranea* after 30 days of growth (Table 1). Thus, although there is no significant difference between $0 \mu\text{M P}_i$ ($0.028 \pm 0.002^a \text{ g.cm}^{-2}$) and $1000 \mu\text{M P}_i$ ($0.027 \pm 0.002^a \text{ g.cm}^{-2}$) in the red variety (V2), the specific leaf weight of white and burgundy varieties is greater in $0 \mu\text{M P}_i$ than $1000 \mu\text{M P}_i$. From 0 to $1000 \mu\text{M P}_i$ (Table 1), a significant decrease of 14.82% and 12.50% is observed in the white variety and burgundy variety respectively.

Table 1. Effect of phosphate concentrations on specific leaf weight and plant water content of *Vigna subterranea* (L.) Verdc. after 30 days of growth.

Varieties	Phosphate (P_i) concentration	Plant water content (%)	Specific leaf weight (g.cm^{-2})
V1	$0 \mu\text{M P}_i$	$85,006 \pm 1,351 \text{ a}$	$0,027 \pm 0,006 \text{ a}$
V2		$85,049 \pm 0,949 \text{ a}$	$0,028 \pm 0,002 \text{ a}$
V3		$85,943 \pm 0,904 \text{ a}$	$0,024 \pm 0,005 \text{ ab}$
V1	$1000 \mu\text{M P}_i$	$86,006 \pm 1,475 \text{ a}$	$0,023 \pm 0,002 \text{ bc}$
V2		$84,296 \pm 1,443 \text{ a}$	$0,027 \pm 0,002 \text{ a}$
V3		$86,400 \pm 4,040 \text{ a}$	$0,021 \pm 0,003 \text{ c}$

V1: white variety, V2: red variety, V3: burgundy variety. The averages with the same letter are not significantly different at $p < 0.05$ with Newman-Keuls test.

3.5 Phosphorus content in plants and Phosphorus use efficiency

Table 2 shows the effect of phosphate (P_i) deficiency on the total phosphorus (P) content of leaves, the total P content of roots and the P use efficiency in *Vigna subterranea* (L.) Verdc. The effect of different concentrations of P_i on the total P content in leaves of *V. subterranea* was very marked. The total P content in leaves of *V. subterranea* significantly increased ($p < 0.001$) by 75.23%, 38.24% and 43.59% for V1, V2 and V3 respectively from 0 to $1000 \mu\text{M P}_i$ (Table 2). The total P content in leaves of *V. subterranea* was significantly ($p < 0.001$) higher in variety 1 (V1). Increase of the P_i concentrations in the watering Hoagland solution caused an increase of the total P content in roots of *V. subterranea* (Table 2). From 0 to $1000 \mu\text{M P}_i$, the total P content in the roots increased by 37.96% for V1, 19.39% for V2 and 20.36% for V3. V1 and V3 accumulated more P in their roots than V2.

Table 2. Effect of phosphate deficiency on leaf phosphorus content, root phosphorus content and phosphorus use efficiency of *Vigna subterranea* (L.) after 30 days of growth.

Phosphate Concentration ($\mu\text{M P}_i$)	Varieties	Total phosphorus content in leaves (%)	Total phosphorus content in roots (%)	PUE (g/mg P)
0	V1	0.109 e	0.108 e	0.919 b
0	V2	0.102 f	0.098 f	0.987 a
0	V3	0.117 d	0.113 d	0.861 c
1000	V1	0.191 a	0.149 a	0.546 f
1000	V2	0.141 c	0.117 c	0.730 d
1000	V3	0.168 b	0.136 b	0.614 e

P_i : phosphate, PUE: phosphorus use efficiency. V1: white variety, V2: red variety, V3: burgundy variety. The averages with the same letter are not significantly different at $p < 0.05$ with Newman-Keuls test.

Vigna subterranea plants deficient in P_i had the best phosphorus use efficiency. The phosphorus use efficiency (PUE) of *V. subterranea* gradually decreased and was very highly significant ($P < 0.001$) with the increase of the P_i concentration in the nutrient solution (Table 2). Compared to the variety 3 (V3), V2 and V1 used P more effectively.

3.6 Correlation test

The number of leaves is negatively and significantly correlated (Table 3) to the root fresh biomass ($p < 0.05$), the root / shoot ratio ($p < 0.01$) and the phosphorus use efficiency ($p < 0.01$). Positive and significant correlations are observed between shoot fresh biomass, roots fresh biomass and total fresh biomass of plants. The specific leaf weight is correlated to the water content of *V. subterranea* plants. The phosphorus use efficiency (PUE) is negatively correlated ($p < 0.01$) to the number of leaves, total phosphorus (P) content in leaves and total P content in roots; it is positively correlated ($p < 0.01$) to the specific leaf weight (Table 3). The total P content of the leaves and the total P content of the roots are very highly correlated ($p < 0.001$). These parameters are also positively correlated to the shoot fresh biomass ($p < 0.01$) and negatively correlated to the specific leaf weight ($p < 0.01$).

Table 3. Pearson correlation test between growth parameters and phosphorus content in plant.

	LN	SFW (g)	RFW (g)	TFW (g.plant ⁻¹)	RTSR	PWC (%)	SLW (g.cm ⁻²)	LPC (% DW)	RPC (% DW)	PUE (g/mg P)
LN	1									
SFW (g)	-0.176 ns	1								
RFW (g)	-0.513 *	0.717 **	1							
TFW (g.plant ⁻¹)	-0.373 ns	0.926 ***	0.927 ***	1						
RTSR	-0.614 **	0.127 ns	0.776 ***	0.489 *	1					
PWC (%)	0.326 ns	-0.159 ns	-0.093 ns	-0.136 ns	0.012 ns	1				
SLW (g.cm ⁻²)	-0.467 ns	-0.154 ns	0.036 ns	-0.063 ns	0.188 ns	-0.489 *	1			
LPC (% DW)	0.417 ns	0.615 **	0.162 ns	0.418 ns	-0.326 ns	0.349 ns	-0.647 **	1		
RPC (% DW)	0.374 ns	0.603 **	0.247 ns	0.458 ns	-0.192 ns	0.406 ns	-0.700 **	0.982 ***	1	
PUE (g.plant ⁻¹ / % DW P)	-0.684 **	-0.007 ns	0.434 ns	0.232 ns	0.623 **	-0.451 ns	0.700 **	-0.724 **	-0.709 **	1

P: phosphorus, PUE: phosphorus use efficiency, FW: fresh weight, DW: Dry weight. LN: Leaves number; SFW: Shoot fresh weight; RFW: Root fresh weight; TFW: Total fresh weight; RTSR: Root/Shoot ratio; PWC: Plant water content; SLW: Specific leaf weight; LPC: Leaves P content; RPC: Root P content. *: p<0.05 (significant difference), **: p<0.01 (highly significant difference), ***: p<0.001 (very high significant difference), ns: non-significant.

IV. Discussion

Shoot fresh biomass and total fresh biomass of *Vigna subterranea* reduced while its root/shoot ratio increased and did not affect its roots fresh biomass under phosphate (P_i) deficiency in our experimental conditions. The decrease of *V. subterranea* shoot biomass in P_i deficiency condition was due to the reduction of the number of leaves [17], leaf expansion and the leaf area [12]. Investigations on the effect of the P_i deficiency on corn stem development in hydroponics and on the field by a weaning experiment [24] in P_i, showed that P_i deficiency greatly reduced leaf growth. The misuse of the most part of resources of the plant by the roots under P_i deficiency [48] could also explain the decrease in shoot fresh biomass of *V. subterranea* under P_i deficient conditions. Similar results were obtained by López-Bucio et al. [16] on *Arabidopsis thaliana*, Cierieszko et al. [9] and Yaseen and Malhi [46] on wheat, Cierieszko et al. [10] on barley, and by Poiré et al. [28] on *Brachypodium distachyon*.

Under P_i limitation, *V. subterranea* did not reduce its root fresh biomass but maintained and even increased it. The maintenance and/or increase of the *V. subterranea* root fresh biomass under P_i deficiency could be attributed to the increase of the dimensions of root architecture traits [15]. Plants react most often to P_i deficiency by allocating more resources to root growth. This preferential allocation of resources under P_i deficiency also explains the increase in root fresh biomass of *V. subterranea*. The increase of root biomass under P_i deficiency was also observed on bean [29], *Arabidopsis thaliana* [16] and on wheat [45]. The reduction of total fresh biomass accumulation by *V. subterranea* plants under P_i deficiency is the consequence of the decrease of the shoot fresh weight. It was also found [48] that maize responds to P_i deficiency by reducing the total plant biomass and diverting resources to root growth. Yaseen and Malhi [46] also found similar results for wheat under P_i deficiency. The decrease of the total fresh biomass of plants could be according to Mollier and Pellerin [23] mainly explained by the reduction of leaf area. These results corroborate those obtained by Watt and Evans [39] on the white lupine and soybean under P_i deficiency.

The increase of *V. subterranea* root/shoot ratio under limited P_i availability is the result of the decrease in shoot fresh biomass and the increase of root fresh biomass. The increase in this roots/shoot ratio under P_i deficiency is due to the use of the most part of the resources for roots production to the detriment of shoot parts [20][37]. This therefore explains the increase of root fresh biomass and roots/shoot ratio. Similar results were observed on common bean [3], *Arabidopsis thaliana* [16], wheat [9], on barley [10], and on rice [47]. The total P content in leaves and roots of *Vigna subterranea* deficient in P_i decreases, while P use efficiency increases. *V. subterranea* plants deficient in P_i have the best P use efficiency. It has been pointed out [31] that there is a loss of use of P by plants when nutrients are abundant. Plants watered with P_i (1000 µM P_i) sufficient nutrient solution do not effectively use the P because they have a luxury consumption. They do not provide any effort to

get the nutrients they need, since they are not subject to stress. These results corroborate those of Yan et al. [42] on bean.

The total P content in leaves and roots of *V. subterranea* decreases under P_i deprivation. However, growth and morphology of plants are very less affected by the P_i deficiency at this growth stage. In fact, the plants would develop to protect themselves against the P_i starvation with varied responses to conserve, remobilize and improve the acquisition of the internal P_i [8]. According to White and Veneklaas [40], the seeds P reserves can support the maximum growth of cereal seedlings for several weeks after germination until the plant has three or more leaves with an extensive root system. Reducing the P content of the leaves and roots under P_i deprivation was observed on soybean [39], common bean [3], barley [10], wheat [9], rice [47], *Arabidopsis thaliana* [41] and *Brachypodium distachyon* [28][35]. According to Yao et al. [44], increasing the ability of the roots to uptake P_i is dependent not only of the increase of the nutrient absorption surface but also of the physiological and metabolic ability to improve the organic acids secretion or enzymes such as acid phosphatase.

V. Conclusion

The study of the effect of P_i deficiency on the growth and the phosphorus (P) content of three *V. subterranea* varieties was conducted for 30 days of growth in the greenhouse. On the one hand the P_i deficiency caused a reduction of the shoot fresh biomass and the total fresh biomass of *V. subterranea* plants. On the other hand, it caused a significant increase of the roots/shoot ratio as well as the root fresh biomass. These biomass variables are highly correlated. P_i deficiency led to a significant reduction of total P content in leaves and roots of *V. subterranea*. But the deficient plants had a better P use efficiency. In fact, they produced a greater amount of biomass for a specific amount of P present in the tissues. The total P content in leaves and roots as well as the P use efficiency are highly correlated. P_i -deficiency symptoms are very pronounced in severe conditions (0 μ M P_i). Under P_i deficiency, the vegetative growth of the white variety was the most important. The total P content in leaves and roots in the burgundy variety was the highest and the red variety had the best P use efficiency. The findings from this study could be exploited for fertilizer recommendation and efficient crop management of *V. subterranea* and other crops.

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