MONOPOTASSIUM PHOSPHATE (KH_2PO_4) AND SALICYLIC ACID (SA) AS SEED PRIMING IN *Vicia faba* L. AND *Vicia sativa* L.

FOSFATO DE MONOPOTÁSSIO (KH2PO4) E ÁCIDO SALICÍLICO (SA) COMO PRIMAGEM DE SEMENTES EM Vicia faba L. E Vicia sativa L.

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ABSTRACT: The first experiment was conducted to evaluate the impact of seed priming on germination behavior and seedling establishment in *Vicia faba* and *Vicia sativa*, for that, seeds priming was done using SA (100 µM) and KH_2PO_4. In order to determine the optimal concentration of KH_2PO_4 for improving germination, different concentrations were used: 25 µM, 50 µM, and 100 µM. The best germination behavior and seedling establishment were obtained with 25 and 50 µM KH_2PO_4, respectively for *Vicia faba* and *Vicia sativa*. Moreover, data showed that 100 µM of SA improved seed germination as well as the seedling establishment for both species. The second experiment was carried out to investigate the influence of seed priming for improving phosphorous (P) deficiency tolerance. To do, seedling obtained from primed and nonprimed seeds were grown in a hydroponic culture system with three different treatments: control (C, medium containing sufficient P concentration: 360 µM KH_2PO_4), direct phosphorus-deficient (DD, medium containing only 10 µM KH_2PO_4), and induced P deficiency by bicarbonate (ID, medium containing sufficient P concentration: 360 µM KH_2PO_4 + 0.5 g L^{-1} $CaCO_3$ + 10 mM $NaHCO_3$). Furthermore, the role of exogenous SA applied to P deficiency tolerance enhancement was explored. Seed priming or the exogenous application of SA significantly reduced the severity effect of P deficiency. In fact, the pretreated plants were observed more tolerant to P deficiency as reflected from the significant increase in plant biomass, P uptake, and an efficient antioxidant system. Overall, this paper highlights the beneficial effect of seeds priming or the exogenous application of SA in the improvement of plant tolerance to phosphorus deficiency.

KEYWORDS: Abiotic Stress. KH_2PO_4. P deficiency. Seed Physiology**.** Seed priming.

INTRODUCTION

Phosphorus (P) is among the most crucial macronutrient required for plant growth and development. However, owing to its limited abundance in the soil and by its adsorption into various soil minerals, P is frequently inaccessible and restricts plant growth (CABEZA et al., 2017). Consequently, P deficiency is one of the most abiotic stresses negatively influencing the productivity of crop legumes over the world mainly in developing countries (GRAHAM, 2003).

To solve this nutritional disturbance, the application of fertilizers and foliar sprays are necessary approaches. Nevertheless, these methods are too expensive to be practiced by farmers, particularly in developing countries. Alternatively, sustainable agricultural practices will be necessary to increase crop productivity and quality under stressful conditions. One promising technique is seed priming. Several works had tested the effectiveness of seed priming for improving plant nutrition in deficient soils. MUHAMMAD et al. (2017) demonstrated that nutrient seed priming (Zn and Mn) improve soybean seed quality for early seedling development under limited nutrient supply or availability. In addition, AJOURI et al. (2004) concluded that P and Zn application through seed priming enhanced barley seeds germination and early growth stage under low nutrient availability.

A various of signal molecules and hormones are being used as exogenous sources to improve plant tolerance to different stresses (JANDA et al., 2017; SALAHUDDIN et al., 2017). Among these chemical substances, salicylic acid (SA) has been identified as an important stress-signaling molecule in plant stress response. In this context, HAYAT et al. (2010) showed that SA was accumulated in plants confronted with various types of environmental stresses. Moreover, numerous researches demonstrated that seed priming with SA enhances plant defense against water stress (HOSSEIN et al., 2015), heat stress (KHAN et al., 2013), and copper accumulation (MOSTOFA;

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FUJITA, 2013). LI et al. (2017) revealed that the exogenous application of ABA, GABA, and SA enhances drought tolerance in *Agrostis stolonifera* by stimulating amino acids and carbohydrates accumulation. The promotive effects of seed priming with SA in abiotic stress tolerance were also reported by MIURA; TADA (2014), who found that exogenous application of SA could improve cold tolerance by regulating antioxidant enzyme activities.

As mentioned above, several researches have highlighted the beneficial role of seed priming technique on plant tolerance to various abiotic stresses. Nevertheless, the improvement of legumes tolerance to P deficiency by seed priming technique has not been well investigated. In addition, to our knowledge, there is no research that has been interested in the effect of SA on P deficiency tolerance on the plant as well as the comparative effects between KH_2PO_4 and SA as priming agents on P deficiency tolerance. For this purpose, the assessment of the contribution of seed priming with KH_2PO_4 and SA (seed priming or exogenous application) on seed germination behavior and P deficiency tolerance in two legumes *Vicia faba* and *Vicia sativa* has been tested in the present research.

MATERIAL AND METHODS

Seed materials and priming

Common vetch *Vicia sativa* L (commercial variety introduced from France many years ago to be used by farmers for forage and hay purpose) and *Vicia faba* L (minor variety) were immersed for 2 min in a 30% $CaCl_2$ solution (commercial substance). After that, the seeds were rinsed 10 times with demineralized sterile water in order to ensure the safe removal of any sterilizing agent. Seeds were then blotted on sterile Whatman filter paper sheets. The seeds of each specie were subjected to seed priming, the first group was nutrient primed (soaked in 25, 50, and 100 µM KH_2PO_4 for 24 h), while the second group was hormonal primed (soaked in 100 µM salicylic acid (SA) for 24 h). Following priming, seeds were washed in distilled water. Whereas the non-primed seeds (soaked in distilled H_2O for 24 h) were used as the controls.

Experiment 1: seed priming effect on germination behavior and early growth stage

Seeds were then placed to germinate in Petri dishes (90 mm diameter) containing a sheet of filter paper, saturated with distilled at 25 °C in the dark. In each treatment, three replicates (each one contained 15 seeds) were used. Radical emergence was checked daily, and germination was defined as radical emergence of ≥ 2 mm (SMITH; COBB, 1991), and primary root length and shoot were measured for 5 days.

The measurements of physiological characteristics such as total seed germination (TG), germination index (GI), mean germination time (MGT), and vigor index (VI) were determined using those formulas:

TG (%) = $100 \times \frac{n}{N}$ where, n is the total germinated seeds and N is the total seeds sowed.

Germination index (GI) was calculated as explained by MAGUIRE (1962): GI= $\sum(\frac{Gt}{Tt})$ where Gt= number of germinated seeds on Day t, Tt= time corresponding to Gt in days.

Mean germination time (MGT) was determined using (EDMOND; DRAPALA, 2012) method:

MGT= $\frac{\sum(Tini)}{\sum ni}$ where n_i= the number of germinated seeds on the i^{th} day and Ti= the rank order of day i (number of days counted from the beginning of germination).

Vigor index (VI) was measured following RAZMI et al. (2013): VI= (RL+SL) *TG where RL= radicle length; SL=shoot length and TG= total seed germination.

Germinated seeds were transferred to square plates filled with sterile sand and irrigated with deionized water. After 15 days of growth, shoot, and root lengths of six randomly selected seedlings were measured. The dried shoots and roots (at 70 °C) were then weighted.

For the conductivity test, ten seeds of each treatment were placed in 10 mL distilled H_2O for 24 h at 25 °C after which the initial conductance (C_0) was measured with a conductivity meter. Seeds were then exposed to 80 °C for 30 min then returned to 25 °C for 30 min before measuring the total conductance (Ct). Percent of electrolyte leakage was expressed as (C_0/Ct) x 100 (BECWAR et al., 1982).

Experiment 2: effects of seed priming with KH_2PO_4, SA and the exogenous application of SA on P deficiency tolerance in *Vicia faba* and *Vicia sativa*

Growth conditions

Seeds of *V. faba* and *V. sativa*: non-primed, primed with KH_2PO_4 (25 µM for *Vicia faba* and 50 µM for *Vicia sativa*) or primed with 100 µM SA were germinated for 6 days in Petri dishes. Six-day-old seedlings were transferred in half-strength VADEZ (1996) modified nutrient solution, continuously aerated, for 7 days. After that, seedlings having similar size were selected and cultured (8 seedlings) in 5 L of full strength nutrient solution containing macronutrients with following concentrations: 1 mM $MgSO_4$, 2 mM KNO_3, 0.7 mM K_2SO_4, 1.65 mM $CaCl_2$ and micronutrients as a mixture of salts: 6.6 µM $MnSO_4$, 1.56 µM $CuSO_4$, 1.55 µM $ZnSO_4$, 0.12 µM $(Na)_2M_oO_4$, 0.12 µM $COSO_4$ and 4 µM H_3BO_3. Three treatments were established as follows: C = control (medium containing sufficient P concentration: 360 µM KH_2PO_4), DD = direct P deficiency treatment (medium containing only 10 µM KH_2PO_4), and ID = induced P deficiency (medium containing sufficient P concentration: 360 µM KH_2PO_4 + 0.5 g L^{-1} $CaCO_3$ + 10 mM $NaHCO_3$). This last treatment was considered to simulate the natural conditions of calcareous soils where P deficiency is widespread. In this part of the experiment, the direct addition of 100 µM SA in the hydroponic box was also investigated (expressed as SA. B).

The experiments were achieved in a glasshouse under controlled conditions (the temperature varies between 24 °C during the day and 16 ºC overnight, a 14 h photoperiod and with the relative humidity of 70 ± 5%). The nutrient solution was continuously aerated and was changed every 5 days. The treatment lasted 27 days. At the end of the experiment, leaves and roots were separated, rinsed with distilled water, and dried in a stove for 48 h at 60 °C. Afterward, the dry matter weights were determined. Moreover, leaves and roots were frozen in liquid nitrogen and kept at −80 °C to be used for enzyme activity assays.

Determination of phosphorous concentrations and acid phosphatase activity

Phosphorus concentration was assayed following FLEURY; LECLERC (1943) method using vanado-molybdate. Roots acid phosphatase activity was measured spectrophotometrically by monitoring the p-nitrophenol released following the protocol described by TALBI ZRIBI et al. (2015).

Lipid peroxidation (MDA) assay

To assay leaves and roots MDA content, the protocol described by CAKMAK; HORST (1991) was adopted.

Enzyme assays

Roots and leaves (200 mg) were homogenized with 10% (v/v) polyvinylpolypyrrolidone and 1 ml phosphate buffer (50 mM; pH=7.8) containing 0,1% (v/v) triton x-100, 1 mM phenylmethylsulphonyl fluoride. After that, the homogenate was centrifuged at 12 000 g for 30 min at 4 °C. The supernatant was used to investigate enzyme activities. SOD and GPOX activities were determined as previously described by MHADHBI et al. (2005). The protein content of each sample was measured by adopting the method of BRADFORD (1976).

Total phenolic compounds analysis

Roots and leaves (1 g) were extracted using 10 ml pure methanol as solvent (M'SEHLI et al., 2008). For the total phenolic compounds' determination method of (M'SEHLI et al., 2008) was followed using Folin–Ciocalteu as a reagent. Total flavonoids analysis was carried out following DEWANTO et al. (2002) method.

Statistical analysis

In experiment 1, all studied parameters for primed seeds were statistically compared with those from non-primed seeds using one-way ANOVA. A two-way analysis of variance (ANOVA) was performed for the whole data in experiment 2 using the STATI-CF statistical software. Means were compared using the Newman Keuls test at $P<0.05$ when significant differences were found.

RESULTS

Experiment 1. Seed priming effect on germination process and seedling growth

The analysis of the data presented in Table 1 shows that, in *Vicia sativa*, seed priming treatments significantly improved total seed germination. Hormonal priming (100 µM SA) enhanced germination percentage as compared to KH_2PO_4 priming treatment. Optimum germination percentage (100%) was observed in 100 µM SA primed seed followed by 50 µM KH_2PO_4 primed seed (99%). Minimum germination percentage (92%) was observed in seeds primed with 100 µM KH_2PO_4.

Table 1. Effect of seed priming with three concentrations of KH_2PO_4 (25, 50 and 100 µM) and 100 µM SA on germination related parameters (TG: total seed germination; %; MGT: mean germination time; days; VI: vigor index; %; GI: germination index; %; conductivity test; %) in *Vicia sativa*. Untreated (immersed in H_2O) seeds were used as the control. Values followed by different letters are significantly different at $P<0.05$ according to the Newman-Keuls test.

Priming Treatments	TG	MGT	VI	GI	Conductivity test
Unprimed seeds (H_2O, control)	97±0.3 c	16±0.08 b	1080±22 ab	76.4±3.4 ab	11±0.7 b
Seeds primed with 25 µM KH_2PO_4	97±0.4 c	15.6±0.1 c	1047±42 ab	69.7±3 b	10.8±0.3 b
Seeds primed with 50 µM KH_2PO_4	99±0.6 b	16.5±0.1 a	1108±32 ab	76.3±1.2 ab	11.5±0.6 b
Seeds primed with 100 µM KH_2PO_4	92±0.2 d	15.3±0.07 d	614±54 c	43.5±5 c	16±1.3 a
Seeds primed with 100 µM SA	100±0.3 a	16.6±0.09 a	1145±68 a	78.8±2 ab	8.6±1 c

Seeds primed with 100 KH_2PO_4 had the lowest values in mean germination time (MGT), vigor index (VI), and germination index (GI). The highest values were noted in SA primed seeds followed by those primed with 50 µM KH_2PO_4. Seeds primed with 100 µM KH_2PO_4 practically showed the highest conductivity test values contrary to those primed with other treatments.

In *Vicia faba*, maximum seed germination percentage was related to seeds primed by SA. Seeds primed with 50 and 100 µM KH_2PO_4 resulted in lower MGT and GI than that of control. Furthermore, SA and 25 µM KH_2PO_4 had a positive effect on the vigor index (VI). But seed priming treatment with 50 and 100 µM KH_2PO_4 had not positive effects on VI and conductivity tests. Overall, seed priming with 100 µM SA was suitable compared to others (Table 2).

Table 2. Effect of seed priming with three concentrations KH_2PO_4 (25, 50 and 100 µM) and 100 µM SA on germination related parameters (TG: total seed germination; %; MGT: mean germination time; days; VI: vigor index; %; GI: germination index; %; conductivity test; %) in *Vicia faba*. Untreated (immersed in H_2O) seeds were used as the control. Values followed by different letters are significantly different at $P<0.05$ according to the Newman-Keuls test.

Priming Treatments	TG	MGT	VI	GI	Conductivity test
Unprimed seeds (H_2O, control)	99±0.2 b	16.5±0.1 b	989±34 b	51.08±2.8 a	13±0.5 b
Seeds primed with 25 µM KH_2PO_4	99±0.3 b	16.5±0.09 b	1073±38 a	52.1±2 a	13.3±0.7 b
Seeds primed with 50 µM KH_2PO_4	96±0.4 c	16±0.2 c	539.5±27 c	45.8±1.3 b	16. 9±0.4 a
Seeds primed with 100 µM KH_2PO_4	94±0.5 d	15.6±0.08 d	533.7±34 c	42.3±2.1 bc	17.7±1 a
Seeds primed with 100 µM SA	100±0.2 a	16.7±0.1 a	1068±47 a	50.7±1.6 a	8.4±0.7 c

Seed priming significantly influenced seedling shoot and root related parameters (length and biomass) in both studied species (Figure 1). For *Vicia sativa*, the maximum length and biomass were observed in seedlings from seeds primed with 100 µM SA and 50 µM KH_2PO_4. However, 100 µM KH_2PO_4 significantly decreased seedlings length (-40%) and biomass (-60%) as compared to the control seedlings (from non-primed seeds) (Figure 1A, B and Figure 2).

Data presented in Figure 1C and D indicates that, in *Vicia faba*, the biomass was increased up to 44% in seedlings primed with 100 µM SA and 25 µM KH_2PO_4 as compared to controls. However, seedling growth parameters were inhibited by a high concentration of KH_2PO_4 (50 and 100 µM) (Figure 2).

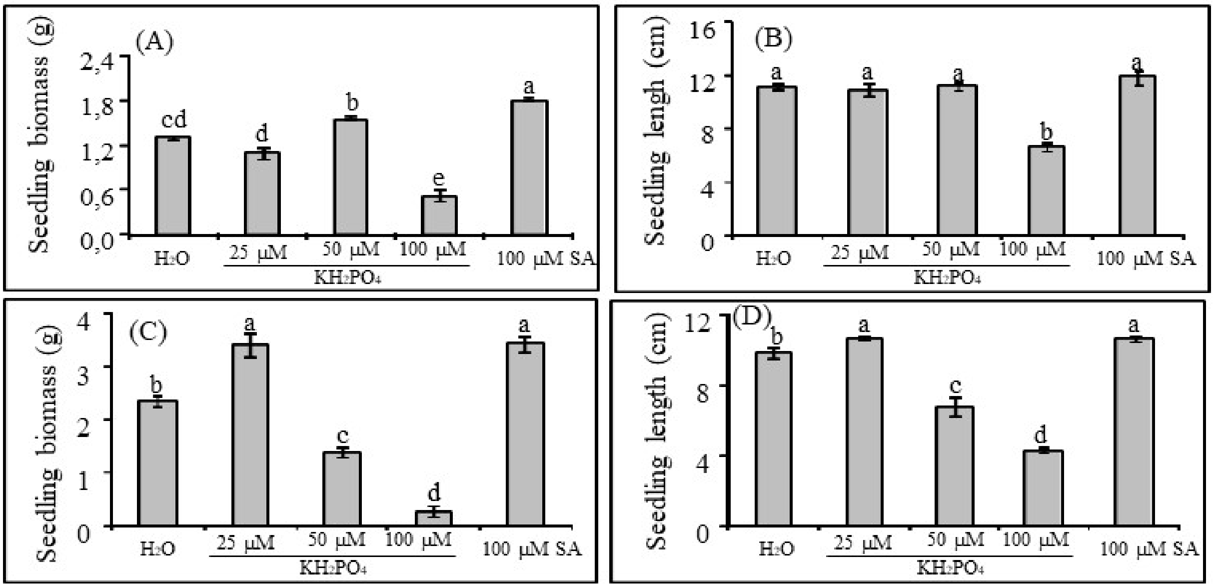

Figure 1. Effect of seed priming with three concentrations of KH_2PO_4 (25, 50, and 100 µM) and 100 µM SA on seedlings growth parameters (biomass and length) of *Vicia sativa* (A and B) and *Vicia faba* (C and D). Untreated (immersed in H_2O) seeds were used as the control. Values followed by different letters are significantly different at $P<0.05$ according to the Newman-Keuls test.

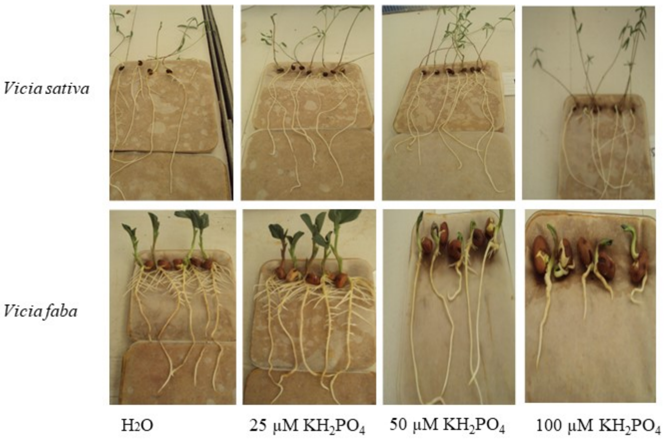

Figure 2. Effect of seed priming with three concentrations of KH_2PO_4 (25, 50, and 100 µM) on seedling growth (15-day-old) in *Vicia sativa* and *Vicia faba*. Untreated (immersed in H_2O) seedling was used as the control.

Experiment 2: seed priming effect on P deficiency tolerance improvement

Considering the main results found in experiment 1, 50 µM KH_2PO_4 and 25 µM KH_2PO_4 were defined as the optimal concentration for seed priming in *Vicia sativa* and *Vicia faba*, respectively. Additionally, in this part of the experiment, the impact of the direct addition of SA in the nutrient solution (expressed as SA. B) in P deficiency tolerance enhancement was considered.

Plant growth: it can be noticed from Figure 3 (A) and (B) that P deficiency (DD or ID) reduced significantly the total dry weight in both species. The seed priming with KH_2PO_4 and SA showed to be helpful in alleviating the depressive effect of P deficiency on plant growth. The results presented in Figure 3(A) and (B) demonstrated that the addition of 100 µM of SA directly in the hydroponic solution (SA. B) improve plant response to P deficiency in both species. For example, under ID treatment, we can see that the decrease in dry biomass which can reach up to 31% in control plants was less than 10% in plants received an exogenous application of SA.

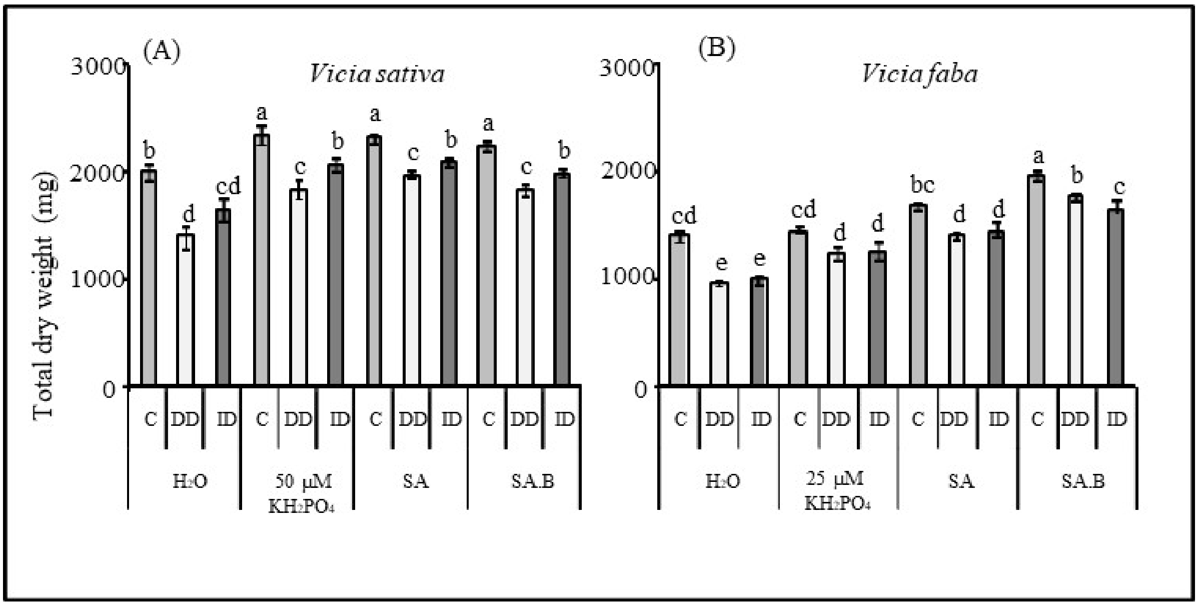

Figure 3. Effect of seed priming (KH_2PO_4 and SA) or the exogenous application of SA (SA. B) on total dry weight in *Vicia sativa* (A) and *Vicia faba* (B) under P deficiency conditions. Untreated (immersed in H_2O) seeds were used as the control. Values followed by different letters are significantly different at $P<0.05$ according to the Newman-Keuls test.

Plant phosphorus status and root acid phosphatase activity: the data presented in Figure 4 (A) and (B) showed that under phosphorus deficiency, a noticeable reduction in P content was showed in both species. The observed decrease became evident in plants from non-primed seeds; while it was less pronounced in P-deficient plants from seeds primed with KH_2PO_4, SA, or received an exogenous SA in hydroponic box.

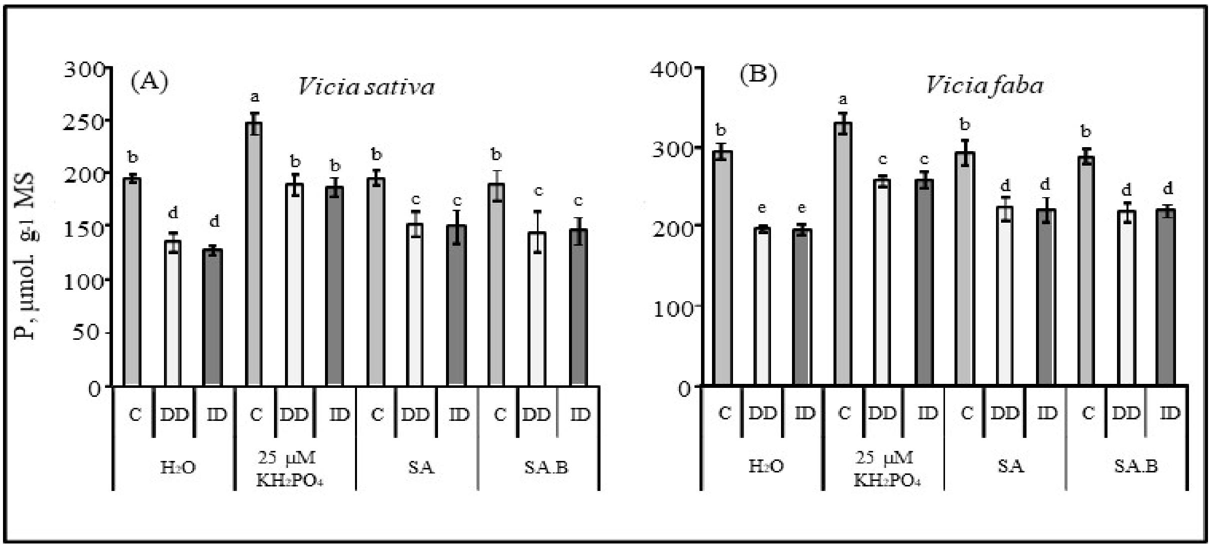

Figure 4. Effect of seed priming (KH_2PO_4 and SA) or the exogenous application of SA (SA. B) on plant P concentrations under P deficiency conditions in *Vicia sativa* (A) and *Vicia faba* (B). Untreated (immersed in H_2O) seeds were used as the control. Values followed by different letters are significantly different at $P<0.05$ according to the Newman-Keuls test.

The analysis of Figure 5 (A) and (B) showed that P deficiency led to a significant enhancement of acid phosphatase activity. The observed increase was more spectacular in plants from primed seeds with KH_2PO_4 and SA or those received an exogenous application of SA.

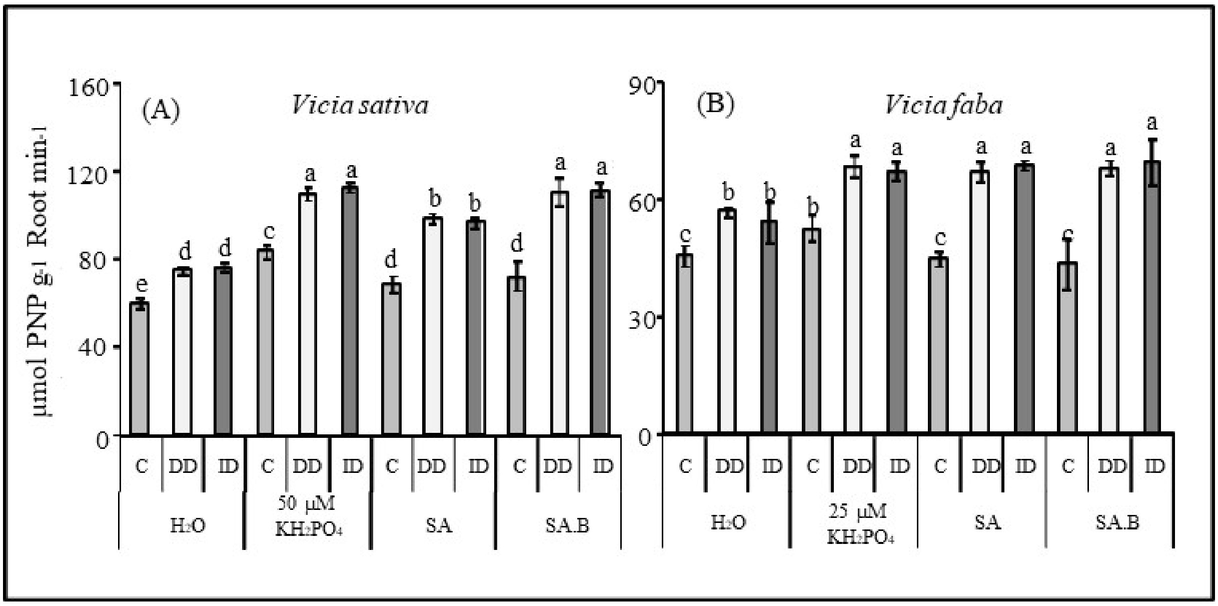

Figure 5. Effect of seed priming (KH_2PO_4 and SA) or the exogenous application of SA (SA. B) on roots acid phosphatase activity under P deficiency conditions in *Vicia sativa* (A) and *Vicia faba* (B). Untreated (immersed in H_2O) seeds were used as the control. Values followed by different letters are significantly different at $P<0.05$ according to the Newman-Keuls test.

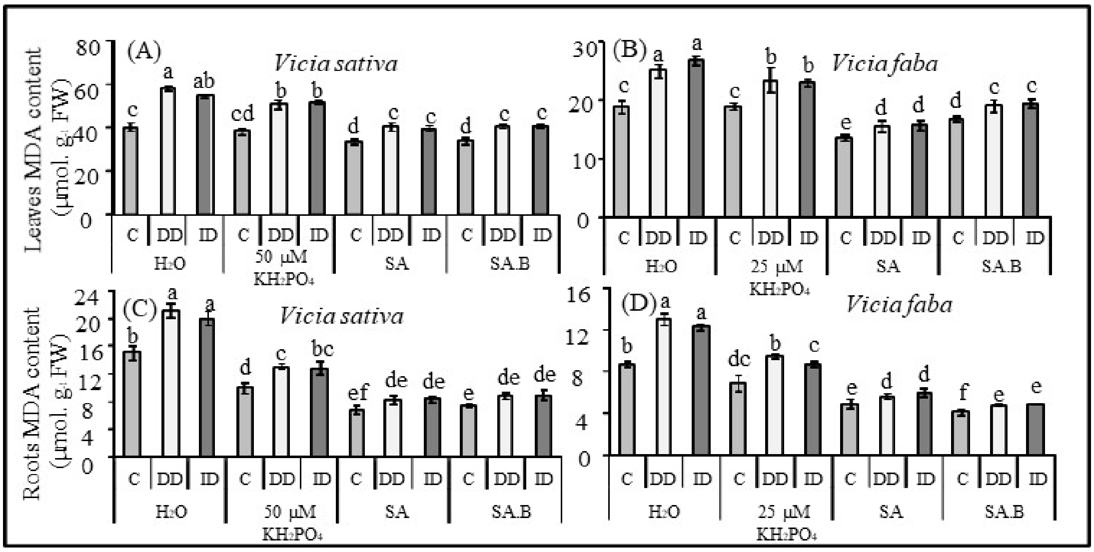

Figure 6. Effect of seed priming (KH_2PO_4 and SA) or the exogenous application of SA (SA. B) on roots and leaves malondialdehyde (MDA) concentration under P deficiency conditions in *Vicia sativa* and *Vicia faba*. Untreated (immersed in H_2O) seeds were used as the control. Values followed by different letters are significantly different at $P<0.05$ according to the Newman-Keuls test.

Leaves and roots MDA concentration: in *Vicia sativa*, P deficiency significantly induced MDA accumulation by 43% in leaves, as well as 40% in roots under DD treatment; and by 40% in leaves and 32% in roots under ID treatment (Figure 6 A and C). Both KH_2PO_4 and SA have a significant inhibitory effect on MDA accumulation under P deficiency conditions. The same applies to the exogenous application of SA. Similarly, in P-deficient plants of *Vicia faba*, the same inhibitor effect of seed primed with KH_2PO_4 and SA or the exogenous application of SA on MDA accumulation was illustrated (Figure 6 B and D).

Antioxidant defense system: the results presented in Table 3 and Table 4 revealed that P deficiency causes a significant increase of all antioxidant enzyme activities (SOD, GPOX, and CAT) in leaves and roots of P-deficient plants. This stimulator effect was significantly more pronounced in P-deficient plants from seeds primed with SA or those received an exogenous application of SA.

The same applies to the secondary metabolites; seed priming significantly influenced the average of secondary metabolites accumulation (polyphenols and flavonoids) in P-deficient plants. The highest concentration was detected in plants treated by SA (seed primed or exogenous application) whereas the lowest was observed in plants from non-primed seeds (Tables 3 and 4).

Table 3. Effect of seed priming with 50 µM KH_2PO_4, 100 µM SA or its exogenous application (SA.B) on SOD (USOD mg^{-1} Proteins), CAT (µmol H_2O_2 $min^{-1}mg^{-1}$ Proteins), GPOX (µmol H_2O_2 $min^{-1}mg^{-1}$ Proteins) activities and secondary metabolites production (polyphenols: mg GAE g^{-1} DW, flavonoids: mg CE g^{-1} DW) in leaves (L) and roots (R) of *Vicia sativa* under P deficiency conditions. Untreated (immersed in H_2O) seeds were used as the control. Values followed by different letters are significantly different at $P<0.05$ according to the Newman-Keuls test.

		SOD		**GPOX**		**CAT**		**Polyphenols**		**Flavonoids**	
Vicia sativa		L	R	L	R	L	R	L	R	L	R
H_20	C	6.30^{f}	11.5^{e}	0.31^{e}	1.72^{e}	3.3^{de}	1.89^{d}	12.54^{d}	10.75^{de}	13.78^{e}	9.07^{f}
	DD	7.59^{e}	12.82^{d}	0.4^{d}	2.06^{d}	3.96^{c}	1.91^{d}	13.76^{c}	11.54^{d}	14.89^{d}	10.64^{e}
	ID	7.92^{e}	12.87^{d}	0.42^{d}	2.57^{c}	4.1^{c}	1.9^{d}	13.9^{c}	11.89^{d}	14.67^{d}	11.74^{d}
KH_2PO_4	C	6.60^{f}	12.94^{d}	0.2^{f}	1.9d^{e}	3.98^{c}	1.98^{d}	12.78^{d}	11.82^{d}	13.76^{e}	10.67^{e}
	DD	8.32^{e}	14.06^{c}	0.34^{e}	2.2^{d}	4.83^{b}	2.09^{d}	13.65^{c}	12.08^{d}	15.08^{d}	11.69^{d}
	ID	8.47^{e}	13.95^{c}	0.47^{c}	2.5^{d}	4.75^{b}	1.93^{d}	13.79^{c}	12.12^{d}	14.98^{d}	11.93^{d}
SA	C	10.04^{d}	11.27^{e}	0.34^{e}	2.69^{c}	4.23^{c}	2.67^{c}	15.63^{b}	14.76^{c}	15.89c	13.56c
	DD	11.73^{c}	16.81^{a}	0.48^{c}	3.23^{c}	4.98^{b}	2.98^{b}	17.83^{a}	16.64^{b}	17.43b	15.53b
	ID	14.32^{b}	15.78^{b}	0.45^{d}	3.37^{b}	4.78^{b}	3.04^{b}	18.03^{a}	16.98^{b}	18.65a	15.96b
SA. B	C	8.51^{e}	13.17^{d}	0.33^{e}	3.04^{bc}	4.1^{c}	3.12^{b}	16.62^{b}	15.09^{c}	16.12^{c}	13.67^{c}
	DD	11.32^{c}	15.71^{b}	0.57^{b}	3.76^{a}	5.4^{a}	3.78^{a}	18.34^{a}	18.25^{a}	18.98^{a}	17.03^{a}
	ID	17.38^{a}	16.41^{b}	0.94^{a}	3.6^{a}	5.32^{a}	3.67^{a}	18.65^{a}	17.43^{b}	18.32^{a}	17.17^{a}

Table 4. Effect of seed priming with 25 µM KH_2PO_4, 100 µM SA or its exogenous application (SA.B) on SOD (USOD mg^{-1} Proteins), CAT (µmol H_2O_2 $min^{-1}mg^{-1}$ Proteins), GPOX (µmol H_2O_2 $min^{-1}mg^{-1}$ Proteins) activities and secondary metabolites production (polyphenols: mg GAE g^{-1} DW, flavonoids: mg CE g^{-1} DW) in leaves (L) and roots (R) of *Vicia faba* under P deficiency conditions. Untreated (immersed in H_2O) seeds were used as the control. Values followed by different letters are significantly different at $P<0.05$ according to the Newman-Keuls test.

		SOD		**GPOX**		**CAT**		**Polyphenols**		**Flavonoids**	
Vicia faba		L	R	L	R	L	R	L	R	L	R
H_20	C	7.02^{f}	31.92^{cd}	0.25^{b}	3.1^{d}	4.6^{c}	2.56^{d}	10.65^{d}	9.15^{f}	11.18^{e}	8^{f}
	DD	8.31^{e}	33.89^{c}	0.25^{b}	3.4^{d}	4.89^{c}	2.89^{cd}	12.58^{cd}	10.24^{e}	11.24^{e}	9.39^{e}
	ID	8.50^{e}	35.94^{b}	0.24^{b}	3.1^{d}	4.8^{c}	3.11^{c}	12.62^{cd}	10.89^{e}	11.67^{e}	9.74^{e}
KH_2PO_4	C	10.01^{d}	34.00^{c}	0.26^{b}	3.7^{c}	4.23^{d}	2.68^{d}	11.98^{d}	10.12^{e}	12.57^{d}	9.97^{e}
	DD	11.14^{c}	37.66^{a}	0.28^{ab}	4.4^{ab}	5.19^{b}	3.72^{bc}	13.65^{c}	13.78^{c}	14.74^{c}	12.89^{d}
	ID	11.32^{c}	37.05^{a}	0.29^{ab}	4.1^{b}	4.84^{c}	3.92^{bc}	13.79^{c}	13.72^{c}	14.98^{c}	13.13^{d}
SA	C	11.55^{c}	27.87^{d}	0.31^{a}	3.1^{d}	4.73^{c}	3.21^{c}	16.43^{b}	13.45^{c}	14.83^{c}	13.02^{d}
	DD	14.64^{a}	37.82^{a}	0.32^{a}	4.7^{a}	5.28^{b}	4.18^{b}	18.13^{a}	15.98^{a}	16.14^{ab}	14.93^{b}
	ID	14.06^{ab}	37.56^{a}	0.33^{a}	4^{b}	5.78^{a}	4.2^{b}	19.02^{a}	15.78^{a}	16.21^{ab}	15.16b
SA. B	C	11.02^{c}	24.96^{e}	0.31^{a}	2.7^{de}	4.71^{c}	4.1^{b}	17.22^{b}	14.29^{b}	15.92^{b}	14.07^{c}
	DD	13.15^{b}	38.38^{a}	0.3^{a}	4.6^{a}	6.03^{a}	4.67^{ab}	19.44^{a}	16.05^{a}	16.9^{a}	15.49^{b}
	ID	13.11^{b}	38.09^{a}	0.32^{a}	3.9^{b}	5.96^{a}	4.79^{a}	19.5^{a}	16.13^{a}	16.92^{a}	16.17^{a}

DISCUSSION

Influence of salicylic acid on germination behavior, early growth stage and P deficiency tolerance

The obtained results depicted that *Vicia sativa* and *Vicia faba* seeds primed with 100 µM SA exhibited a higher germination percentage, germination index (GI), and vigor index (VI) than the non-primed ones. In addition, SA-primed seeds are characterized by a lowest conductivity test testifying a high seed quality. The current findings are confirmed with those of ROYCHOUDHURY et al. (2016) and AHMAD et al. (2017) who highlighted the promotive effects of SA in germination behavior under abiotic stress. LEE et al. (2010) stated that SA could stimulate or inhibit seed germination as a function of the concentration used. In fact, those researchers found that higher concentrations of SA (SA > 100 µM) inhibited Arabidopsis seeds germination. Most previous reports investigated the effect of SA concentrations in seed germination. They deduced that the restrain effects of high SA concentrations may be caused by the toxic effects (RAO et al., 1997; ALONSO-RAMIREZ et al., 2009; LEE et al., 2010).

Several reports have described the beneficial effects of seed priming with SA on early seedling growth. In the present investigation, results showed that seeds primed with 100 µM SA caused considerable enhancement of early growth of seedlings (dry weight, length) compared to controls, in both species. The findings were corroborative of the early reports of SAKHABUTDINOVA et al. (2003) in wheat and REHMAN et al. (2015) in maize. The better seed germination/ seedling growth depicted in seed primed with SA might be attributed to higher α-amylase activity and total soluble sugar contents in primed seeds (WANG et al., 2016).

To date, the effectiveness of seed priming with SA on P deficiency tolerance improvement has not been thoroughly investigated. This part of the present work was conducted to check the influence of SA-seed priming on P deficiency tolerance improvement in *Vicia faba* and *Vicia sativa*.

The present study clearly indicates that plant growth was reduced in both species when they were grown under P deficiency conditions. Our findings pointed out also that SA treatment (seed priming or exogenous application) could be quenched this dramatic effect of stressful conditions on plant biomass. The observations are consistent with earlier findings indicating the ameliorative effect exerted by SA treatment on plant growth potential confronted with different abiotic stresses (KHAN et al., 2015; NOREEN et al., 2017). Interesting research found that P, K, Mg, and Mn concentrations of SA-treated plants were increased under stressed conditions and these findings proposed that SA could be used to improve plant growth and mineral status under stress conditions (PER et al., 2017). Additionally, KONG et al., (2014) reported that low concentrations of SA could alleviate chlorosis by improving Fe absorption and increasing chlorophyll concentrations.

In this experiment, P deficiency significantly decreased P concentration compared with control treatment. This inhibitory effect was significantly alleviated by SA treatments (seed priming or its exogenous application in nutrient solution). The role of SA treatment in plant P status promotion under stressful conditions could be explained by the higher stimulation of acid phosphatases activity (APase) in SA-treated plants. These enzymes are induced by P deficiency and they are involved in Pi acquisition in plants (MEHRA et al., 2017).

Various researchers suggested the implication of SA in the modulation of antioxidant metabolism to stimulate plant-tolerance to abiotic stresses (HASANUZZAMAN et al., 2014). In a recent study, KOHLI et al. (2017) have reported that salicylic acid enhanced the level of plant tolerance to heavy metal by up-regulating the antioxidative system defense.

Data from this study indicated that exogenous applications of SA or its use in seed priming were effective in decreasing MDA concentration under P deficiency treatments in both species. This fact was positively correlated with the stimulation of antioxidant system defense (antioxidant enzymes and accumulation of secondary metabolites) that increased membrane stability and tolerance for *Vicia faba* and *Vicia sativa* to P deficiency. The results corroborated well the early findings of HUSSAIN et al. (2016) who observed that oxidative stress caused by abiotic stress including P deprivation was effectively mitigated with selenium- or salicylic acid-priming in rice. Summarizing the findings, it might be concluded that SA treatment whether by its exogenous application or by seed priming may enhance plant tolerance to P deficiency.

Influence of KH_2PO_4 on germination behavior, early growth stage and P deficiency tolerance

Our findings showed that KH_2PO_4, depending on its concentration, decreases or enhances germination processes, seed quality, and early seedlings growth of *Vicia sativa* and *Vicia faba*. Out of three different concentrations used for KH_2PO_4 as a priming agent in the present experiment, 25 µM, and 50 µM KH_2PO_4 were found to have the best results in *Vicia faba* and *Vicia sativa*, respectively. These concentrations were selected to examine the possibility of using KH_2PO_4 as a priming agent to improve P deficiency tolerance in *Vicia sativa* and *Vicia faba*.

Previously, it was been reported that seed priming with the limited nutrient element was more effective in overcoming the nutrient deficiencies problem and improving plant growth on deficient soil, comparing to soil or foliar applications (FAROOQ et al., 2012). To date, the contribution of KH_2PO_4 to improve plant tolerance to phosphorus deficiency was not well explored. To our knowledge, AJOURI et al. (2004) are the only researchers who have investigated the implication of seed priming with KH_2PO_4 in the enhancement of germination performance and seedling growth in barley under P deficiency and they found that this technique can improve the germination of barley and increase the seed nutrient content. The present work provided further confirmation of their conclusion. The analysis of our results showed that, in both species, KH_2PO_4-treated seedlings have higher growth compared to control ones under P deficiency conditions.

Besides, the same pattern was observed for plant P nutrition illustrating that the highest P content was detected in seedling treated with KH_2PO_4 by stimulating acid phosphatases activity (APase).

Taken together, the maintenance of plant biomass and P uptake improvement made seedlings treated by KH_2PO_4 more efficient to overcome P deficiency conditions.

Data on lipid peroxidation showed that MDA production was intensified under P deficiency in both species. However, our results revealed that P-deficient plants that emerged from KH_2PO_4-primed seeds manifested significantly lower MDA contents.

The observed reduction in MDA content by KH_2PO_4-priming suggests an effective antioxidative mechanism. In the present research, the highest activities of antioxidant enzymes and secondary metabolites (polyphenols and flavonoids) contents were recorded in KH_2PO_4-treated plants. In this paper, we highlight the first time the ameliorative effect of seed priming with KH_2PO_4 on P-deficiency tolerance by alleviating oxidative stress in *Vicia sativa* and *Vicia faba*.

CONCLUSION

Results showed that seed priming with KH_2PO_4, SA, or the exogenous application of SA could enhance P deficiency tolerance in *Vicia sativa* and *Vicia faba* without affecting the performance of seeds germination. This mainly results from the enhancement of plant growth, P nutrition, and alleviates oxidative stress in treated plants under P deprivation conditions. Surprisingly, the comparison of KH_2PO_4 and SA effects on P deficiency tolerance lets us deduce that KH_2PO_4 aided the plants to overcome P deficiency by supporting a suitable P acquisition, whereas, SA by stimulating the antioxidant system defense to scavenging ROS. Overall, we propose the potential application of seed priming procedure by KH_2PO_4 and SA for improving plant tolerance to P deficient soils.

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RESUMO: A deficiência de fósforo (P) é um fator ambiental adverso comum que limita a produção agrícola em todo o mundo. Este estudo é uma avaliação do efeito benéfico da técnica de priming de sementes para tolerância à deficiência de P em *Vicia faba* e *Vicia sativa*. Para avaliar o impacto do condicionamento das sementes no comportamento germinativo de Vicia faba e Vicia sativa, suas sementes foram imersas em diferentes concentrações de KH_2PO_4 (25 µM, 50 µM e 100 µM) e em 100 µM de ácido salicílico (SA) por 24 h. Os resultados obtidos definiram KH_2PO_4 50 µM (para *Vicia sativa*) e KH_2PO_4 25 µM (para *Vicia faba*) como as concentrações ótimas que garantem uma melhor germinação das sementes. Além disso, os dados mostraram que a SA melhora a germinação de sementes e o estabelecimento de mudas. Posteriormente, para investigar a contribuição dessa técnica no aumento da tolerância à deficiência de P, sementes preparadas e não preparadas foram cultivadas em solução hidropônica com três tratamentos diferentes: controle (C, meio contendo concentração suficiente de P: KH_2PO_4 360 µM), deficiente em fósforo direto (DD, meio contendo apenas 10 µM de KH_2PO_4) e deficiência induzida de P por bicarbonato (ID, meio contendo concentração suficiente de P: 360 µM de KH_2PO_4 + 0,5 g l-1 de CaCO3 + 10 mM de NaHCO3). Além disso, o papel da SA exógeno aplicada no aumento da tolerância à deficiência de P foi explorado. A preparação das sementes ou a aplicação exógena de SA reduziu significativamente o efeito da severidade da deficiência de P. De fato, as plantas pré-tratadas foram observadas mais tolerantes à deficiência de P, refletidas no aumento significativo da biomassa da planta, na absorção de P e em um eficiente sistema antioxidante. No geral, este artigo destaca o efeito benéfico da priming de sementes ou a aplicação exógena de SA na melhoria da tolerância das plantas à deficiência de fósforo.

PALAVRAS-CHAVE: Deficiência de P. KH2PO4. Estresse abiótico. Preparação de sementes.

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