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## Identifying roles of the scion and the rootstock in regulating plant development and functioning under different phosphorus supplies in grapevine

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1       **Identifying roles of the scion and the rootstock in regulating plant**  
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3       **development and functioning under different phosphorus supplies in**  
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5       **grapevine**  
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## Abstract

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4 Phosphorus is essential for plant life and plants have developed numerous strategies to maximise  
5 phosphate (Pi) acquisition and use under limited Pi supply. Here we have used reciprocal grafting to  
6 determine whether the shoot and root have different roles in regulating some of these strategies.  
7 Reciprocal grafts of two grapevine genotypes (*Vitis vinifera* cv. Pinot noir and *V. berlandieri* x *V.*  
8 *rupestris* cv. 1103 Paulsen) were produced as well as the corresponding homo-graft controls; the  
9 plants were grown in hydroponic culture and subjected to two levels of Pi supply (high (0.6 mM) or  
10 low (0.001 mM)). Biomass accumulation, root morphology, and root, stem and leaf organic acid,  
11 phosphate, nitrate and sulphate concentrations were measured. The transcript abundance of  
12 orthologues of known phosphate starvation induced (PSI) genes from Arabidopsis was also  
13 quantified. Under high Pi, the scion genotype had a large impact on plant growth, but the  
14 morphology of roots (such as root tip density) was not affected. Low Pi supply affected growth, tissue  
15 organic acid concentration, the activity of acid phosphatases released by the roots and the  
16 expression of PSI genes. Rootstock genotypes showed differences in root responses to low Pi supply,  
17 but the scion also exerted long-distance regulation of rootstock responses to low Pi, for example,  
18 modifying PSI gene expression, sulphate acquisition efficiency, the activity of acid phosphatases  
19 released by the roots and root organic acid concentrations. This work shows for the first time that  
20 the grapevine genotypes differ in their response to low Pi supply and that the scion can modify  
21 rootstock responses to the nutrient availability. This work highlights that genetic variation in shoot-  
22 borne signals can regulate root responses to Pi supply and that understanding rootstock responses to  
23 the environment must be done considering scion influence.  
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## Introduction

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2 Phosphorus is an essential macronutrient for plants, it is part of essential macromolecules (e.g.  
3 nucleic acids or phospholipids) and involved in energy transfer (Marschner, 1995). Phosphorus is  
4 taken up from the soil solution by roots in its inorganic form (Pi, orthophosphate) by transporters of  
5 the INORGANIC PHOSPHATE TRANSPORTER1 (PHT1) family (Nussaume *et al.*, 2011). *INORGANIC*  
6 *PHOSPHATE TRANSPORTER1* transcript levels are controlled by different regulators such as  
7 PHOSPHATE STARVATION RESPONSE1 (PHR1, which also regulates the expression of other  
8 PHOSPHATE STARVATION INDUCED (PSI) genes), microRNA 399 and SYG/PHO81/XPR1 (SPX) proteins  
9 (Duan *et al.*, 2008; Rubio *et al.*, 2001). Furthermore, PHT1 transporters are regulated at the post-  
10 transcriptional level by degradation by PHOSPHATE2 (PHO2), a protein with an E2 ubiquitin  
11 conjugase domain (Bari *et al.*, 2006).

12 Available Pi accounts for a small fraction of total phosphorus present in the soil. Phosphate  
13 concentrations in the soil solution are low, whilst a large proportion of phosphorus is held in the soil.  
14 For example, it can be bound to iron and aluminium-(hydr)oxides in acid soils, or it can occur in  
15 calcium-phosphate forms in neutral to alkaline soils (Hinsinger, 2001). In addition, a high  
16 concentration of organic phosphorus is found in soil, which requires hydrolysis before being taken up  
17 by plants (Robinson *et al.*, 2012). Root-rhizosphere interactions can modify soil properties to increase  
18 Pi availability via the following mechanisms: (1) releasing protons or hydroxide ions, resulting a  
19 change of rhizosphere pH (Hinsinger, 2001; Hinsinger *et al.*, 2003; Liu *et al.*, 2016); (2) releasing  
20 organic acids (e.g. malate or citrate) (Bolan *et al.*, 1994; Gahoonia *et al.*, 2000), resulting a  
21 complexation of ions such as iron or aluminium involved in phosphorus sorption (Jones, 1998); or (3)  
22 releasing acid phosphatases (APases; EC 3.1.3.2, which are encoded by *PURPLE ACID PHOSPHATASE*  
23 (*PAP*) genes), enzymes which catalyse the hydrolysis of organic phosphorus into Pi (Lu *et al.*, 2016;  
24 Robinson *et al.*, 2012).

25 In addition to modifying the rhizosphere to increase Pi availability, under low Pi supply plants can  
26 also modify biomass allocation, root system architecture and morphology, gene expression and Pi  
27 transport to increase root foraging capacity (Lan *et al.*, 2015; Lynch, 2011; Morcuende *et al.*, 2007;  
28 Pant *et al.*, 2015). This is often mediated by the preferential allocation of biomass to the roots rather  
29 than the shoots, by the increase in the degree of lateral root branching, and by the increase in the  
30 number and length of root hairs (Lynch, 2011). Low Pi supply induces the expression of many PSI  
31 genes, for example, genes coding for PHT1s, which can increase root Pi transport capacity (Lan *et al.*,  
32 2015). As low Pi reduces plant growth, metabolites such as starch, sugars and many amino acids  
33 increase in concentration, while phosphorylated metabolites such as hexose phosphates, ATP and  
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1 ADP decrease in concentration (Morcuende *et al.*, 2007). Tissue organic acid concentrations often  
2 change in response to Pi supply to maintain internal pH balance (Vance *et al.* 2003).  
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4 One method to determine the roles of the shoot and root in regulating plant responses to the  
5 environment is by grafting two different genotypes together. Grafting is an old horticultural  
6 technique used for the cultivation of perennial fruit crops (*e.g.* grape, apple, citrus, etc.) and annual  
7 vegetables and fruits (*e.g.* tomatoes, cucumber and pepper) (Mudge *et al.* 2009; Bie *et al.* 2017). In  
8 horticulture, grafting allows us to combine desirable traits of the shoot and the root of two different  
9 genotypes in a single plant. Rootstocks have been selected for various agronomical traits such as  
10 controlling scion growth (i.e. inducing dwarfing and modifying scion architecture), improving water  
11 use efficiency/drought tolerance, altering mineral element status, improving yield and fruit quality,  
12 and providing resistance to root pathogens (Nawaz *et al.* 2016; Warschefsky *et al.* 2016). Since the  
13 19<sup>th</sup> century, the Eurasian grapevine species (*Vitis vinifera*) is cultivated grafted using North American  
14 *Vitis spp.* as rootstocks because of their tolerance to the American aphid pest Phylloxera. In addition  
15 to providing Phylloxera resistance, grapevine rootstocks are also chosen based on their known  
16 adaptation to soil conditions and ability to control scion vigour (Ollat *et al.*, 2016). Grapevine  
17 rootstocks are known to modify the mineral element composition in the scion (Cordeau 1998;  
18 Bavaresco *et al.* 2003) and these changes are linked to the genetic background of the rootstock  
19 (Gautier *et al.*, 2020b). Grapevine rootstocks modify plant responses to nutrient supply, such as  
20 nitrogen (Lecourt *et al.* 2015; Cochetel *et al.* 2017), potassium (Ruhl 1989, 1991), phosphorus (Grant  
21 and Matthews 1996b, a; Gautier *et al.* 2018) and iron (Jiménez *et al.* 2007; Covarrubias and Rombolà  
22 2015; Covarrubias *et al.* 2016).  
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38 As grapevine rootstocks are known to modify scion phosphorus content and plant responses to Pi  
39 supply, we used reciprocal grafting of two widely used grapevine genotypes to explore the roles of  
40 both the shoot and the root in regulating Pi content and plant responses to Pi supply in grafted  
41 grapevine. The genotypes chosen were a *V. vinifera* scion obtained from the sexual reproduction  
42 from self-pollination of Pinot Noir, named 40024 (PN), which grows well in *in vitro* culture and grafts  
43 easily, and a rootstock genotype of American origin, *V. berlandieri* x *V. rupestris* cv. 1103 Paulsen  
44 clone 198 (1103P), which confers a high scion vigour and petiole phosphorus concentration in the  
45 vineyard (Gautier *et al.*, 2020b). These two genotypes were also chosen because they differ in their  
46 phosphorus acquisition efficiency in the first 21 d of development when grown as woody cuttings  
47 (Gautier *et al.*, 2018). Firstly, we aimed to determine if the scion and/or rootstock alters plant  
48 growth, biomass allocation, Pi content and phosphate acquisition efficiency (PAE) in non-limited Pi  
49 supply. Secondly, we assessed the growth response of grafted grapevines to low Pi supply and  
50 quantified the expression of different *PHT1* genes. In addition, because Pi acquisition interacts with  
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1 both sulphate and nitrate uptake, both sulphate and nitrate acquisition efficiencies were calculated.  
2 Thirdly we asked does the scion/rootstock combination affect the exudation of APases and the  
3 abundance of *PAP* transcripts. And finally we asked whether the scion/rootstock combinations  
4 differed in tissue organic acid concentration and whether organic acid concentrations respond to Pi  
5 supply. This was achieved by comparing all possible scion/rootstock combinations of two grapevine  
6 genotypes grown under two contrasted Pi supplies.  
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## 10 **Materials and methods**

### 11 *Plant material and growing conditions*

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13 All four possible scion/rootstock combinations of 1103P and PN were micro-grafted using the cleft  
14 grafting system, i.e. 1103P/1103P, 1103P/PN, PN/1103P and PN/PN and grown as previously  
15 described (Gautier *et al.*, 2020a). Both genotypes were introduced into *in vitro* culture after surface  
16 sterilisation. Plants were cultivated *in vitro* on McCown Woody Plant Medium (Duchefa)  
17 supplemented with 30 g L<sup>-1</sup> sucrose, 0.27 μM 1-naphthalene acetic acid and 0.4 % agar in a growth  
18 chamber at 22 °C and with a photoperiod of 16 h light/8 h dark at a photon flux density of 55 μmol  
19 m<sup>-2</sup> s<sup>-1</sup> (Supplementary Fig. 1A). Six-week-old plantlets were then acclimated for four weeks to  
20 perlite-filled pots, irrigated with water, in a growth chamber at 26 °C and with a photoperiod of 16 h  
21 light/8 h dark at a photon flux density of 145 μmol m<sup>-2</sup> s<sup>-1</sup> at plant level (Supplementary Fig. 1B).  
22 Plants were then transferred into hydroponic culture with an aerated solution; each pot contained 2  
23 plants of the same scion/rootstock combination with 700 mL of high P solution (HP, 0.6 mM P). Four  
24 days later, half the pots continued receiving the HP solution and the other half were subjected to a  
25 low P (LP, 0.001 mM P) treatment (Supplementary Fig. 1C and D). The macronutrient composition  
26 was 2.45 mM KNO<sub>3</sub>, 0.69 mM MgSO<sub>4</sub> and 1.27 mM CaCl<sub>2</sub> for both the HP and LP solutions; HP  
27 solution also contained 0.6 mM KH<sub>2</sub>PO<sub>4</sub> and 0.6 mM CaSO<sub>4</sub>, whereas the LP solution contained 0.3  
28 mM K<sub>2</sub>SO<sub>4</sub> and 0.3 mM CaSO<sub>4</sub>. Micronutrients were supplied as 46 μmol H<sub>3</sub>BO<sub>3</sub>, 9.1 μmol MnCl<sub>2</sub>, 2.4  
29 μmol ZnSO<sub>4</sub>, 0.5 μmol CuSO<sub>4</sub> and 14 nmol (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>, and iron was supplied as 8.5 mg L<sup>-1</sup>  
30 Sequestrene 138 (i.e. 31.3 μmol ethylenediamine-N,N'-bis (2-hydroxyphenylacetic acid) NaFe,  
31 Syngenta Agro S.A.S.). The solutions were changed once a week for 28 d.  
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### 50 *Plant growth measurements*

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52 After 28 d of either LP or HP treatment, five plants were used for root system morphology analysis,  
53 five plants were used the quantification of acid phosphatases released by the roots and five plants  
54 were harvested for metabolite profiling and the quantification of transcript abundance. For each  
55 plant, leaves, stems and roots were weighed (fresh weight, FW).  
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### *Root system morphology analysis*

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2 Images of the whole root system were captured using an Epson 1640XL scanner and 2D images were  
3 analysed using WinRHIZO software (Regent Instruments Inc., 1996). The total number of root tips  
4 (both primary and lateral roots), root average diameter and total root length were measured.  
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### *Activity of APases released by the roots*

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10 Plants were transferred to 40 mL of aqueous solution containing 10 mmol of p-nitrophenyl  
11 phosphate (pNPP), covered with aluminium foil for 2 h in the growth chamber. The reaction was  
12 stopped by the addition of 1 mL of NaOH 1M, the volume of solution remaining and root FW were  
13 measured. p-nitrophenyl (pNP) concentration was measured by spectrophotometer at 405 nm and  
14 APase activity was calculated as mmol of pNP produced per g root FW per h.  
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### *Metabolite profile of plant tissues*

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22 Root, stem and leaf samples were frozen in liquid nitrogen and kept at -80 °C for metabolite  
23 measurements. Samples were ground using a Retsch™ MM400 cooled with liquid nitrogen.  
24 Metabolites were extracted from 40 mg FW in aqueous ethanol at 80 °C in three incubation steps  
25 each lasting 20 min (step 1: 700 µL 80 % ethanol; step 2: 700 µL 50 % ethanol; and step 3: 300 µL 50  
26 % ethanol) and then centrifuged for 10 min at 4800 g. Supernatants were pooled. The ethanol was  
27 allowed to evaporate using speed-vac and the dry extracts were re-suspended in 1.7 mL of distilled  
28 water. Phosphate, nitrate, sulphate and organic acids (malate, citrate and tartrate) were identified  
29 and quantified by ion exchange chromatography ICS-5000 HPIC system (Thermo Scientific Dionex),  
30 using an IonPac® AS11 analytical column (250 mm × 4.0 mm) with an isocratic gradient of KOH from 4  
31 to 28 mM for 16 min, with a volume of sample injected of 20 µL. Phosphate, sulphate and nitrate  
32 acquisition efficiencies were calculated using the whole plant Pi, sulphate and nitrate contents  
33 respectively by root FW (we assume that the Pi, sulphate and nitrate contents, and root biomass at  
34 the beginning of the experiment are negligible).  
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### *Quantification of transcript abundance*

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48 Three root tips (~15 mm in length) from each plant that was used for metabolite measurement were  
49 harvested and immediately snap-frozen in liquid nitrogen. Total RNA of samples was extracted using  
50 the Spectrum Plant Total RNA kit (Sigma-Aldrich) with some modifications as described by Cookson *et*  
51 *al.* (2013). Total RNA (1.5 µg) was reverse transcribed into cDNA using the SuperScript III First-Strand  
52 Synthesis System (Invitrogen). Quantitative reverse transcription polymerase chain reactions (RT-  
53 qPCRs) were performed using SYBR Green on an iCycler iQH (Bio-Rad) according to the procedure  
54 described by the supplier, with 0.2 µM of primers for each gene. Transcript abundance was  
55 calculated as normalized relative quantities (NRQs), with the reference genes ACTIN, GAPDH and  
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SAND3' for normalization (Hellemans et al. 2007). Primer sequences are listed in Supplementary Table 1.

The identification of *PHT1*, *PHO2*, *SPX DOMAIN CONTAIN PROTEINS* and *PAP* genes in grapevine was based on protein sequence similarity with *Arabidopsis thaliana* (L.). Analysis was performed using the Arabidopsis protein sequence and the putative grapevine proteins were selected on the basis of the highest sequence homology value of their predicted coding sequences (the threshold value for sequence homology was set at 85 %). Sequences of Arabidopsis genes were obtained from TAIR (<https://www.arabidopsis.org/>), and the predicted sequences in grapevine were identified using CRIBI (<http://genomes.cribi.unipd.it/grape/>).

### *Statistical analysis*

All statistical analyses were performed using the software R (R Core Team, 2018). The effect of Pi supply and scion or rootstock genotype on plant biomass and biomass allocation was tested using t-test or Wilcoxon non-parametric tests respectively at *P* value < 0.05 with the Bonferroni correction, when assumptions for parametric tests were met. For multivariable analysis, a multiple comparison test after a Kruskal-Wallis test at *P* value < 0.05 was used when assumptions for parametric tests were not respected, using the function *kruskalmc* from the *pgirmess* R package. Letters to indicate significant differences among multiple comparisons were obtained using the function *multcompLetters* from the R package *multcompView*. In the case of assumptions for parametric tests were respected, data were analysed using two-way or three-way analysis of variance (ANOVA *P* value < 0.05, with Tukey's Honest Significant Difference test), with combination and P supply, scion and/or rootstock as factors.

## **Results**

### ***Growth of grafted grapevine is mainly controlled by the scion genotype and affected by phosphate supply***

Four scion/rootstock combinations of the grapevine genotypes PN and 1103P (1103P/1103P, 1103P/PN, PN/1103P and PN/PN) were grown in aerated hydroponic culture on high phosphate (HP) supply, and then half of them were transferred to low phosphate (LP) supply (whilst the remaining half continued to receive HP). Stem, leaf and root biomass was measured (Fig. 1A-C). Effect of scion and rootstock genotypes on biomass, biomass allocation and root morphology was analysed by grouping together data from plants grown on HP by common scion or rootstock genotypes (Supplementary Table 2); this is because the data did not meet the assumptions for a 3-way ANOVA. This analysis showed that the scion genotype had a significant impact on leaf, stem and root biomass; leaf, stem and root biomasses were over 2-fold higher in plants with an 1103P scion compared to PN

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regardless of rootstock genotype. Root mass fraction (root:whole plant biomass ratio) was not affected by either the scion or rootstock genotype (Fig. 1D, Supplementary Table 2). However, the scion genotype did modify leaf mass fraction (leaf:whole plant biomass ratio, Fig. 1E, Supplementary Table 2), which was higher in grafts with 1103P scions, and both the scion and rootstock modified the stem to whole shoot mass fraction (stem:whole shoot biomass ratio, Fig. 1G) (Supplementary Table 2).

After 28 d of LP supply, stem biomass was lower in all scion/rootstock combinations than the HP treatment (Fig. 1.A); whereas leaf biomass was only lower in LP than HP in plants with an 1103P scion (Fig. 1.B). Root biomass was not affected by P supply (Fig. 1.C); consequently root mass fraction was higher in plants grown on LP than HP for all scion/rootstock combinations (Fig. 1.D). Leaf mass fraction was only modified by LP in 1103P/1103P (Fig. 1.E) and stem to whole shoot mass fraction was lower in plants growing on LP than HP in all scion rootstock combinations (Fig. 1.F).

The total number of tips per root system and the average diameter of roots were measured, and root tip density (i.e. the number of tips per cm of root) was calculated (Fig. 2.). This analysis showed that the 1103P scion increased the total number of root tips relative to PN (Fig. 2A, Supplementary Table 2), which was associated with the higher root biomass induced by 1103P scions (Fig. 1C). The PN rootstock had a higher number of tips than 1103P (Fig. 2A, Supplementary Table 2), without necessarily being associated with a higher root biomass. The root tip density was affected by the rootstock; PN roots had a higher root density compared to 1103P (particularly with an 1103P scion) (Fig. 2B, Table 1). Root diameter was not affected by either the scion or the rootstock genotype (Fig. 2C).

The total number of root tips was not different between the LP and HP treatments (Fig. 2A). However, the roots of the two hetero-grafts (i.e. 1103P/PN and PN/1103P) had a higher tip density in plants grown in HP than LP (Fig. 2.B) and average root diameter was lower in plant growing on LP than HP (Fig. 2.C).

### ***Homo-grafted grapevines have differences in phosphate and sulphate, but not nitrate acquisition efficiencies***

At the end of the 28 d treatment, the concentrations of Pi, nitrate and sulphate in leaves, stems and roots were quantified; the LP treatment reduced Pi concentration in both the leaves and the roots in all scion/rootstock combinations, but there were no significant differences between the different scion/rootstock combinations (Fig. 3). Nutrient acquisition efficiencies were estimated (assuming that the concentrations of Pi, nitrate and sulphate were negligible at the beginning of the treatment) by dividing total plant nutrient content by root FW (Fig. 4). The homo-grafts had differences in PAE

1 under HP, with PN/PN having a higher efficiency than 1103P/1103P, while the hetero-grafts had  
2 intermediate values; PAE was very low and there were no differences in PAE between the  
3 scion/rootstock combinations grown under LP (data not shown). Nitrate acquisition efficiency (NAE)  
4 was not affected by LP supply or the scion/rootstock combination (Fig. 4.B). Under HP, sulphate  
5 acquisition efficiency (SAE) in 1103P/1103P was higher than in PN/PN, while the hetero-grafts had  
6 intermediate values (Fig. 4.C), i.e. the opposite of what was found for PAE (Fig. 4.A). Sulphate  
7 acquisition efficiency was not affected by LP supply in any scion/rootstock combination, although  
8 there was a tendency for SAE to decrease in response to LP when the grafts had an 1103P scion (Fig  
9 4.C).

### 16 ***Activity of APases released by the roots is higher under LP than HP***

18 After 28 d of LP or HP treatment, the activity of APases released into the media by the roots was  
19 measured and normalized by root FW (Fig. 5). The activity of APases released by the roots was two-  
20 fold higher under LP compared to HP in all scion/rootstock combinations. The activity of APases  
21 released by the roots was affected by both the scion and rootstock genotypes, and the interaction  
22 between the scion and rootstock. Under HP, the PN scion increased APase release relative to the  
23 1103P scion when the rootstock was 1103P.

### 29 ***The scion genotype modified organic acid concentrations in the root, while the rootstock genotype only affected root organic acid concentrations***

33 Malate, tartrate and citrate concentrations were measured in leaves, stems and roots of grafted  
34 grapevine grown under HP and LP (Fig. 6). Effect of scion and rootstock genotypes on tissue organic  
35 acid concentrations of plants grown on HP was analysed by grouping together data from the grafted  
36 plants by common scion or rootstock genotypes (Supplementary Table 3); this is because the data did  
37 not meet the assumptions for a 3-way ANOVA.

43 In leaves, total organic acid concentration (i.e. the sum of malate, tartrate and citrate concentration)  
44 was not affected by the scion or rootstock genotype (Fig. 6.A); however, the proportion of malate  
45 and tartrate was affected by the scion genotype (Fig. 6.B; Fig. 6.C). Grafts with an 1103P scion had  
46 approximately 10-fold more tartrate compared to malate, while combinations with a PN scion had  
47 approximately equal malate and tartrate concentrations. Leaf citrate concentrations were higher in  
48 PN than 1103P scions (Fig. 6.D). In stems, tartrate was the main organic acid in all scion/rootstock  
49 combinations (approximately 70 %, Fig. 6.B) and was higher in grafts with an 1103P scion (Fig. 6.B,  
50 Supplementary Table 3). However, grafts with a PN scion had higher citrate concentrations in the  
51 leaves and the stems (Fig. 6.D, Supplementary Table 3). The rootstock did not affect scion organic  
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1 acid concentrations although the interaction between scion and rootstock genotype affected stem  
2 citrate concentrations (Supplementary Table 3).

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4 In the root, both scion and rootstock affected total organic acid concentration; the concentration  
5 was lowest for 1103P/1103P and highest for PN/PN with the hetero-grafts having intermediate  
6 concentrations (Fig. 6.A, Supplementary Table 3). Root tartrate concentration was only affected by  
7 rootstock genotype (root tartrate concentration was higher in PN than 1103P), but the interaction  
8 between the scion and rootstock genotype was significant. Both the scion and rootstock genotype  
9 affected root malate and citrate concentrations (Supplementary Table 3). Citrate concentration was  
10 highest in PN/PN and lowest in 1103P/1103P with the hetero-grafts having intermediate values  
11 (Supplementary Table 3).

### 12 ***Tissue organic acid concentrations respond to LP***

13  
14 In leaves, total organic acid concentration was significantly higher in 1103P/1103P, 1103P/PN and  
15 PN/PN grown under LP than HP (Fig. 6.A), mainly due to an increase of malate in 1103P scions (Fig  
16 5B) and tartrate in PN scions (Fig. 6.B). In the stem, total organic acid concentration was not affected  
17 by Pi supply (Fig. 6.A). Total root organic acid and malate concentration was higher in plants grown  
18 under LP than HP in scion/rootstock combinations with an 1103P rootstock. The concentration of  
19 citrate was higher in plants grown on LP in scion/rootstock combinations with an 1103P scion (Fig. 6).  
20 The root concentration of tartrate was higher in LP than HP only for 1103P/1103P.

### 21 ***The abundance of transcripts encoding PHT1 transporters, PAPs, PHO2 and SPX domain containing 22 proteins was quantified in the roots of grafted grapevine grown under LP and HP***

23  
24 The abundance of the transcripts of seven Pi transporters from *PHT1* family was analysed in root tips  
25 of four scion/rootstock combinations of grapevine after 28 d of LP or HP (Fig. 7, Supplementary Table  
26 4). Under HP, the abundance of several *PHT1* transcripts was different between the scion/rootstock  
27 combinations: *PHT1.3a* transcripts were more abundant in 1103P rootstocks, whereas the  
28 abundance of *PHT1.3b* and *PHT1.4d* transcripts were affected by both the scion and rootstock, and  
29 the abundance of *PHT1.4a* transcripts were affected by the scion genotype. The abundance of all the  
30 *PHT1* transcripts measured was higher under LP than HP. The abundance of *PHT1.3b* and *PHT1.4d*  
31 showed significant interactions between both the scion or rootstock genotype and P supply,  
32 indicating a difference in regulation of transcript abundance in response to P depending on the  
33 scion/rootstock combination for these genes.

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35 The transcript abundance of *PAP10* and *PAP12* was analysed in root tips after 28 d of LP or HP (Fig.  
36 7). The transcript abundance of *PAP10* was not affected by the scion or rootstock genotype under  
37 HP, but was higher in plants grown in LP than HP. The increase of abundance of *PAP10* transcripts  
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1 was higher in scion/rootstock combinations with an 1103P than a PN rootstock (Fig. 7). The  
2 abundance of *PAP12* transcripts was higher in plants with a PN than an 1103P rootstock under HP  
3 and was higher in plants grown under LP than HP all scion/rootstock combinations.  
4

5 The transcript abundance of *PHO2* was analysed in root tips after 28 d of LP and HP (Fig. 7); the  
6 abundance of *PHO2* transcripts was not affected by the scion or rootstock genotype or P supply,  
7 however, there was an interaction between the rootstock genotype and P supply: the abundance of  
8 *PHO2* transcripts decreased in scion/rootstock combinations with an 1103P rootstock, but increased  
9 in scion/rootstock combinations with a PN rootstock. The transcript abundance of the SPX domain  
10 containing genes studied was higher under LP than HP, but was not affected by either the scion or  
11 rootstock genotype.  
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## 18 Discussion

### 19 ***Growth of young grapevines is scion dependant under non-limiting phosphate conditions***

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21 In our study, biomass of grafted grapevines growing under HP was affected by the scion genotype,  
22 grapevines grafted with 1103P as scion, regardless of the rootstock genotype, had a higher shoot and  
23 root FW compared to grapevines with a PN scion. Our results agree with previous studies showing  
24 that scion regulates the growth of young grapevines more than the rootstock genotype (Lefort and  
25 Legisle, 1977; Rives, 1971; Tandonnet *et al.*, 2010). In terms of root development, a few studies have  
26 reported an effect of the scion genotype on root system characteristics in young perennial fruit  
27 crops; these studies were limited to measurements of shoot:root ratio, root biomass or total root  
28 (Harrison *et al.*, 2016; Oslobeanu, 1978; Tandonnet *et al.*, 2010). In the present study, we also  
29 quantified the root diameter, and the number and the density of tips. Under HP, grapevines grafted  
30 with 1103P as scion has a higher number of tips than those with a PN scion, and this was related to a  
31 higher total root length. The number of tips was also higher in the rootstock PN than 1103P without  
32 necessarily being associated with increased root biomass suggesting that a high degree of branching  
33 of the PN rootstock, which was also confirmed by the tip density data. This is in agreement with the  
34 literature as grapevine genotypes have been shown to differ in root branching patterns (Dumont *et*  
35 *al.*, 2016; Smart *et al.*, 2006; Southey, 1992). The scion genotype affected tip density particularly  
36 when the rootstock was PN; tip density was higher in 1103P/PN compared to PN/PN; this could  
37 suggest that the scion can exert long-distance regulation of root branching in grapevine. This  
38 hypothesis is supported by a study on the effect of the scion on the root system of mature plants in a  
39 17 year-old vineyard, which showed that the scion could affect root biomass, shoot:root ratio, root  
40 density (per m<sup>2</sup> of soil) and root distribution in the soil (Ferlito *et al.*, 2020).  
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2 In summary, the scion genotype exerted control over whole plant development in the reciprocal  
3 grafts of grapevine studied, which is largely in agreement with the literature.

#### 4 ***LP alters biomass allocation and root morphology in grafted grapevine***

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6 Many studies shown a drastic reduction in shoot growth in response to limited Pi supply, this is  
7 related to a decrease of photosynthetic activity and carbon assimilation (Vance et al. 2003; Warren  
8 2011), as well as the preferential allocation of carbon resources to root system in order to decrease  
9 the shoot:root biomass ratio (Mollier and Pellerin 1999; Wen et al. 2017). These modifications have  
10 previously been observed in grafted grapevines (Grant and Matthews 1996b) and in un-grafted  
11 woody cuttings (Gautier et al. 2018). In our present study, shoot biomass was lower in plants treated  
12 with LP; grapevines grafted with an 1103P scion had a larger response. Stem biomass relative to total  
13 shoot biomass was lower in plants grown under LP than HP, suggesting that under limited Pi  
14 availability carbon is diverted away from the formation of stems (which support photosynthetic  
15 organs and conducting sap, and constitute carbon reserves) towards carbon capture (i.e. leaves).  
16 Root biomass was not affected by the LP treatment in all scion/rootstock combinations, resulting in a  
17 lower root mass fraction (i.e. root to total plant biomass ratio) in plants grown in LP as often  
18 observed in other species (Fernandez and Rubio 2015).

19  
20 Increased root branching (here quantified as tip density) is an important factor for Pi acquisition and  
21 for many species the enhancement of emergence of lateral roots is a common response to LP supply  
22 (Péret et al. 2011, 2014). However, some species (e.g. *Phaseolous vulgaris*) shown the opposite  
23 response, i.e. a decrease in the emergence of lateral roots has been shown (Borch et al. 1999). In this  
24 study on young grafted grapevines, it seems that tip density is also reduced in response to LP. Root  
25 diameter decreased in all scion/rootstock combinations in response to LP, suggesting that grapevine  
26 adapts its root system architecture to forage for Pi by reducing carbon demand per unit of root  
27 length rather than increasing branching.

28  
29 In summary, in response to LP grafted grapevines modify biomass allocation and growth to relatively  
30 increase soil exploration by roots and economize carbon use in the stems.

#### 31 ***Scion/rootstock combination and phosphate supply effects PAE, the abundance of PHT1 transcripts*** 32 ***and SAE***

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34 The genotypes studied in this work are known to differ in their PAE, when 1103P is cultivated as un-  
35 grafted, woody cuttings, it has a higher PAE than PN (Gautier et al. 2018). However, in this study on  
36 herbaceous micro-grafts, PAE was higher for PN/PN homo-grafts compared to 1103P/1103P homo-  
37 grafts, with intermediate values for the two hetero-grafts PN/1103P and 1103P/PN. The methods  
38 used in these studies were not the same; in Gautier et al. (2018) PAE was defined by slope of linear  
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1 regression between  $^{32}\text{P}$  uptake of the shoot and biomass root FW, whereas in this work PAE was  
2 estimated by total plant Pi divided by root FW (assuming that Pi content at the beginning of the  
3 treatment was negligible). This could suggest that plant growth conditions and/or plant  
4 developmental stage alters PAE in grapevine and/or that the method used to calculate PAE affects  
5 the results.  
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9 The abundance of seven *PHT1* transcripts in the root was quantified under both HP and LP. Under  
10 HP, several transcripts showed scion and/or rootstock specific accumulation patterns, suggesting an  
11 intrinsic difference in *PHT1* transcript abundance between the two rootstock genotypes studied and  
12 that it is altered by shoot-derived signals. All transcripts were strongly up-regulated under LP supply,  
13 suggesting a higher capacity to take up Pi from the soil under LP, but given only 0.001 mM Pi was  
14 provided to the plants PAE could not be quantified. *PHOSPHATE2* abundance was affected by LP in a  
15 rootstock dependent manner, suggesting differences in post-transcriptional regulation and  
16 potentially in the quantity of PHT1 protein in the different rootstock genotypes.  
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20 Under HP, the different scion/rootstock combinations differed in SAE, which was highest in  
21 1103P/1103P and lowest in PN/PN with the hetero-grafts having intermediate values, suggesting that  
22 under HP both the scion and rootstock genotype regulate SAE. Genotypic differences in sulphur  
23 accumulation have been reported in different scion/rootstock combinations of grapevine and 1103P  
24 has been shown to confer higher sulphur contents than other rootstock genotypes (Gautier *et al.*,  
25 2020b; Lecourt *et al.*, 2015). Under HP, there was an inverse relationship between PAE and SAE;  
26 there are well-described interactions between Pi and sulphate nutrition in plants with phospholipids  
27 being replaced by sulpholipids in times of Pi limitation and vice versa, although the molecular  
28 mechanisms are poorly understood (Bouain *et al.*, 2019; Rouached *et al.*, 2011). In grafted plants  
29 with an 1103P scion, SAE was lower under LP than HP (whereas SAE was not affected by Pi supply  
30 when the scion was PN); this suggests that the scion genotype can modify SAE in response to LP in  
31 grafted grapevine. The magnitude of differences in SAE in this study suggest that the differences  
32 reported here are due to more than subtle differences in the composition of lipids and may be  
33 related to the accumulation of other sulphur containing molecules such as glutathione.  
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37 Nitrate acquisition efficiency was affected by the scion genotype, it was higher with a PN scion  
38 (largely due to higher shoot N concentration), and its response to LP was scion genotype dependent  
39 (remaining unchanged with a PN scion but lower with an 1103P scion). In summary, this suggests that  
40 in response to LP, the 1103P scion reduces NAE and SAE, whereas the PN scion does not.  
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57 ***The release of APases by roots in response to LP is associated with the increase in abundance of***  
58 ***PAP10 and PAP12***  
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1 The activity of APases released into the media was approximately two-fold higher for each  
2 scion/rootstock combination grown under LP compared to HP. Related to this increase of APase  
3 activity, the relative abundances of *PAP10*, and *PAP12* transcripts were higher in plants grown under  
4 LP supply. *PURPLE ACID PHOSPHATASE10* and *PAP12* encode secreted APases, which are known to be  
5 increased in response to Pi starvation in species such as Arabidopsis, tomato or rice (Tian et al. 2012).  
6 These results suggest that grapevine is also able to use organic phosphorus from the soil. The activity  
7 of APases released into the media was affected by the scion and rootstock genotype and their  
8 interaction, whereas only the rootstock genotype affected the abundance of *PAP12* and only the  
9 interaction between the rootstock genotype and Pi supply affected the abundance of *PAP10*  
10 suggesting that other APase genes may be underlying the activity of APases released by the roots.

### 11 ***Organic acid profile of grapevine tissues varies between genotypes and can be modified by grafting*** 12 ***and phosphate supply***

13 In many species, the major organic acids are citrate and malate (Neumann and Römheld 1999),  
14 whereas tartrate is an organic acid restricted to a small number of species, including the *Vitaceae*  
15 (Bennet-Clark 1933). In the present study, organic acids were mainly found in the highest  
16 concentrations in the leaves without differences in total concentration between the four  
17 scion/rootstock combinations although the concentration of individual organic acids varied. Tartrate  
18 and malate concentrations have been shown to differ between different *V. vinifera* varieties,  
19 different leaf ages and during the growing season (Attia *et al.*, 2007). The rootstock did not affect  
20 organic acid concentration in scion in our study although the rootstock has been shown to alter  
21 organic acid concentration in 5-year old grapevines grown in an inert substrate and irrigated with a  
22 nutrient solution (Attia *et al.*, 2007). The stem organic concentrations were lower than those of the  
23 leaves and were different between the different scion genotypes. Root total organic acid  
24 concentration was different between the different rootstock genotypes studied as has been shown in  
25 other work (Covarrubias *et al.*, 2016) and was affected by the scion genotype. This could suggest that  
26 the shoots provide roots with some organic acids, in particularly malate which is the major organic  
27 acid in the phloem (Keller, 2010). We have previously shown that grafting an 1103P rootstock with a  
28 PN scion (i.e. PN/1103P) reduces the expression of *NADP-MALIC ENZYME2/Vitvi04g00009* (a key  
29 enzyme regulating malate metabolism) in comparison to the 1103P/1103P homograft control further  
30 suggesting that scion could provide malate to the rootstock in grapevine.

31 Tissue organic acid concentrations often change in response to Pi supply due to a number of reasons,  
32 such as, under HP supply, the co-transport of Pi with protons can lead to metabolic adjustments  
33 (such as a reduction in organic acid concentration) to maintain internal pH, whereas under LP supply,  
34 organic acids may be synthesized to be released by the roots to increase the availability of

1 phosphorus in the soil (Vance et al. 2003). In grapevine, differences in organic acid concentration in  
2 root tips were previously observed under iron deficiency and in response to different nitrogen  
3 sources (Jiménez et al. 2007; Covarrubias and Rombolà 2015). In response to LP, scion/rootstock  
4 combinations with an 1103P rootstock showed an increase of total organic acid concentration in  
5 root, which was particularly associated with an increase in malate concentration. Recently, the  
6 capacity of grapevine to release organic acids into the rhizosphere in responses to alkalinity were  
7 demonstrated (Xiang et al. 2019). Unfortunately, due to technical difficulties we were unable to  
8 measure root organic acid exudation in these experiments.  
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### 14 **The role of long-distance signalling in regulating responses to P supply in grafted grapevine**

16 We have previously characterised the early root transcriptome response to LP of the same reciprocal  
17 scion/rootstock combinations of grapevine; we showed that the transcriptome response of PN/PN is  
18 more extensive than that of 1103P/1103P and that many of the genes showing genotype-specific  
19 responses are related to iron, organic acid and sulphate transport, carbon metabolism and hormone  
20 signalling (Gautier et al. 2020a). We also found that the scion could modify the root transcriptome  
21 response to LP and the genes differentially expressed suggest that phloem mobile metabolites,  
22 cysteine rich peptides, hormones such as strigolactones and ethylene, and microRNAs could be  
23 involved (Gautier et al. 2020a). In this manuscript we have characterised the long-term physiological  
24 responses to LP in the same scion/rootstock combinations, some root traits are regulated by both  
25 the scion and rootstock tissues (such as tissue Pi and organic acid concentrations, PAE and SAE)  
26 suggesting that these traits are regulated by long-distance signalling.  
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### 37 **Conclusion**

39 The objective of this study was to determine the roles of both the shoot and the root in employing  
40 strategies to cope with LP supply in grapevine. Under HP, the homograft PN/PN has higher tissue Pi  
41 concentrations and higher PAE than 1103P, which was associated with a higher constitutive  
42 expression of a number of *PHT1* genes, a lower SAE, and reduced growth. Reciprocal grafting showed  
43 that growth was regulated by the scion genotype, presumably via its capacity of assimilation and/or  
44 translocation of carbon. Whereas the heterografts, PN/1103P and 1103P/PN, had intermediate  
45 phenotypes in terms of tissue Pi concentrations, PAE and SAE suggesting that these parameters were  
46 regulated by both the scion and rootstock. In response to LP, all scion/rootstock combinations  
47 altered growth to favour root foraging, increase the expression of PSI genes and increased the  
48 exudation of APases (with the concomitant increase in the abundance of *PAP10* and *PAP12*  
49 transcripts). The organic acid concentration in the different tissues was affected by the  
50 scion/rootstock combination and the response to LP supply showed an effect on scion and rootstock  
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1 genotype. Many signals are known to move between the shoot and the root (and vice versa) to  
2 regulate plant responses to P supply (Zhang *et al.*, 2014), by grafting different genotypes together we  
3 have shown that genetic variation in the shoot exerts long-distance control over a number of these  
4 responses.  
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## 6 **Data and Materials availability**

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9 Experimental data and materials available to third party academic researchers upon reasonable  
10 request.  
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## 12 **Supplementary Data**

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16 Supplementary Figure 1. Photographs of the plants used in the manuscript at different stages A. in  
17 vitro grafted grapevines, B. acclimation in perlite, C. and D. plants growing in hydroponic culture.  
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20 Supplementary Table 1. List of primers used for RT-qPCR experiments.  
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Supplementary Table 2. Statistical analysis of biomass, biomass allocation and root morphology  
measurements of reciprocal grafts of *Vitis berlandieri* x *V. rupestris* cv. 1103 Paulsen (1103P) and *V.*  
*vinifera* cv. Pinot noir (PN) grown under high phosphate supply grouped by common scion or  
rootstock genotype.

Supplementary Table 3. Statistical analysis of malate, tartrate and citrate concentrations and the sum  
of these three organic acids in the leaves, stems and roots of reciprocal grafts of *Vitis berlandieri* x *V.*  
*rupestris* cv. 1103 Paulsen (1103P) and *V. vinifera* cv. Pinot noir (PN) grown under high phosphate  
supply grouped by common scion or rootstock genotype.

Supplementary Table 4. Results of a 3-way analysis of variance of the abundance of transcripts  
encoding various *PHOSPHATE TRANSPORTER1 (PHT1)*, *PURPLE ACID PHOSPHATASE (PAP)*,  
*PHOSPHATE2 (PHO2)* and *SPX DOMAIN CONTAINING PROTEINS (SPX)* genes in the roots of reciprocal  
grafts of *Vitis berlandieri* x *V. rupestris* cv. 1103 Paulsen and *V. vinifera* cv. Pinot noir after 28 d of  
either high or low phosphate (Pi) supply (with scion genotype, rootstock genotype and P supply as  
factors).

## 66 **Conflict of interest**

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None declared.

## 101 **Funding**

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## **Authors' contributions**

AG, IM, BP and PD executed the experiments and collected data, AG and NC analysed the data, VL, AM, PV and SJC acquired the funding, SJC drafted the manuscript, and all authors made significant intellectual contributions to the design and execution of the work, and revised the manuscript.

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## List of Figures

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2 Figure 1. (A) Stem, (B) leaf and (C) root fresh weight (FW), and (D) root:whole plant FW, (E)  
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4 *rupestris* cv. 1103 Paulsen (1103P) and *V. vinifera* cv. Pinot noir (PN) after 28 d of either high (dark  
5 colours) or low phosphate supply (light colours). The scion genotype is presented above the  
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7 an effect of phosphate supply using Wilcoxon test with the Bonferroni correction (\*P value < 0.05,  
8 \*\* P value < 0.01, \*\*\* P value < 0.001).

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15 Figure 2. (A) Total number of root tips, (B) tip density and (C) average diameter of roots of reciprocal  
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18 presented above the rootstock genotype. Means and standard errors shown (n = 5). For each  
19 combination, stars indicate an effect of phosphate supply using t-test with the Bonferroni correction  
20 (\*P value < 0.05, \*\* P value < 0.01, \*\*\* P value < 0.001).

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26 Figure 3. (A) Leaf and (B) root phosphate concentration of reciprocal grafts of *Vitis berlandieri* x *V.*  
27 *rupestris* cv. 1103 Paulsen (1103P) and *V. vinifera* cv. Pinot noir (PN) after 28 d of either high (dark  
28 colours) or low phosphate (light colours) supply. The scion genotype is presented above the  
29 rootstock genotype. Means and standard errors shown (n = 5). For each combination, stars indicate  
30 an effect of phosphate supply using t-test with the Bonferroni correction (\*\*\* P value < 0.001).

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36 Figure 4. Estimates of (A) phosphate, (B) nitrate and (C) sulphate acquisition efficiencies (PAE, NAE  
37 and SAE respectively) of reciprocal grafts of *Vitis berlandieri* x *V. rupestris* cv. 1103 Paulsen (1103P)  
38 and *V. vinifera* cv. Pinot noir (PN) after 28 d of either high (dark colours) or low phosphate supply  
39 (light colours). The scion genotype is presented above the rootstock genotype. Means and standard  
40 errors shown (n = 5). Letters indicate significant differences using a multiple comparison after  
41 Kruskal-Wallis tests at P value < 0.05.

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47 Figure 5. Acid phosphatase (APase) activity measured in root exudates of reciprocal grafts of *Vitis*  
48 *berlandieri* x *V. rupestris* cv. 1103 Paulsen (1103P) and *V. vinifera* cv. Pinot noir (PN) after 28 d of  
49 either high (dark colours) or low phosphate supply (light colours). The scion genotype is presented  
50 above the rootstock genotype. Means and standard errors shown (n = 5). Letters indicate significant  
51 differences (P value < 0.05) using a Tukey test after a two-way ANOVA with scion/rootstock  
52 combination and phosphate supply as factors.

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58 Figure 6. Histograms of (B) malate, (C) tartrate, (D) citrate concentrations and (A) the sum of these  
59 three organic acids in leaves, stems and roots of reciprocal grafts of *Vitis berlandieri* x *V. rupestris* cv.

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1103 Paulsen (1103P) and *V. vinifera* cv. Pinot noir (PN) grown under high (dark colours) or low (light colours) phosphate supply. The scion genotype is presented before the rootstock genotype. Stars indicate a significant change in concentration in between phosphate supplies tested using a t-test with the Bonferroni correction (\*P value < 0.05, \*\* P value < 0.01, \*\*\* P value < 0.001).

Figure 7. Heatmaps of the abundance of transcripts encoding various PHOSPHATE TRANSPORTER1 (PHT1), PURPLE ACID PHOSPHATASE (PAP), PHOSPHATE2 (PHO2) and SPX DOMAIN CONTAINING PROTEINS genes in the roots of reciprocal grafts of *Vitis berlandieri* x *V. rupestris* cv. 1103 Paulsen (1103P) and *V. vinifera* cv. Pinot noir (PN) after 28 d of either high (HP) or low phosphate (LP) supply. The scion genotype is presented above the rootstock genotype. For transcript abundance under HP, green shade indicates the level of expression relative to the lowest value. For transcript abundance changes in response to LP, orange and purple shades indicates the extent up- or down-regulation respectively (n = 3).

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**Supplementary Table 1.** List of primers used for RT-qPCR experiments.

<b>Gene name</b>	<b>Accession number</b>	<b>Forward primer</b>	<b>Reverse primer</b>	<b>Efficiency</b>	<b>Amplicon size</b>
PHT 1.3a	VIT_13s0067g03280	GGGCAATTGTGGCTTTGTCT	TGGTCCTCCCAGAGAGTTG	96.5	106
PHT 1.3b	VIT_16s0050g02380	CCCCACTAAAGATAAGCCTGGG	TCCTACTAGGGCAACCCCAA	103.2	73
PHT 1.4a	VIT_05s0049g00920	TTGTGCTCGGGGTAGTCAAC	CCAGTCCTGGTAGAAGGGGA	90	148
PHT 1.4b	VIT_05s0049g00930	CCCACCTGGTATTGGAATGAGA	TAGTAGGAGGCTGCATGTCCA	85	166
PHT 1.4c	VIT_05s0049g00940	CCCAGTCCAAGGGAAAGTC	GGAACAGTCCTAGCCTGCTG	99.1	98
PHT 1.4d	VIT_16s0050g02370	TTGGTGACGGAGACCAAAGG	GTGTGCTAGGCATCTGGGTT	94.5	90
PHT 1.9	VIT_18s0122g00780	CCCGTGAAACCAAGGGAAGA	GAATCCGCGATGAGTTTCGC	95.8	167
PHO2	VIT_00s0265g00070	TTGTGCTGTGGAAGCAGGAT	GTGCAGGCAAACCTCAAACCA	80.2	80
PAP10	VIT_03s0038g00220	AGCCAGTTTTGGACACGCTA	GTGGGTCCACAAACCTTCCA	96.9	136
PAP12	VIT_18s0001g13340	GGACCTCTTCAGAAAAATCCTCT	ACCAAATGGGACAGTGAACAT	100.1	114
SPX1/SPX2	VIT_11s0016g05330	GATGGACAGGAAGGGTGTGG	TTTCCTTCAGAGCCCGCAAT	91	151
SPX3	VIT_15s0048g00190	CAGGAACACAGTTGCAGCAC	TGGGGATGGGAGAGTGAAT	91.5	140
ACTIN	VIT_04s0044g00580	CTTGCATCCCTCAGCACCTT	TCCTGTGGACAATGGATGGA	93.6	82
GAPDH	VIT_17s0000g10430	CCACAGACTTCATCGGTGACA	TTCTCGTTGAGGGCTATTCCA	91	70
SAND3'	VIT_06s0004g02820	TGCTGGGTTACCCCGAGTTTGA	CAGACCCGGTTGCACGTCCG	89.9	89

**Supplementary Table 2.** Statistical analysis of biomass, biomass allocation and root morphology measurements of reciprocal grafts of *Vitis berlandieri* x *V. rupestris* cv. 1103 Paulsen (1103P) and *V. vinifera* cv. Pinot noir (PN) grown under high phosphate supply grouped by common scion or rootstock genotype. Means and standard errors shown (n = 30 for biomass measurements and n = 10 for root morphology measurements). Stars indicate an effect of either the scion or rootstock genotype, tested using Wilcoxon tests for biomass measurements and t-tests for root morphology (\**P* value < 0.05, \*\* *P* value < 0.01, \*\*\* *P* value < 0.001).

	Scion			Rootstock		
	1103P	PN	Significance	1103P	PN	Significance
<b>Leaf FW (g)</b>	3.11 ± 0.22	1.07 ± 0.12	***	2.01 ± 0.25	2.17 ± 0.27	NS
<b>Stem FW (g)</b>	2.42 ± 0.17	0.91 ± 0.10	***	1.58 ± 0.18	1.73 ± 0.21	NS
<b>Root FW (g)</b>	2.56 ± 0.13	1.10 ± 0.08	***	1.74 ± 0.17	1.90 ± 0.18	NS
<b>Root:plant FW ratio</b>	0.33 ± 0.01	0.37 ± 0.01	NS	0.34 ± 0.01	0.34 ± 0.02	NS
<b>Leaf:plant FW ratio</b>	0.38 ± 0.01	0.34 ± 0.01	***	0.36 ± 0.01	0.36 ± 0.02	NS
<b>Stem:shoot FW ratio</b>	0.79 ± 0.02	0.85 ± 0.03	**	0.85 ± 0.03	0.79 ± 0.02	**
<b>Number of tips</b>	823 ± 92	389 ± 52	***	485 ± 85	726 ± 105	NS
<b>Tip density (cm<sup>-1</sup>)</b>	1.03 ± 0.05	0.93 ± 0.03	NS	0.90 ± 0.02	1.07 ± 0.05	**
<b>Average root diameter (mm)</b>	0.95 ± 0.02	0.97 ± 0.03	NS	0.94 ± 0.01	0.98 ± 0.02	NS

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**Supplementary Table 3.** Statistical analysis of malate, tartrate and citrate concentrations and the sum of these three organic acids in the leaves, stems and roots of reciprocal grafts of *Vitis berlandieri* x *V. rupestris* cv. 1103 Paulsen (1103P) and *V. vinifera* cv. Pinot noir (PN) grown under high phosphate supply grouped by common scion or rootstock genotype. Stars indicate an effect of the scion or rootstock genotype tested using a two-way analysis of variance with scion and rootstock genotype as factors (\**P* value < 0.05, \*\* *P* value < 0.01, \*\*\* *P* value < 0.001).

Organic acid concentration (mg g <sup>-1</sup> FW)	Scion			Rootstock			Scion x Rootstock Interaction
	1103P	PN	Significance	1103P	PN	Significance	Significance
<b>Shoot</b>							
Organic acids	14.11 ± 0.93	13.02 ± 0.45	NS	14.63 ± 0.63	12.93 ± 0.80	NS	NS
Malate	1.36 ± 0.19	6.01 ± 0.20	***	3.89 ± 0.75	3.48 ± 0.83	NS	NS
Tartrate	12.48 ± 0.81	6.27 ± 0.43	***	9.94 ± 1.20	8.82 ± 1.20	NS	NS
Citrate	0.21 ± 0.02	0.29 ± 0.02	*	0.26 ± 0.02	0.24 ± 0.63	NS	NS
<b>Stem</b>							
Organic acids	8.08 ± 0.37	7.08 ± 0.30	*	7.85 ± 0.42	7.30 ± 0.30	NS	NS
Malate	2.15 ± 0.15	2.02 ± 0.12	NS	2.15 ± 0.13	2.02 ± 0.15	NS	NS
Tartrate	5.50 ± 0.21	4.45 ± 0.17	***	5.16 ± 0.29	4.80 ± 0.20	NS	NS
Citrate	0.29 ± 0.03	0.46 ± 0.03	***	0.38 ± 0.02	0.36 ± 0.05	NS	***
<b>Root</b>							
Organic acids	1.61 ± 0.17	2.66 ± 0.28	***	1.54 ± 0.16	2.74 ± 0.25	***	NS
Malate	0.41 ± 0.06	0.62 ± 0.10	*	0.34 ± 0.03	0.70 ± 0.08	***	NS
Tartrate	0.71 ± 0.08	0.76 ± 0.04	NS	0.57 ± 0.04	0.90 ± 0.04	***	**
Citrate	0.35 ± 0.03	1.12 ± 0.14	***	0.50 ± 0.09	0.97 ± 0.18	***	**

**Supplementary Table 4.** Results of a 3-way analysis of variance of the abundance of transcripts encoding various *PHOSPHATE TRANSPORTER1 (PHT1)*, *PURPLE ACID PHOSPHATASE (PAP)*, *PHOSPHATE2 (PHO2)* and *SPX DOMAIN CONTAINING PROTEINS (SPX)* genes in the roots of reciprocal grafts of *Vitis berlandieri* x *V. rupestris* cv. 1103 Paulsen and *V. vinifera* cv. Pinot noir after 28 d of either high or low phosphate (Pi) supply (with scion genotype, rootstock genotype and P supply as factors, n = 3). \**P* value < 0.05, \*\* *P* value < 0.01, \*\*\* *P* value < 0.001.

	Scion	Rootstock	Pi supply	Scion x Rootstock	Scion x Pi supply	Rootstock x Pi supply	Scion x Rootstock x Pi supply
PHT1.3a		***	*				
PHT1.3b	**	***	***		***	***	
PHT1.4a	*		***				**
PHT1.4b			***				
PHT1.4c			***				
PHT1.4d	***	***	***		***	***	
PHT1.9			***				
PHO2						***	
PAP10			***			***	
PAP12		***	***				
SPX1/2			***				
SPX 3			***				

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8 **Identifying roles of the scion and the rootstock in regulating plant**  
9 **development and functioning under different phosphorus supplies in**  
10 **grapevine**

11 **Authors:** Antoine T. Gautier<sup>1,2</sup>, Isabelle Merlin<sup>1</sup>, Patrick Doumas<sup>3</sup>, Noé Cochetel<sup>4</sup>, Alain Mollier<sup>5</sup>,  
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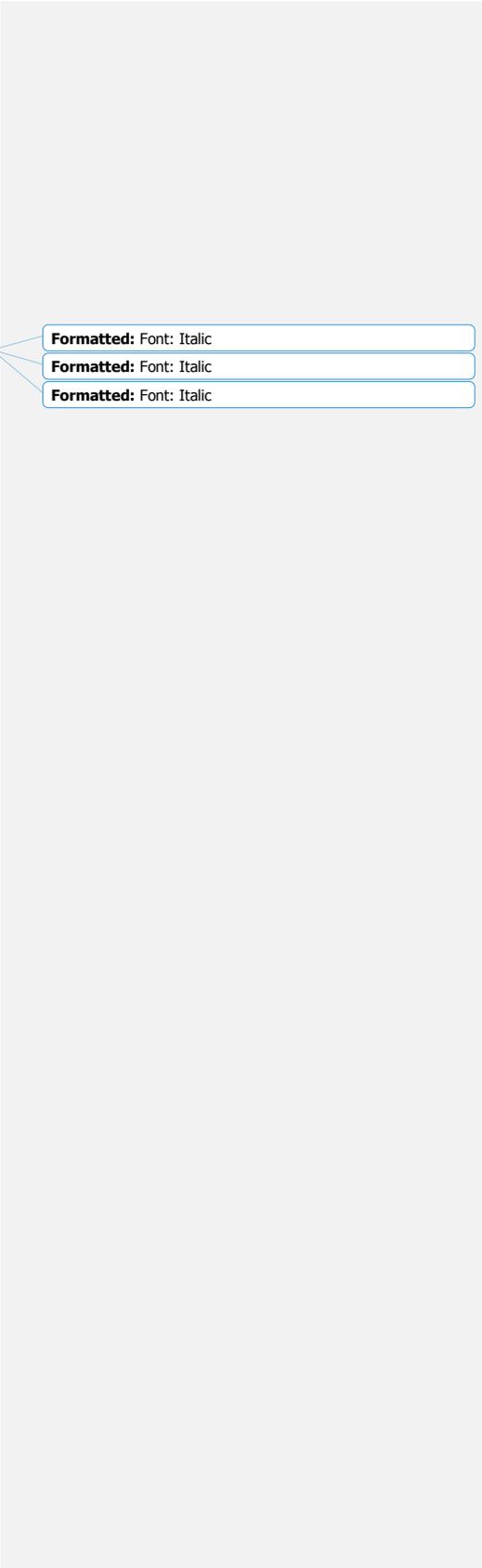
21 **Keywords:** *Vitis spp.*, Root system architecture, Rhizosphere, Root exudation, Gene Expression, RT-  
22 qPCR

23 Running head: Reciprocal grafts under different P supplies  
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**Abstract**

Phosphorus is essential for plant life and plants have developed numerous strategies to maximise phosphate (Pi) acquisition and use under limited Pi supply. Here we have used reciprocal grafting to determine whether the shoot and root have different roles in regulating some of these strategies. Reciprocal grafts of two grapevine genotypes (*Vitis vinifera* cv. Pinot noir and *V. berlandieri* x *V. rupestris* cv. 1103 Paulsen) were produced as well as the corresponding homo-graft controls; the plants were grown in hydroponic culture and subjected to two levels of Pi supply (high (0.6 mM) or low (0.001 mM)). Biomass accumulation, root morphology, and root, stem and leaf organic acid, phosphate, nitrate and sulphate concentrations were measured. The transcript abundance of orthologues of known phosphate starvation induced (PSI) genes from Arabidopsis was also quantified. Under high Pi, the scion genotype had a large impact on plant growth, but the morphology of roots (such as root tip density) was not affected. Low Pi supply affected growth, tissue organic acid concentration, the activity of acid phosphatases released by the roots and the expression of PSI genes. Rootstock genotypes showed differences in root responses to low Pi supply, but the scion also exerted long-distance regulation of rootstock responses to low Pi, for example, modifying PSI gene expression, sulphate acquisition efficiency, the activity of acid phosphatases released by the roots and root organic acid concentrations. This work shows for the first time that the grapevine genotypes differ in their response to low Pi supply and that the scion can modify rootstock responses to the nutrient availability. This work highlights that genetic variation in shoot-borne signals can regulate root responses to Pi supply and that understanding rootstock responses to the environment must be done considering scion influence.



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7 **Introduction**  
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9 Phosphorus is an essential macronutrient for plants, it is part of essential macromolecules (e.g.  
10 nucleic acids or phospholipids) and involved in energy transfer (Marschner, 1995). Phosphorus is  
11 taken up from the soil solution by roots in its inorganic form (Pi, orthophosphate) by transporters of  
12 the INORGANIC PHOSPHATE TRANSPORTER1 (PHT1) family (Nussaume *et al.*, 2011). *INORGANIC*  
13 *PHOSPHATE TRANSPORTER1* transcript levels are controlled by different regulators such as  
14 *PHOSPHATE STARVATION RESPONSE1* (PHR1, which also regulates the expression of other  
15 *PHOSPHATE STARVATION INDUCED* (PSI) genes), microRNA 399 and SYG/PHO81/XPR1 (SPX) proteins  
16 (Duan *et al.*, 2008; Rubio *et al.*, 2001). Furthermore, PHT1 transporters are regulated at the post-  
17 transcriptional level by degradation by *PHOSPHATE2* (PHO2), a protein with an E2 ubiquitin  
18 conjugase domain (Bari *et al.*, 2006).  
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23 Available Pi accounts for a small fraction of total phosphorus present in the soil. Phosphate  
24 concentrations in the soil solution are low, whilst a large proportion of phosphorus is held in the soil.  
25 For example, it can be bound to iron and aluminium-(hydr)oxides in acid soils, or it can occur in  
26 calcium-phosphate forms in neutral to alkaline soils (Hinsinger, 2001). In addition, a high  
27 concentration of organic phosphorus is found in soil, which requires hydrolysis before being taken up  
28 by plants (Robinson *et al.*, 2012). Root-rhizosphere interactions can modify soil properties to increase  
29 Pi availability via the following mechanisms: (1) releasing protons or hydroxide ions, resulting a  
30 change of rhizosphere pH (Hinsinger, 2001; Hinsinger *et al.*, 2003; Liu *et al.*, 2016); (2) releasing  
31 organic acids (e.g. malate or citrate) (Bolan *et al.*, 1994; Gahoonia *et al.*, 2000), resulting a  
32 complexation of ions such as iron or aluminium involved in phosphorus sorption (Jones, 1998); or (3)  
33 releasing acid phosphatases (APases; EC 3.1.3.2, which are encoded by *PURPLE ACID PHOSPHATASE*  
34 (*PAP*) genes), enzymes which catalyse the hydrolysis of organic phosphorus into Pi (Lu *et al.*, 2016;  
35 Robinson *et al.*, 2012).  
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41 In addition to modifying the rhizosphere to increase Pi availability, under low Pi supply plants can  
42 also modify biomass allocation, root system architecture and morphology, gene expression and Pi  
43 transport to increase root foraging capacity (Lan *et al.*, 2015; Lynch, 2011; Morcuende *et al.*, 2007;  
44 Pant *et al.*, 2015). This is often mediated by the preferential allocation of biomass to the roots rather  
45 than the shoots, by the increase in the degree of lateral root branching, and by the increase in the  
46 number and length of root hairs (Lynch, 2011). Low Pi supply induces the expression of many PSI  
47 genes, for example, genes coding for PHT1s, which can increase root Pi transport capacity (Lan *et al.*,  
48 2015). As low Pi reduces plant growth, metabolites such as starch, sugars and many amino acids  
49 increase in concentration, while phosphorylated metabolites such as hexose phosphates, ATP and  
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7 ADP decrease in concentration (Morcuende *et al.*, 2007). Tissue organic acid concentrations often  
8 change in response to Pi supply to maintain internal pH balance (Vance *et al.* 2003).  
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10 One method to determine the roles of the shoot and root in regulating plant responses to the  
11 environment is by grafting two different genotypes together. Grafting is an old horticultural  
12 technique used for the cultivation of perennial fruit crops (*e.g.* grape, apple, citrus, etc.) and annual  
13 vegetables and fruits (*e.g.* tomatoes, cucumber and pepper) (Mudge *et al.* 2009; Bie *et al.* 2017). In  
14 horticulture, grafting allows us to combine desirable traits of the shoot and the root of two different  
15 genotypes in a single plant. Rootstocks have been selected for various agronomical traits such as  
16 controlling scion growth (i.e. inducing dwarfing and modifying scion architecture), improving water  
17 use efficiency/drought tolerance, altering mineral element status, improving yield and fruit quality,  
18 and providing resistance to root pathogens (Nawaz *et al.* 2016; Warschefsky *et al.* 2016). Since the  
19 19<sup>th</sup> century, the Eurasian grapevine species (*Vitis vinifera*) is cultivated grafted using North American  
20 *Vitis spp.* as rootstocks because of their tolerance to the American aphid pest Phylloxera. In addition  
21 to providing Phylloxera resistance, grapevine rootstocks are also chosen based on their known  
22 adaptation to soil conditions and ability to control scion vigour (Ollat *et al.*, 2016). Grapevine  
23 rootstocks are known to modify the mineral element composition in the scion (Cordeau 1998;  
24 Bavaresco *et al.* 2003) and these changes are linked to the genetic background of the rootstock  
25 (Gautier *et al.*, 2020b). Grapevine rootstocks modify plant responses to nutrient supply, such as  
26 nitrogen (Lecourt *et al.* 2015; Cochetel *et al.* 2017), potassium (Ruhl 1989, 1991), phosphorus (Grant  
27 and Matthews 1996b, a; Gautier *et al.* 2018) and iron (Jiménez *et al.* 2007; Covarrubias and Rombolà  
28 2015; Covarrubias *et al.* 2016).  
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36 As grapevine rootstocks are known to modify scion phosphorus content and plant responses to Pi  
37 supply, we used reciprocal grafting of two [widely used](#) grapevine genotypes to explore the roles of  
38 both the shoot and the root in regulating Pi content and plant responses to Pi supply in grafted  
39 grapevine. The genotypes chosen were a *V. vinifera* scion obtained from the sexual reproduction  
40 from self-pollination of Pinot Noir, named 40024 (PN), which grows well in *in vitro* culture and grafts  
41 easily, and a rootstock genotype of American origin, *V. berlandieri* x *V. rupestris* cv. 1103 Paulsen  
42 clone 198 (1103P), which confers a high scion vigour and petiole phosphorus concentration in the  
43 vineyard (Gautier *et al.*, 2020b). [These two genotypes were also chosen because they differ in their  
44 phosphorus acquisition efficiency in the first 21 d of development when grown as woody cuttings  
45 \(Gautier \*et al.\*, 2018\).](#) Firstly, we aimed to determine if the scion and/or rootstock alters plant  
46 growth, biomass allocation, Pi content and phosphate acquisition efficiency (PAE) in non-limited Pi  
47 supply. Secondly, we assessed the growth response of grafted grapevines to low Pi supply and  
48 quantified the expression of different *PHT1* genes. In addition, because Pi acquisition interacts with  
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7 both sulphate and nitrate uptake, both sulphate and nitrate acquisition efficiencies were calculated.  
8 Thirdly we asked does the scion/rootstock combination affect the exudation of APases and the  
9 abundance of *PAP* transcripts. And finally we asked whether the scion/rootstock combinations  
10 differed in tissue organic acid concentration and whether organic acid concentrations respond to Pi  
11 supply. This was achieved by comparing all possible scion/rootstock combinations of two grapevine  
12 genotypes grown under two contrasted Pi supplies.  
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## 15 **Materials and methods**

### 16 *Plant material and growing conditions*

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18 All four possible scion/rootstock combinations of 1103P and PN were micro-grafted using the cleft  
19 grafting system, i.e. 1103P/1103P, 1103P/PN, PN/1103P and PN/PN and grown as previously  
20 described (Gautier *et al.*, 2020a). Both genotypes were introduced into *in vitro* culture after surface  
21 sterilisation. Plants were cultivated *in vitro* on McCown Woody Plant Medium (Duchefa)  
22 supplemented with 30 g L<sup>-1</sup> sucrose, 0.27 µM 1-naphthalene acetic acid and 0.4 % agar in a growth  
23 chamber at 22 °C and with a photoperiod of 16 h light/8 h dark at a photon flux density of 55 µmol  
24 m<sup>-2</sup> s<sup>-1</sup> (Supplementary Fig. 1A). Six-week-old plantlets were then acclimated for four weeks to  
25 perlite-filled pots, irrigated with water, in a growth chamber at 26 °C and with a photoperiod of 16 h  
26 light/8 h dark at a photon flux density of 145 µmol m<sup>-2</sup> s<sup>-1</sup> at plant level (Supplementary Fig. 1B).  
27 Plants were then transferred into hydroponic culture with an aerated solution; each pot contained 2  
28 plants of the same scion/rootstock combination with 700 mL of high P solution (HP, 0.6 mM P). Four  
29 days later, half the pots continued receiving the HP solution and the other half were subjected to a  
30 low P (LP, 0.001 mM P) treatment (Supplementary Fig. 1C and D). The macronutrient composition  
31 was 2.45 mM KNO<sub>3</sub>, 0.69 mM MgSO<sub>4</sub> and 1.27 mM CaCl<sub>2</sub> for both the HP and LP solutions; HP  
32 solution also contained 0.6 mM KH<sub>2</sub>PO<sub>4</sub> and 0.6 mM CaSO<sub>4</sub>, whereas the LP solution contained 0.3  
33 mM K<sub>2</sub>SO<sub>4</sub> and 0.3 mM CaSO<sub>4</sub>. Micronutrients were supplied as 46 µmol H<sub>3</sub>BO<sub>3</sub>, 9.1 µmol MnCl<sub>2</sub>, 2.4  
34 µmol ZnSO<sub>4</sub>, 0.5 µmol CuSO<sub>4</sub> and 14 nmol (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>, and iron was supplied as 8.5 mg L<sup>-1</sup>  
35 Sequestrene 138 (i.e. 31.3 µmol ethylenediamine-N,N'-bis (2-hydroxyphenylacetic acid) NaFe,  
36 Syngenta Agro S.A.S.). The solutions were changed once a week for 28 d.  
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### 45 *Plant growth measurements*

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47 After 28 d of either LP or HP treatment, five plants were used for root system morphology analysis,  
48 five plants were used the quantification of acid phosphatases released by the roots and five plants  
49 were harvested for metabolite profiling and the quantification of transcript abundance. For each  
50 plant, leaves, stems and roots were weighed (fresh weight, FW).  
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7 *Root system morphology analysis*

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9 Images of the whole root system were captured using an Epson 1640XL scanner and 2D images were  
10 analysed using WinRHIZO software (Regent Instruments Inc., 1996). The total number of root tips  
11 (both primary and lateral roots), root average diameter and total root length were measured.  
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13 *Activity of APases released by the roots*

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15 Plants were transferred to 40 mL of aqueous solution containing 10 mmol of p-nitrophenyl  
16 phosphate (pNPP), covered with aluminium foil for 2 h in the growth chamber. The reaction was  
17 stopped by the addition of 1 mL of NaOH 1M, the volume of solution remaining and root FW were  
18 measured. p-nitrophenyl (pNP) concentration was measured by spectrophotometer at 405 nm and  
19 APase activity was calculated as mmol of pNP produced per g root FW per h.  
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22 *Metabolite profile of plant tissues*

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24 Root, stem and leaf samples were frozen in liquid nitrogen and kept at -80 °C for metabolite  
25 measurements. Samples were ground using a Retsch™ MM400 cooled with liquid nitrogen.  
26 Metabolites were extracted from 40 mg FW in aqueous ethanol at 80 °C in three incubation steps  
27 each lasting 20 min (step 1: 700 µL 80 % ethanol; step 2: 700 µL 50 % ethanol; and step 3: 300 µL 50  
28 % ethanol) and then centrifuged for 10 min at 4800 g. Supernatants were pooled. The ethanol was  
29 allowed to evaporate using speed-vac and the dry extracts were re-suspended in 1.7 mL of distilled  
30 water. Phosphate, nitrate, sulphate and organic acids (malate, citrate and tartrate) were identified  
31 and quantified by ion exchange chromatography ICS-5000 HPIC system (Thermo Scientific Dionex),  
32 using an IonPac® AS11 analytical column (250 mm × 4.0 mm) with an isocratic gradient of KOH from 4  
33 to 28 mM for 16 min, with a volume of sample injected of 20 µL. Phosphate, sulphate and nitrate  
34 acquisition efficiencies were calculated using the whole plant Pi, sulphate and nitrate contents  
35 respectively by root FW (we assume that the Pi, sulphate and nitrate contents, and root biomass at  
36 the beginning of the experiment are negligible).  
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41 *Quantification of transcript abundance*

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43 Three root tips (~15 mm in length) from each plant that was used for metabolite measurement were  
44 harvested and immediately snap-frozen in liquid nitrogen. Total RNA of samples was extracted using  
45 the Spectrum Plant Total RNA kit (Sigma-Aldrich) with some modifications as described by Cookson *et*  
46 *al.* (2013). Total RNA (1.5 µg) was reverse transcribed into cDNA using the SuperScript III First-Strand  
47 Synthesis System (Invitrogen). Quantitative reverse transcription polymerase chain reactions (RT-  
48 qPCRs) were performed using SYBR Green on an iCycler iQH (Bio-Rad) according to the procedure  
49 described by the supplier, with 0.2 µM of primers for each gene. Transcript abundance was  
50 calculated as normalized relative quantities (NRQs), with the reference genes ACTIN, GAPDH and  
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7 SAND3' for normalization (Hellemans et al. 2007). Primer sequences are listed in Supplementary  
8 Table 1.  
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10 The identification of *PHT1*, *PHO2*, *SPX DOMAIN CONTAIN PROTEINS* and *PAP* genes in grapevine was  
11 based on protein sequence similarity with *Arabidopsis thaliana* (L.). Analysis was performed using the  
12 Arabidopsis protein sequence and the putative grapevine proteins were selected on the basis of the  
13 highest sequence homology value of their predicted coding sequences (the threshold value for  
14 sequence homology was set at 85 %). Sequences of Arabidopsis genes were obtained from TAIR  
15 (<https://www.arabidopsis.org/>), and the predicted sequences in grapevine were identified using  
16 CRIBI (<http://genomes.cribi.unipd.it/grape/>).  
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#### 20 *Statistical analysis*

21 All statistical analyses were performed using the software R (R Core Team, 2018). The effect of Pi  
22 supply and scion or rootstock genotype on plant biomass and biomass allocation was tested using t-  
23 test or Wilcoxon non-parametric tests respectively at *P* value < 0.05 with the Bonferroni correction,  
24 when assumptions for parametric tests were met. For multivariable analysis, a multiple comparison  
25 test after a Kruskal-Wallis test at *P* value < 0.05 was used when assumptions for parametric tests  
26 were not respected, using the function *kruskalmc* from the *pgirmess* R package. Letters to indicate  
27 significant differences among multiple comparisons were obtained using the function  
28 *multcompLetters* from the R package *multcompView*. In the case of assumptions for parametric tests  
29 were respected, data were analysed using two-way or three-way analysis of variance (ANOVA *P* value  
30 < 0.05, with Tukey's Honest Significant Difference test), with combination and P supply, scion and/or  
31 rootstock as factors.  
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## 37 **Results**

### 38 ***Growth of grafted grapevine is mainly controlled by the scion genotype and affected by phosphate*** 39 ***supply*** 40

41 Four scion/rootstock combinations of the grapevine genotypes PN and 1103P (1103P/1103P,  
42 1103P/PN, PN/1103P and PN/PN) were grown in aerated hydroponic culture on high phosphate (HP)  
43 supply, and then half of them were transferred to low phosphate (LP) supply (whilst the remaining  
44 half continued to receive HP. Stem, leaf and root biomass was measured (Fig. 1A-C). Effect of scion  
45 and rootstock genotypes on biomass, biomass allocation and root morphology was analysed by  
46 grouping together data from plants grown on HP by common scion or rootstock genotypes  
47 (Supplementary Table 2); this is because the data did not meet the assumptions for a 3-way ANOVA.  
48 This analysis showed that the scion genotype had a significant impact on leaf, stem and root biomass;  
49 leaf, stem and root biomasses were over 2-fold higher in plants with an 1103P scion compared to PN  
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7 regardless of rootstock genotype. Root mass fraction (root:whole plant biomass ratio) was not  
8 affected by either the scion or rootstock genotype (Fig. 1D, Supplementary Table 2). However, the  
9 scion genotype did modify leaf mass fraction (leaf:whole plant biomass ratio, Fig. 1E, Supplementary  
10 Table 2), which was higher in grafts with 1103P scions, and both the scion and rootstock modified the  
11 stem to whole shoot mass fraction (stem:whole shoot biomass ratio, Fig. 1G) (Supplementary Table  
12 2).  
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15 After 28 d of LP supply, stem biomass was lower in all scion/rootstock combinations than the HP  
16 treatment (Fig. 1.A); whereas leaf biomass was only lower in LP than HP in plants with an 1103P scion  
17 (Fig. 1.B). Root biomass was not affected by P supply (Fig. 1.C); consequently root mass fraction was  
18 higher in plants grown on LP than HP for all scion/rootstock combinations (Fig. 1.D). Leaf mass  
19 fraction was only modified by LP in 1103P/1103P (Fig. 1.E) and stem to whole shoot mass fraction  
20 was lower in plants growing on LP than HP in all scion rootstock combinations (Fig. 1.F).  
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23 The total number of tips per root system and the average diameter of roots were measured, and root  
24 tip density (i.e. the number of tips per cm of root) was calculated (Fig. 2.). This analysis showed that  
25 the 1103P scion increased the total number of root tips relative to PN (Fig. 2A, Supplementary Table  
26 2), which was associated with the higher root biomass induced by 1103P scions (Fig. 1C). The PN  
27 rootstock had a higher number of tips than 1103P (Fig. 2A, Supplementary Table 2), without  
28 necessarily being associated with a higher root biomass. The root tip density was affected by the  
29 rootstock; PN roots had a higher root density compared to 1103P (particularly with an 1103P scion)  
30 (Fig. 2B, Table 1). Root diameter was not affected by either the scion or the rootstock genotype (Fig.  
31 2C).  
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34 The total number of root tips was not different between the LP and HP treatments (Fig. 2A).  
35 However, the roots of the two hetero-grafts (i.e. 1103P/PN and PN/1103P) had a higher tip density in  
36 plants grown in HP than LP (Fig. 2.B) and average root diameter was lower in plant growing on LP  
37 than HP (Fig. 2.C).  
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#### 39 ***Homo-grafted grapevines have differences in phosphate and sulphate, but not nitrate acquisition*** 40 ***efficiencies*** 41

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43 At the end of the 28 d treatment, the concentrations of Pi, nitrate and sulphate in leaves, stems and  
44 roots were quantified; the LP treatment reduced Pi concentration in both the leaves and the roots in  
45 all scion/rootstock combinations, but there were no significant differences between the different  
46 scion/rootstock combinations (Fig. 3). Nutrient acquisition efficiencies were estimated (assuming  
47 that the concentrations of Pi, nitrate and sulphate were negligible at the beginning of the treatment)  
48 by dividing total plant nutrient content by root FW (Fig. 4). The homo-grafts had differences in PAE  
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7 under HP, with PN/PN having a higher efficiency than 1103P/1103P, while the hetero-grafts had  
8 intermediate values; PAE was very low and there were no differences in PAE between the  
9 scion/rootstock combinations grown under LP (data not shown). Nitrate acquisition efficiency (NAE)  
10 was not affected by LP supply or the scion/rootstock combination (Fig. 4.B). Under HP, sulphate  
11 acquisition efficiency (SAE) in 1103P/1103P was higher than in PN/PN, while the hetero-grafts had  
12 intermediate values (Fig. 4.C), i.e. the opposite of what was found for PAE (Fig. 4.A). Sulphate  
13 acquisition efficiency was not affected by LP supply in any scion/rootstock combination, although  
14 there was a tendency for SAE to decrease in response to LP when the grafts had an 1103P scion (Fig  
15 4.C).  
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19 ***Activity of APases released by the roots is higher under LP than HP***

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21 After 28 d of LP or HP treatment, the activity of APases released into the media by the roots was  
22 measured and normalized by root FW (Fig. 5). The activity of APases released by the roots was two-  
23 fold higher under LP compared to HP in all scion/rootstock combinations. The activity of APases  
24 released by the roots was affected by both the scion and rootstock genotypes, and the interaction  
25 between the scion and rootstock. Under HP, the PN scion increased APase release relative to the  
26 1103P scion when the rootstock was 1103P.  
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30 ***The scion genotype modified organic acid concentrations in the root, while the rootstock genotype***  
31 ***only affected root organic acid concentrations***

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33 Malate, tartrate and citrate concentrations were measured in leaves, stems and roots of grafted  
34 grapevine grown under HP and LP (Fig. 6). Effect of scion and rootstock genotypes on tissue organic  
35 acid concentrations of plants grown on HP was analysed by grouping together data from the grafted  
36 plants by common scion or rootstock genotypes (Supplementary Table 3); this is because the data did  
37 not meet the assumptions for a 3-way ANOVA.  
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40 In leaves, total organic acid concentration (i.e. the sum of malate, tartrate and citrate concentration)  
41 was not affected by the scion or rootstock genotype (Fig. 6.A); however, the proportion of malate  
42 and tartrate was affected by the scion genotype (Fig. 6.B; Fig. 6.C). Grafts with an 1103P scion had  
43 approximately 10-fold more tartrate compared to malate, while combinations with a PN scion had  
44 approximately equal malate and tartrate concentrations. Leaf citrate concentrations were higher in  
45 PN than 1103P scions (Fig. 6.D). In stems, tartrate was the main organic acid in all scion/rootstock  
46 combinations (approximately 70 %, Fig. 6.B) and was higher in grafts with an 1103P scion (Fig. 6.B,  
47 Supplementary Table 3). However, grafts with a PN scion had higher citrate concentrations in the  
48 leaves and the stems (Fig. 6.D, Supplementary Table 3). The rootstock did not affect scion organic  
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7 acid concentrations although the interaction between scion and rootstock genotype affected stem  
8 citrate concentrations (Supplementary Table 3).

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10 In the root, both scion and rootstock affected total organic acid concentration; the concentration  
11 was lowest for 1103P/1103P and highest for PN/PN with the hetero-grafts having intermediate  
12 concentrations (Fig. 6.A, Supplementary Table 3). Root tartrate concentration was only affected by  
13 rootstock genotype (root tartrate concentration was higher in PN than 1103P), but the interaction  
14 between the scion and rootstock genotype was significant. Both the scion and rootstock genotype  
15 affected root malate and citrate concentrations (Supplementary Table 3). Citrate concentration was  
16 highest in PN/PN and lowest in 1103P/1103P with the hetero-grafts having intermediate values  
17 (Supplementary Table 3).

#### 21 ***Tissue organic acid concentrations respond to LP***

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23 In leaves, total organic acid concentration was significantly higher in 1103P/1103P, 1103P/PN and  
24 PN/PN grown under LP than HP (Fig. 6.A), mainly due to an increase of malate in 1103P scions (Fig  
25 5B) and tartrate in PN scions (Fig. 6.B). In the stem, total organic acid concentration was not affected  
26 by Pi supply (Fig. 6.A). Total root organic acid and malate concentration was higher in plants grown  
27 under LP than HP in scion/rootstock combinations with an 1103P rootstock. The concentration of  
28 citrate was higher in plants grown on LP in scion/rootstock combinations with an 1103P scion (Fig. 6).  
29 The root concentration of tartrate was higher in LP than HP only for 1103P/1103P.

#### 33 ***The abundance of transcripts encoding PHT1 transporters, PAPs, PHO2 and SPX domain containing 34 proteins was quantified in the roots of grafted grapevine grown under LP and HP***

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36 The abundance of the transcripts of seven Pi transporters from *PHT1* family was analysed in root tips  
37 of four scion/rootstock combinations of grapevine after 28 d of LP or HP (Fig. 7, Supplementary Table  
38 4). Under HP, the abundance of several *PHT1* transcripts was different between the scion/rootstock  
39 combinations: *PHT1.3a* transcripts were more abundant in 1103P rootstocks, whereas the  
40 abundance of *PHT1.3b* and *PHT1.4d* transcripts were affected by both the scion and rootstock, and  
41 the abundance of *PHT1.4a* transcripts were affected by the scion genotype. The abundance of all the  
42 *PHT1* transcripts measured was higher under LP than HP. The abundance of *PHT1.3b* and *PHT1.4d*  
43 showed significant interactions between both the scion or rootstock genotype and P supply,  
44 indicating a difference in regulation of transcript abundance in response to P depending on the  
45 scion/rootstock combination for these genes.

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47 The transcript abundance of *PAP10* and *PAP12* was analysed in root tips after 28 d of LP or HP (Fig.  
48 7). The transcript abundance of *PAP10* was not affected by the scion or rootstock genotype under  
49 HP, but was higher in plants grown in LP than HP. The increase of abundance of *PAP10* transcripts  
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7 was higher in scion/rootstock combinations with an 1103P than a PN rootstock (Fig. 7). The  
8 abundance of *PAP12* transcripts was higher in plants with a PN than an 1103P rootstock under HP  
9 and was higher in plants grown under LP than HP all scion/rootstock combinations.

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11 The transcript abundance of *PHO2* was analysed in root tips after 28 d of LP and HP (Fig. 7); the  
12 abundance of *PHO2* transcripts was not affected by the scion or rootstock genotype or P supply,  
13 however, there was an interaction between the rootstock genotype and P supply: the abundance of  
14 *PHO2* transcripts decreased in scion/rootstock combinations with an 1103P rootstock, but increased  
15 in scion/rootstock combinations with a PN rootstock. The transcript abundance of the SPX domain  
16 containing genes studied was higher under LP than HP, but was not affected by either the scion or  
17 rootstock genotype.  
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## 21 Discussion

### 22 ***Growth of young grapevines is scion dependant under non-limiting phosphate conditions***

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24 In our study, biomass of grafted grapevines growing under HP was affected by the scion genotype,  
25 grapevines grafted with 1103P as scion, regardless of the rootstock genotype, had a higher shoot and  
26 root FW compared to grapevines with a PN scion. Our results agree with previous studies showing  
27 that scion regulates the growth of young grapevines more than the rootstock genotype (Lefort and  
28 Legisle, 1977; Rives, 1971; Tandonnet *et al.*, 2010). In terms of root development, a few studies have  
29 reported an effect of the scion genotype on root system characteristics in young perennial fruit  
30 crops; these studies were limited to measurements of shoot:root ratio, root biomass or total root  
31 (Harrison *et al.*, 2016; Oslobeanu, 1978; Tandonnet *et al.*, 2010). In the present study, we also  
32 quantified the root diameter, and the number and the density of tips. Under HP, grapevines grafted  
33 with 1103P as scion has a higher number of tips than those with a PN scion, and this was related to a  
34 higher total root length. The number of tips was also higher in the rootstock PN than 1103P without  
35 necessarily being associated with increased root biomass suggesting that a high degree of branching  
36 of the PN rootstock, which was also confirmed by the tip density data. This is in agreement with the  
37 literature as grapevine genotypes have been shown to differ in root branching patterns (Dumont *et*  
38 *al.*, 2016; Smart *et al.*, 2006; Southey, 1992). The scion genotype affected tip density particularly  
39 when the rootstock was PN; tip density was higher in 1103P/PN compared to PN/PN; this could  
40 suggest that the scion can exert long-distance regulation of root branching in grapevine. This  
41 hypothesis is supported by a study on the effect of the scion on the root system of mature plants in a  
42 17 year-old vineyard, which showed that the scion could affect root biomass, shoot:root ratio, root  
43 density (per m<sup>2</sup> of soil) and root distribution in the soil (Ferlito *et al.*, 2020).  
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7 In summary, the scion genotype exerted control over whole plant development in the reciprocal  
8 grafts of grapevine studied, which is largely in agreement with the literature.  
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#### 10 ***LP alters biomass allocation and root morphology in grafted grapevine***

11 Many studies shown a drastic reduction in shoot growth in response to limited Pi supply, this is  
12 related to a decrease of photosynthetic activity and carbon assimilation (Vance et al. 2003; Warren  
13 2011), as well as the preferential allocation of carbon resources to root system in order to decrease  
14 the shoot:root biomass ratio (Mollier and Pellerin 1999; Wen et al. 2017). These modifications have  
15 previously been observed in grafted grapevines (Grant and Matthews 1996b) and in un-grafted  
16 woody cuttings (Gautier et al. 2018). In our present study, shoot biomass was lower in plants treated  
17 with LP; grapevines grafted with an 1103P scion had a larger response. Stem biomass relative to total  
18 shoot biomass was lower in plants grown under LP than HP, suggesting that under limited Pi  
19 availability carbon is diverted away from the formation of stems (which support photosynthetic  
20 organs and conducting sap, and constitute carbon reserves) towards carbon capture (i.e. leaves).  
21 Root biomass was not affected by the LP treatment in all scion/rootstock combinations, resulting in a  
22 lower root mass fraction (i.e. root to total plant biomass ratio) in plants grown in LP as often  
23 observed in other species (Fernandez and Rubio 2015).  
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30 Increased root branching (here quantified as tip density) is an important factor for Pi acquisition and  
31 for many species the enhancement of emergence of lateral roots is a common response to LP supply  
32 (Péret et al. 2011, 2014). However, some species (e.g. *Phaseolous vulgaris*) shown the opposite  
33 response, i.e. a decrease in the emergence of lateral roots has been shown (Borch et al. 1999). In this  
34 study on young grafted grapevines, it seems that tip density is also reduced in response to LP. Root  
35 diameter decreased in all scion/rootstock combinations in response to LP, suggesting that grapevine  
36 adapts its root system architecture to forage for Pi by reducing carbon demand per unit of root  
37 length rather than increasing branching.  
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41 In summary, in response to LP grafted grapevines modify biomass allocation and growth to relatively  
42 increase soil exploration by roots and economize carbon use in the stems.  
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#### 44 ***Scion/rootstock combination and phosphate supply effects PAE, the abundance of PHT1 transcripts*** 45 ***and SAE***

46 The genotypes studied in this work are known to differ in their PAE, when 1103P is cultivated as un-  
47 grafted, woody cuttings, it has a higher PAE than PN (Gautier et al. 2018). However, in this study on  
48 herbaceous micro-grafts, PAE was higher for PN/PN homo-grafts compared to 1103P/1103P homo-  
49 grafts, with intermediate values for the two hetero-grafts PN/1103P and 1103P/PN. The methods  
50 used in these studies were not the same; in Gautier et al. (2018) PAE was defined by slope of linear  
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7 regression between  $^{32}\text{P}$  uptake of the shoot and biomass root FW, whereas in this work PAE was  
8 estimated by total plant Pi divided by root FW (assuming that Pi content at the beginning of the  
9 treatment was negligible). This could suggest that plant growth conditions and/or plant  
10 developmental stage alters PAE in grapevine and/or that the method used to calculate PAE affects  
11 the results.  
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14 The abundance of seven *PHT1* transcripts in the root was quantified under both HP and LP. Under  
15 HP, several transcripts showed scion and/or rootstock specific accumulation patterns, suggesting an  
16 intrinsic difference in *PHT1* transcript abundance between the two rootstock genotypes studied and  
17 that it is altered by shoot-derived signals. All transcripts were strongly up-regulated under LP supply,  
18 suggesting a higher capacity to take up Pi from the soil under LP, but given only 0.001 mM Pi was  
19 provided to the plants PAE could not be quantified. *PHOSPHATE2* abundance was affected by LP in a  
20 rootstock dependent manner, suggesting differences in post-transcriptional regulation and  
21 potentially in the quantity of PHT1 protein in the different rootstock genotypes.  
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25 Under HP, the different scion/rootstock combinations differed in SAE, which was highest in  
26 1103P/1103P and lowest in PN/PN with the hetero-grafts having intermediate values, suggesting that  
27 under HP both the scion and rootstock genotype regulate SAE. Genotypic differences in sulphur  
28 accumulation have been reported in different scion/rootstock combinations of grapevine and 1103P  
29 has been shown to confer higher sulphur contents than other rootstock genotypes (Gautier *et al.*,  
30 2020b; Lecourt *et al.*, 2015). Under HP, there was an inverse relationship between PAE and SAE;  
31 there are well-described interactions between Pi and sulphate nutrition in plants with phospholipids  
32 being replaced by sulpholipids in times of Pi limitation and vice versa, although the molecular  
33 mechanisms are poorly understood (Bouain *et al.*, 2019; Rouached *et al.*, 2011). In grafted plants  
34 with an 1103P scion, SAE was lower under LP than HP (whereas SAE was not affected by Pi supply  
35 when the scion was PN); this suggests that the scion genotype can modify SAE in response to LP in  
36 grafted grapevine. The magnitude of differences in SAE in this study suggest that the differences  
37 reported here are due to more than subtle differences in the composition of lipids and may be  
38 related to the accumulation of other sulphur containing molecules such as glutathione.  
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44 Nitrate acquisition efficiency was affected by the scion genotype, it was higher with a PN scion  
45 (largely due to higher shoot N concentration), and its response to LP was scion genotype dependent  
46 (remaining unchanged with a PN scion but lower with an 1103P scion). In summary, this suggests that  
47 in response to LP, the 1103P scion reduces NAE and SAE, whereas the PN scion does not.  
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50 ***The release of APases by roots in response to LP is associated with the increase in abundance of***  
51 ***PAP10 and PAP12***  
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7 The activity of APases released into the media was approximately two-fold higher for each  
8 scion/rootstock combination grown under LP compared to HP. Related to this increase of APase  
9 activity, the relative abundances of *PAP10*, and *PAP12* transcripts were higher in plants grown under  
10 LP supply. *PURPLE ACID PHOSPHATASE10* and *PAP12* encode secreted APases, which are known to be  
11 increased in response to Pi starvation in species such as Arabidopsis, tomato or rice (Tian et al. 2012).  
12 These results suggest that grapevine is also able to use organic phosphorus from the soil. The activity  
13 of APases released into the media was affected by the scion and rootstock genotype and their  
14 interaction, whereas only the rootstock genotype affected the abundance of *PAP12* and only the  
15 interaction between the rootstock genotype and Pi supply affected the abundance of *PAP10*  
16 suggesting that other APase genes may be underlying the activity of APases released by the roots.  
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21 ***Organic acid profile of grapevine tissues varies between genotypes and can be modified by grafting***  
22 ***and phosphate supply***  
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24 In many species, the major organic acids are citrate and malate (Neumann and Römheld 1999),  
25 whereas tartrate is an organic acid restricted to a small number of species, including the *Vitaceae*  
26 (Bennet-Clark 1933). In the present study, organic acids were mainly found in the highest  
27 concentrations in the leaves without differences in total concentration between the four  
28 scion/rootstock combinations although the concentration of individual organic acids varied. Tartrate  
29 and malate concentrations have been shown to differ between different *V. vinifera* varieties,  
30 different leaf ages and during the growing season (Attia et al., 2007). The rootstock did not affect  
31 organic acid concentration in scion in our study although the rootstock has been shown to alter  
32 organic acid concentration in 5-year old grapevines grown in an inert substrate and irrigated with a  
33 nutrient solution (Attia et al., 2007). The stem organic concentrations were lower than those of the  
34 leaves and were different between the different scion genotypes. Root total organic acid  
35 concentration was different between the different rootstock genotypes studied as has been shown in  
36 other work (Covarrubias et al., 2016) and was affected by the scion genotype. This could suggest that  
37 the shoots provide roots with some organic acids, in particularly malate which is the major organic  
38 acid in the phloem (Keller, 2010). We have previously shown that grafting an 1103P rootstock with a  
39 PN scion (i.e. PN/1103P) reduces the expression of *NADP-MALIC ENZYME2/Vitvi04g00009* (a key  
40 enzyme regulating malate metabolism) in comparison to the 1103P/1103P homograft control further  
41 suggesting that scion could provide malate to the rootstock in grapevine.  
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48 Tissue organic acid concentrations often change in response to Pi supply due to a number of reasons,  
49 such as, under HP supply, the co-transport of Pi with protons can lead to metabolic adjustments  
50 (such as a reduction in organic acid concentration) to maintain internal pH, whereas under LP supply,  
51 organic acids may be synthesized to be released by the roots to increase the availability of  
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7 phosphorus in the soil (Vance et al. 2003). In grapevine, differences in organic acid concentration in  
8 root tips were previously observed under iron deficiency and in response to different nitrogen  
9 sources (Jiménez et al. 2007; Covarrubias and Rombolà 2015). In response to LP, scion/rootstock  
10 combinations with an 1103P rootstock showed an increase of total organic acid concentration in  
11 root, which was particularly associated with an increase in malate concentration. Recently, the  
12 capacity of grapevine to release organic acids into the rhizosphere in responses to alkalinity were  
13 demonstrated (Xiang et al. 2019). Unfortunately, due to technical difficulties we were unable to  
14 measure root organic acid exudation in these experiments.  
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### 18 **The role of long-distance signalling in regulating responses to P supply in grafted grapevine**

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20 We have previously characterised the early root transcriptome response to LP of the same reciprocal  
21 scion/rootstock combinations of grapevine; we showed that the transcriptome response of PN/PN is  
22 more extensive than that of 1103P/1103P and that many of the genes showing genotype-specific  
23 responses are related to iron, organic acid and sulphate transport, carbon metabolism and hormone  
24 signalling (Gautier et al. 2020a). We also found that the scion could modify the root transcriptome  
25 response to LP and the genes differentially expressed suggest that phloem mobile metabolites,  
26 cysteine rich peptides, hormones such as strigolactones and ethylene, and microRNAs could be  
27 involved (Gautier et al. 2020a). In this manuscript we have characterised the long-term physiological  
28 responses to LP in the same scion/rootstock combinations, some root traits are regulated by both  
29 the scion and rootstock tissues (such as tissue Pi and organic acid concentrations, PAE and SAE)  
30 suggesting that these traits are regulated by long-distance signalling.  
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### 35 **Conclusion**

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37 The objective of this study was to determine the roles of both the shoot and the root in employing  
38 strategies to cope with LP supply in grapevine. Under HP, the homograft PN/PN has higher tissue Pi  
39 concentrations and higher PAE than 1103P, which was associated with a higher constitutive  
40 expression of a number of *PHT1* genes, a lower SAE, and reduced growth. Reciprocal grafting showed  
41 that growth was regulated by the scion genotype, presumably via its capacity of assimilation and/or  
42 translocation of carbon. Whereas the heterografts, PN/1103P and 1103P/PN, had intermediate  
43 phenotypes in terms of tissue Pi concentrations, PAE and SAE suggesting that these parameters were  
44 regulated by both the scion and rootstock. In response to LP, all scion/rootstock combinations  
45 altered growth to favour root foraging, increase the expression of PSI genes and increased the  
46 exudation of APases (with the concomitant increase in the abundance of *PAP10* and *PAP12*  
47 transcripts). The organic acid concentration in the different tissues was affected by the  
48 scion/rootstock combination and the response to LP supply showed an effect on scion and rootstock  
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7 genotype. Many signals are known to move between the shoot and the root (and vice versa) to  
8 regulate plant responses to P supply (Zhang *et al.*, 2014), by grafting different genotypes together we  
9 have shown that genetic variation in the shoot exerts long-distance control over a number of these  
10 responses.  
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### 12 **Data and Materials availability**

13 Experimental data and materials available to third party academic researchers upon reasonable  
14 request.  
15

### 16 **Supplementary Data**

17 [Supplementary Figure 1. Photographs of the plants used in the manuscript at different stages A. in](#)  
18 [vitro grafted grapevines, B. acclimation in perlite, C. and D. plants growing in hydroponic culture.](#)

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23 Supplementary Table 1. List of primers used for RT-qPCR experiments.

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29 Supplementary Table 2. Statistical analysis of biomass, biomass allocation and root morphology  
30 measurements of reciprocal grafts of *Vitis berlandieri* x *V. rupestris* cv. 1103 Paulsen (1103P) and *V.*  
31 *vinifera* cv. Pinot noir (PN) grown under high phosphate supply grouped by common scion or  
32 rootstock genotype.

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Supplementary Table 3. Statistical analysis of malate, tartrate and citrate concentrations and the sum  
of these three organic acids in the leaves, stems and roots of reciprocal grafts of *Vitis berlandieri* x *V.*  
*rupestris* cv. 1103 Paulsen (1103P) and *V. vinifera* cv. Pinot noir (PN) grown under high phosphate  
supply grouped by common scion or rootstock genotype.

### Supplementary Table 4. Results of a 3-way analysis of variance of the abundance of transcripts encoding various *PHOSPHATE TRANSPORTER1 (PHT1)*, *PURPLE ACID PHOSPHATASE (PAP)*, *PHOSPHATE2 (PHO2)* and *SPX DOMAIN CONTAINING PROTEINS (SPX)* genes in the roots of reciprocal grafts of *Vitis berlandieri* x *V. rupestris* cv. 1103 Paulsen and *V. vinifera* cv. Pinot noir after 28 d of either high or low phosphate (Pi) supply (with scion genotype, rootstock genotype and P supply as factors).

**Conflict of interest**

None declared.

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(ANR) in the frame of the Investments for the future Program, within the Cluster of Excellence COTE  
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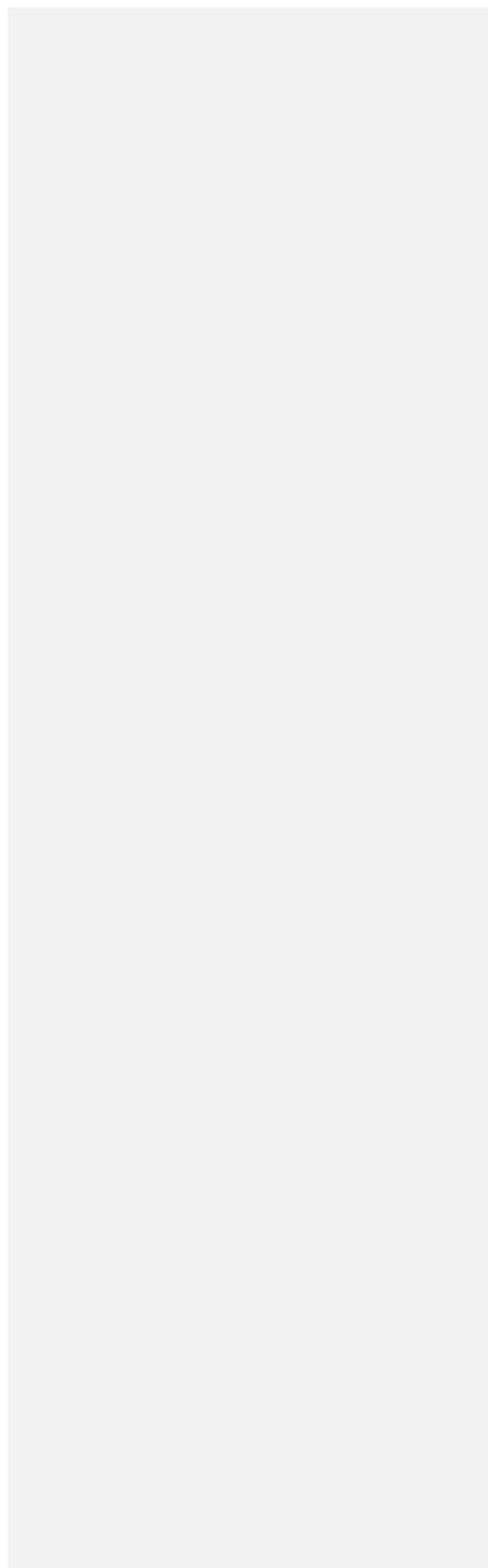
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**Authors' contributions**

AG, IM, BP and PD executed the experiments and collected data, AG and NC analysed the data, VL, AM, PV and SJC acquired the funding, SJC drafted the manuscript, and all authors made significant intellectual contributions to the design and execution of the work, and revised the manuscript.



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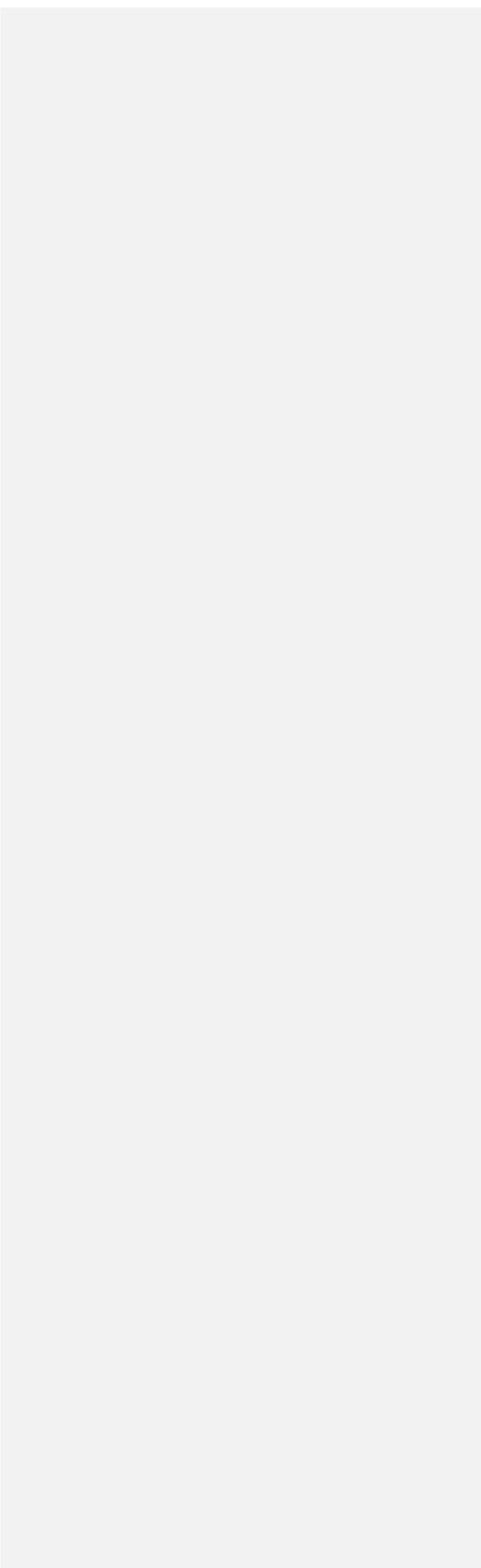
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9 Figure 1. (A) Stem, (B) leaf and (C) root fresh weight (FW), and (D) root:whole plant FW, (E)  
10 leaf:whole plant FW and (F) stem:whole shoot FW ratios of reciprocal grafts of *Vitis berlandieri* x *V.*  
11 *rupestris* cv. 1103 Paulsen (1103P) and *V. vinifera* cv. Pinot noir (PN) after 28 d of either high (dark  
12 colours) or low phosphate supply (light colours). The scion genotype is presented above the  
13 rootstock genotype. Means and standard errors shown (n = 15). For each combination, stars indicate  
14 an effect of phosphate supply using Wilcoxon test with the Bonferroni correction (\*P value < 0.05,  
15 \*\* P value < 0.01, \*\*\* P value < 0.001).  
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18 Figure 2. (A) Total number of root tips, (B) tip density and (C) average diameter of roots of reciprocal  
19 grafts of *Vitis berlandieri* x *V. rupestris* cv. 1103 Paulsen (1103P) and *V. vinifera* cv. Pinot noir (PN)  
20 after 28 d of either high (dark colours) or low phosphate (light colours) supply. The scion genotype is  
21 presented above the rootstock genotype. Means and standard errors shown (n = 5). For each  
22 combination, stars indicate an effect of phosphate supply using t-test with the Bonferroni correction  
23 (\*P value < 0.05, \*\* P value < 0.01, \*\*\* P value < 0.001).  
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27 Figure 3. (A) Leaf and (B) root phosphate concentration of reciprocal grafts of *Vitis berlandieri* x *V.*  
28 *rupestris* cv. 1103 Paulsen (1103P) and *V. vinifera* cv. Pinot noir (PN) after 28 d of either high (dark  
29 colours) or low phosphate (light colours) supply. The scion genotype is presented above the  
30 rootstock genotype. Means and standard errors shown (n = 5). For each combination, stars indicate  
31 an effect of phosphate supply using t-test with the Bonferroni correction (\*\*\* P value < 0.001).  
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34 Figure 4. Estimates of (A) phosphate, (B) nitrate and (C) sulphate acquisition efficiencies (PAE, NAE  
35 and SAE respectively) of reciprocal grafts of *Vitis berlandieri* x *V. rupestris* cv. 1103 Paulsen (1103P)  
36 and *V. vinifera* cv. Pinot noir (PN) after 28 d of either high (dark colours) or low phosphate supply  
37 (light colours). The scion genotype is presented above the rootstock genotype. Means and standard  
38 errors shown (n = 5). Letters indicate significant differences using a multiple comparison after  
39 Kruskal-Wallis tests at P value < 0.05.  
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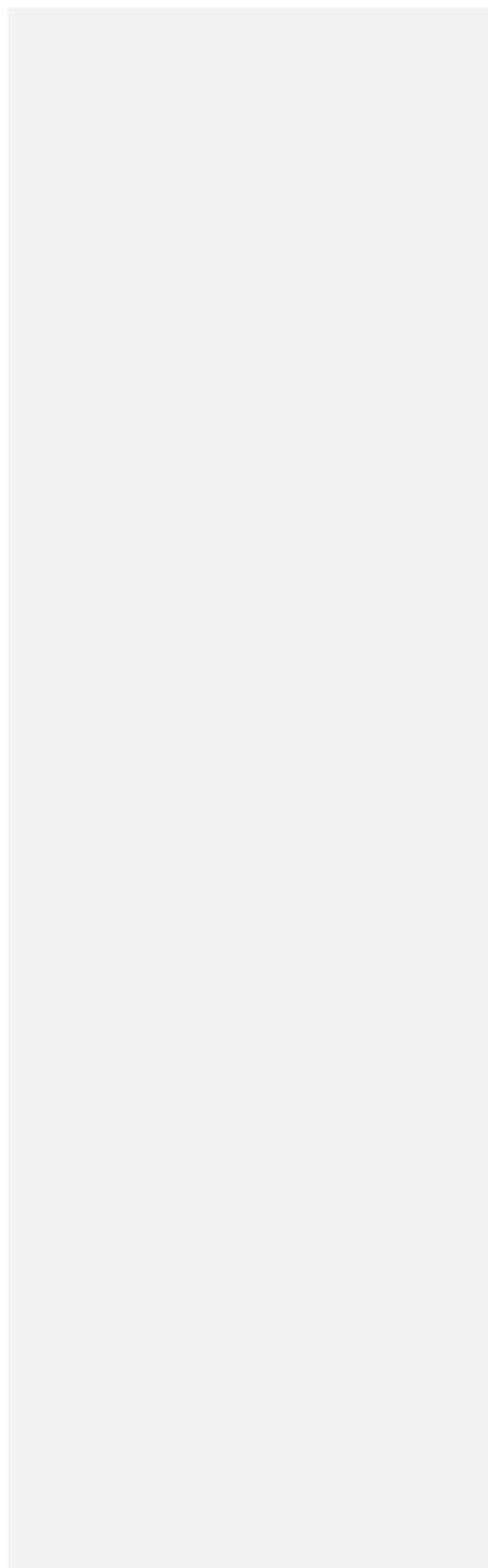
42 Figure 5. Acid phosphatase (APase) activity measured in root exudates of reciprocal grafts of *Vitis*  
43 *berlandieri* x *V. rupestris* cv. 1103 Paulsen (1103P) and *V. vinifera* cv. Pinot noir (PN) after 28 d of  
44 either high (dark colours) or low phosphate supply (light colours). The scion genotype is presented  
45 above the rootstock genotype. Means and standard errors shown (n = 5). Letters indicate significant  
46 differences (P value < 0.05) using a Tukey test after a two-way ANOVA with scion/rootstock  
47 combination and phosphate supply as factors.  
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51 Figure 6. Histograms of (B) malate, (C) tartrate, (D) citrate concentrations and (A) the sum of these  
52 three organic acids in leaves, stems and roots of reciprocal grafts of *Vitis berlandieri* x *V. rupestris* cv.  
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1103 Paulsen (1103P) and *V. vinifera* cv. Pinot noir (PN) grown under high (dark colours) or low (light colours) phosphate supply. The scion genotype is presented before the rootstock genotype. Stars indicate a significant change in concentration in between phosphate supplies tested using a t-test with the Bonferroni correction (\*P value < 0.05, \*\* P value < 0.01, \*\*\* P value < 0.001).

Figure 7. Heatmaps of the abundance of transcripts encoding various PHOSPHATE TRANSPORTER1 (PHT1), PURPLE ACID PHOSPHATASE (PAP), PHOSPHATE2 (PHO2) and SPX DOMAIN CONTAINING PROTEINS genes in the roots of reciprocal grafts of *Vitis berlandieri* x *V. rupestris* cv. 1103 Paulsen (1103P) and *V. vinifera* cv. Pinot noir (PN) after 28 d of either high (HP) or low phosphate (LP) supply. The scion genotype is presented above the rootstock genotype. For transcript abundance under HP, green shade indicates the level of expression relative to the lowest value. For transcript abundance changes in response to LP, orange and purple shades indicates the extent up- or down-regulation respectively (n = 3).



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65**Supplementary Table 1.** List of primers used for RT-qPCR experiments.

Gene name	Accession number	Forward primer	Reverse primer	Efficiency	Amplicon size
PHT 1.3a	VIT_13s0067g03280	GGGCAATTGTGGCTTTGTCT	TGGTCCTCCCCAGAGAGTTG	96.5	106
PHT 1.3b	VIT_16s0050g02380	CCCCACTAAAGATAAGCCTGGG	TCCTACTAGGGCAACCCCAA	103.2	73
PHT 1.4a	VIT_05s0049g00920	TTGTGCTCGGGGTAGTCAAC	CCAGTCCTGGTAGAAGGGGA	90	148
PHT 1.4b	VIT_05s0049g00930	CCCACCTGGTATTGGAATGAGA	TAGTAGGAGGCTGCATGTCCA	85	166
PHT 1.4c	VIT_05s0049g00940	CCCGAGTCCAAGGGAAAGTC	GGAACAGTCCTAGCCTGCTG	99.1	98
PHT 1.4d	VIT_16s0050g02370	TTGGTGACGGAGACCAAAGG	GTGTGCTAGGCATCTGGGTT	94.5	90
PHT 1.9	VIT_18s0122g00780	CCCGTGAAACCAAGGGAAGA	GAATCCGCGATGAGTTTCGC	95.8	167
PHO2	VIT_00s0265g00070	TTGTGCTGTGGAAGCAGGAT	GTGCAGGCAAACCTCAAACCA	80.2	80
PAP10	VIT_03s0038g00220	AGCCAGTTTTGGACACGCTA	GTGGGTCCACAAACCTTCCA	96.9	136
PAP12	VIT_18s0001g13340	GGACCTCTCAGAAAAATCCTCT	ACCAAATGGGACAGTGAACAT	100.1	114
SPX1/SPX2	VIT_11s0016g05330	GATGGACAGGAAGGGTGTGG	TTTCCTTCAGAGCCCGCAAT	91	151
SPX3	VIT_15s0048g00190	CAGGAACACAGTTGCAGCAC	TGGGGATGGGAGAGTGGAAT	91.5	140
ACTIN	VIT_04s0044g00580	CTTGATCCCTCAGCACCTT	TCCTGTGACAATGGATGGA	93.6	82
GAPDH	VIT_17s0000g10430	CCACAGACTTCATCGGTGACA	TTCTCGTTGAGGGCTATTCCA	91	70
SAND3'	VIT_06s0004g02820	TGCTGGGTTACCCCGAGTTTGA	CAGACCCGTTGCACGTCCG	89.9	89

**Supplementary Table 2.** Statistical analysis of biomass, biomass allocation and root morphology measurements of reciprocal grafts of *Vitis berlandieri* x *V. rupestris* cv. 1103 Paulsen (1103P) and *V. vinifera* cv. Pinot noir (PN) grown under high phosphate supply grouped by common scion or rootstock genotype. Means and standard errors shown (n = 30 for biomass measurements and n = 10 for root morphology measurements). Stars indicate an effect of either the scion or rootstock genotype, tested using Wilcoxon tests for biomass measurements and t-tests for root morphology (\**P* value < 0.05, \*\* *P* value < 0.01, \*\*\* *P* value < 0.001).

	Scion			Rootstock		
	1103P	PN	Significance	1103P	PN	Significance
<b>Leaf FW (g)</b>	3.11 ± 0.22	1.07 ± 0.12	***	2.01 ± 0.25	2.17 ± 0.27	NS
<b>Stem FW (g)</b>	2.42 ± 0.17	0.91 ± 0.10	***	1.58 ± 0.18	1.73 ± 0.21	NS
<b>Root FW (g)</b>	2.56 ± 0.13	1.10 ± 0.08	***	1.74 ± 0.17	1.90 ± 0.18	NS
<b>Root:plant FW ratio</b>	0.33 ± 0.01	0.37 ± 0.01	NS	0.34 ± 0.01	0.34 ± 0.02	NS
<b>Leaf:plant FW ratio</b>	0.38 ± 0.01	0.34 ± 0.01	***	0.36 ± 0.01	0.36 ± 0.02	NS
<b>Stem:shoot FW ratio</b>	0.79 ± 0.02	0.85 ± 0.03	**	0.85 ± 0.03	0.79 ± 0.02	**
<b>Number of tips</b>	823 ± 92	389 ± 52	***	485 ± 85	726 ± 105	NS
<b>Tip density (cm<sup>-1</sup>)</b>	1.03 ± 0.05	0.93 ± 0.03	NS	0.90 ± 0.02	1.07 ± 0.05	**
<b>Average root diameter (mm)</b>	0.95 ± 0.02	0.97 ± 0.03	NS	0.94 ± 0.01	0.98 ± 0.02	NS

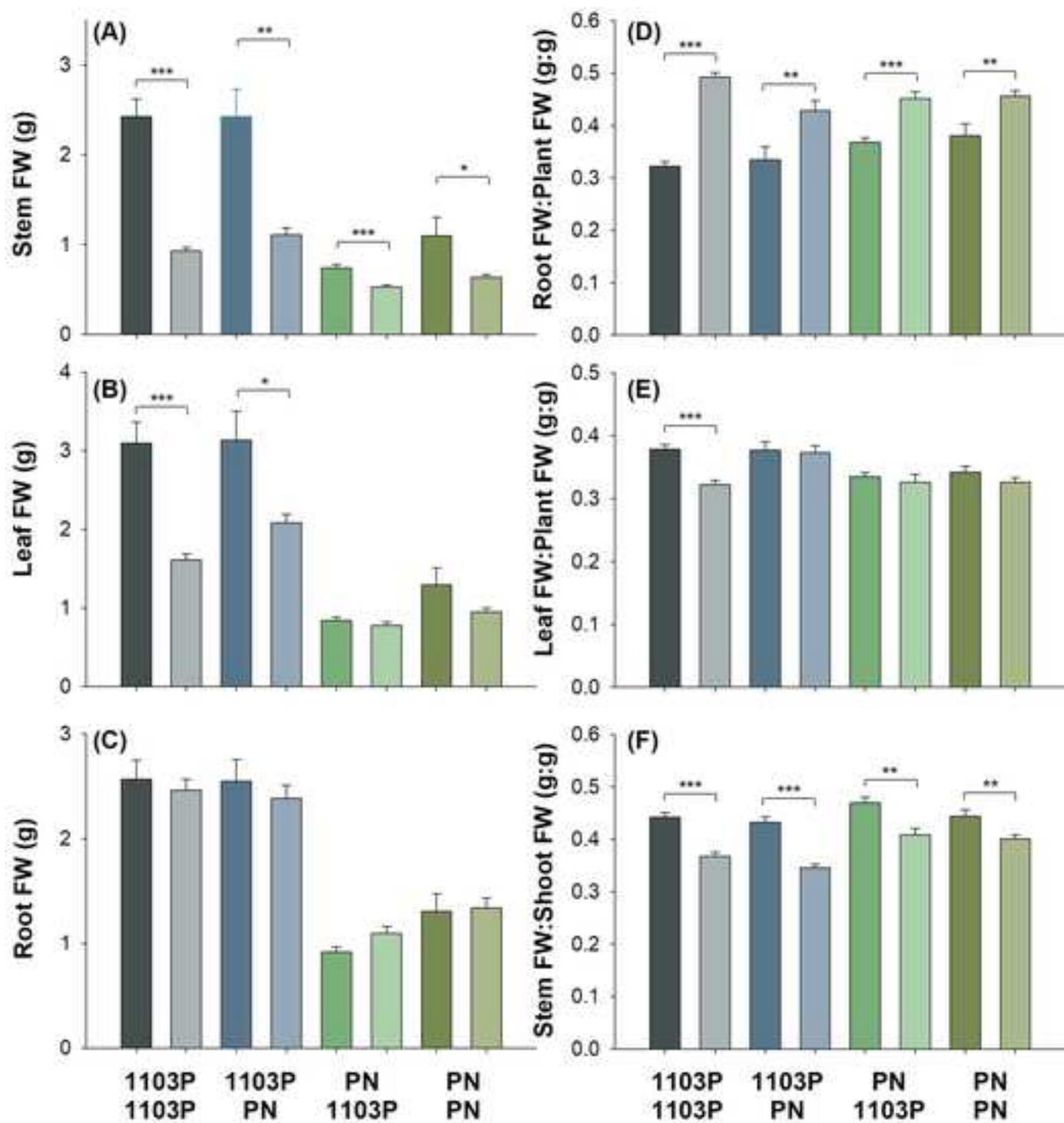
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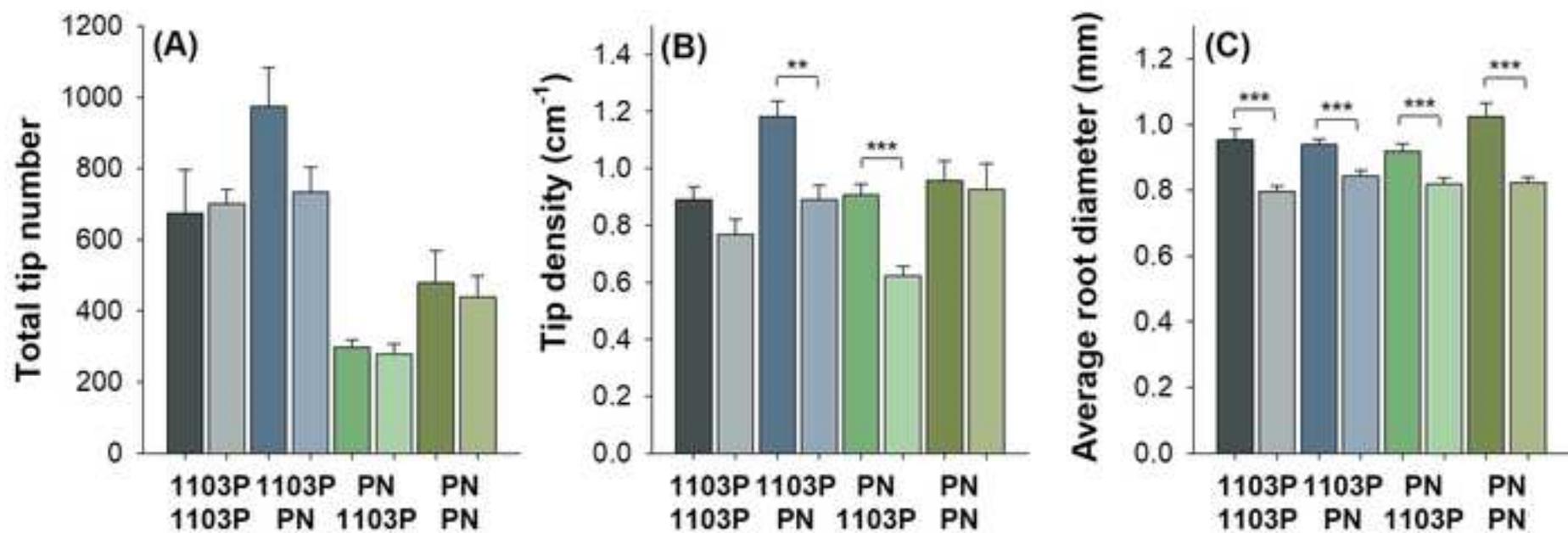
**Supplementary Table 3.** Statistical analysis of malate, tartrate and citrate concentrations and the sum of these three organic acids in the leaves, stems and roots of reciprocal grafts of *Vitis berlandieri* x *V. rupestris* cv. 1103 Paulsen (1103P) and *V. vinifera* cv. Pinot noir (PN) grown under high phosphate supply grouped by common scion or rootstock genotype. Stars indicate an effect of the scion or rootstock genotype tested using a two-way analysis of variance with scion and rootstock genotype as factors (\**P* value < 0.05, \*\* *P* value < 0.01, \*\*\* *P* value < 0.001).

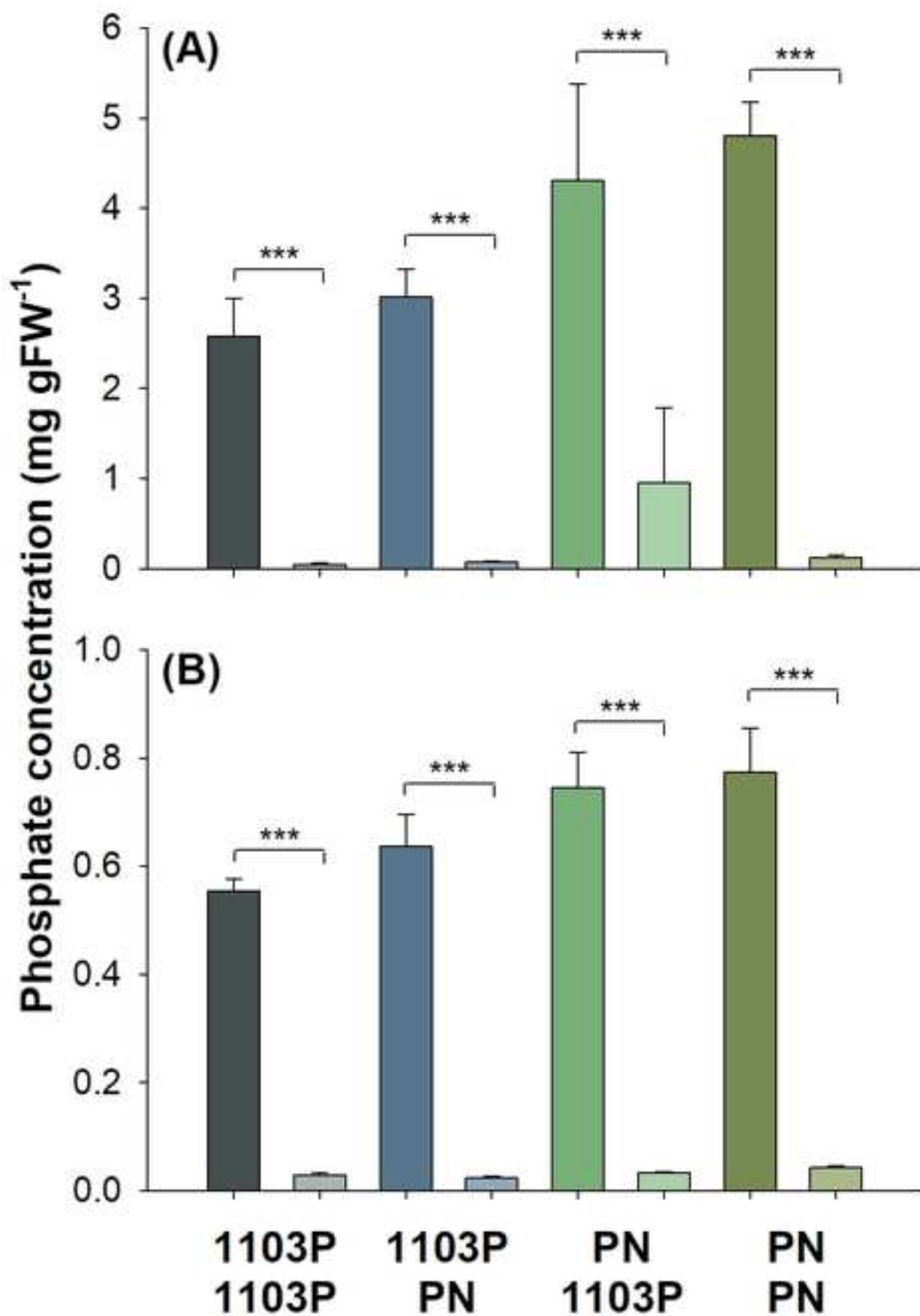
Organic acid concentration (mg g <sup>-1</sup> FW)	Scion			Rootstock			Scion x Rootstock Interaction
	1103P	PN	Significance	1103P	PN	Significance	Significance
<b>Shoot</b>							
Organic acids	14.11 ± 0.93	13.02 ± 0.45	NS	14.63 ± 0.63	12.93 ± 0.80	NS	NS
Malate	1.36 ± 0.19	6.01 ± 0.20	***	3.89 ± 0.75	3.48 ± 0.83	NS	NS
Tartrate	12.48 ± 0.81	6.27 ± 0.43	***	9.94 ± 1.20	8.82 ± 1.20	NS	NS
Citrate	0.21 ± 0.02	0.29 ± 0.02	*	0.26 ± 0.02	0.24 ± 0.63	NS	NS
<b>Stem</b>							
Organic acids	8.08 ± 0.37	7.08 ± 0.30	*	7.85 ± 0.42	7.30 ± 0.30	NS	NS
Malate	2.15 ± 0.15	2.02 ± 0.12	NS	2.15 ± 0.13	2.02 ± 0.15	NS	NS
Tartrate	5.50 ± 0.21	4.45 ± 0.17	***	5.16 ± 0.29	4.80 ± 0.20	NS	NS
Citrate	0.29 ± 0.03	0.46 ± 0.03	***	0.38 ± 0.02	0.36 ± 0.05	NS	***
<b>Root</b>							
Organic acids	1.61 ± 0.17	2.66 ± 0.28	***	1.54 ± 0.16	2.74 ± 0.25	***	NS
Malate	0.41 ± 0.06	0.62 ± 0.10	*	0.34 ± 0.03	0.70 ± 0.08	***	NS
Tartrate	0.71 ± 0.08	0.76 ± 0.04	NS	0.57 ± 0.04	0.90 ± 0.04	***	**
Citrate	0.35 ± 0.03	1.12 ± 0.14	***	0.50 ± 0.09	0.97 ± 0.18	***	**

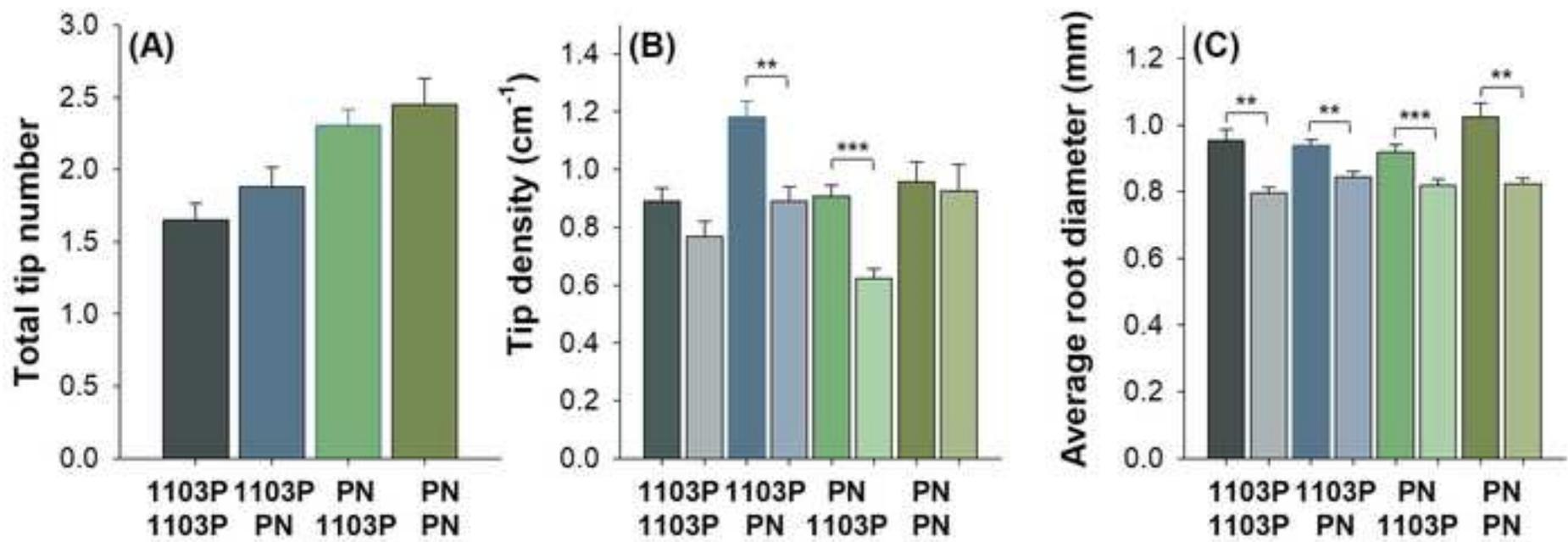
**Supplementary Table 4.** Results of a 3-way analysis of variance of the abundance of transcripts encoding various *PHOSPHATE TRANSPORTER1 (PHT1)*, *PURPLE ACID PHOSPHATASE (PAP)*, *PHOSPHATE2 (PHO2)* and *SPX DOMAIN CONTAINING PROTEINS (SPX)* genes in the roots of reciprocal grafts of *Vitis berlandieri* x *V. rupestris* cv. 1103 Paulsen and *V. vinifera* cv. Pinot noir after 28 d of either high or low phosphate (Pi) supply (with scion genotype, rootstock genotype and P supply as factors, n = 3). \**P* value < 0.05, \*\* *P* value < 0.01, \*\*\* *P* value < 0.001.

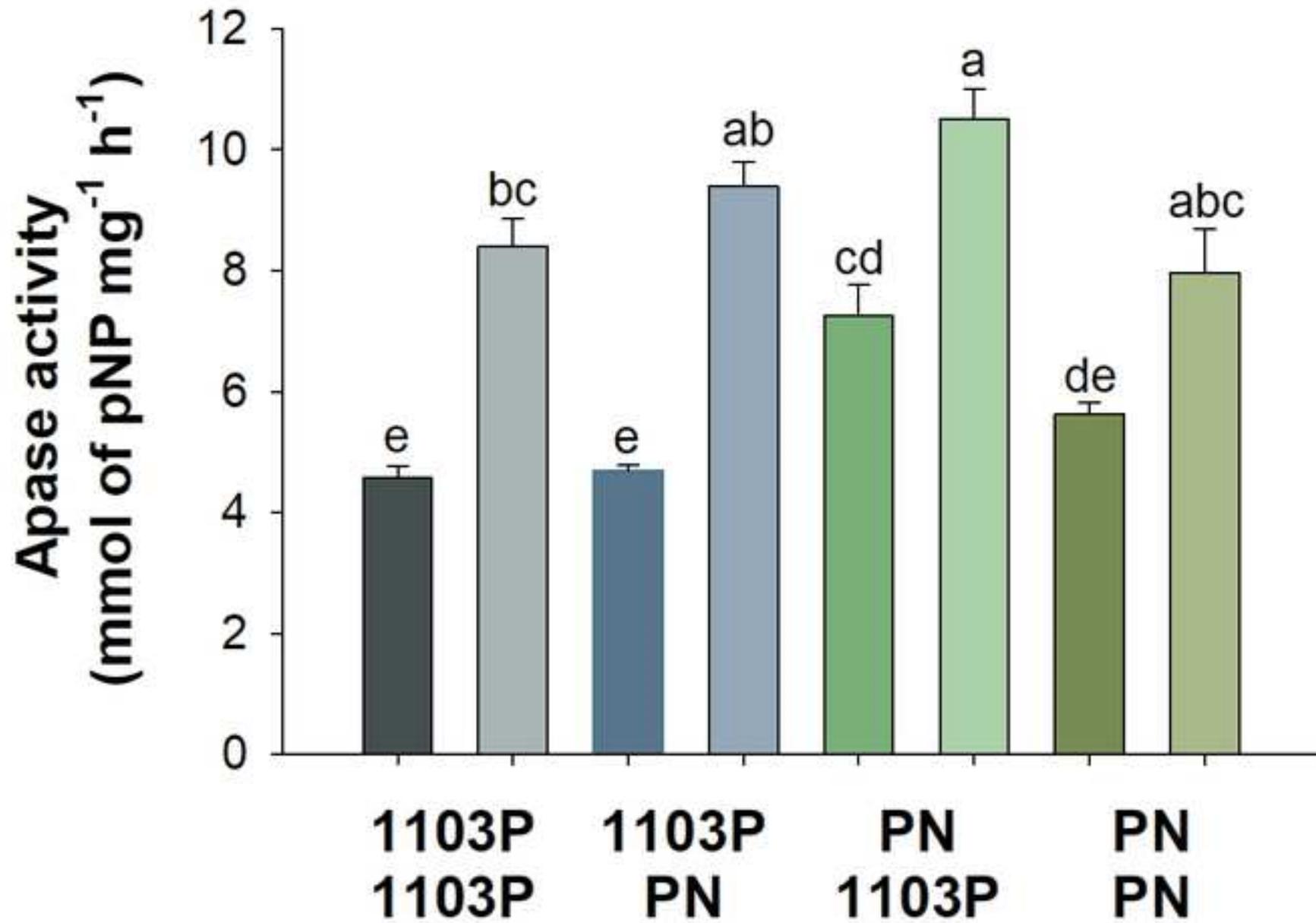
	Scion	Rootstock	Pi supply	Scion x Rootstock	Scion x Pi supply	Rootstock x Pi supply	Scion x Rootstock x Pi supply
PHT1.3a		***	*				
PHT1.3b	**	***	***		***	***	
PHT1.4a	*		***				**
PHT1.4b			***				
PHT1.4c			***				
PHT1.4d	***	***	***		***	***	
PHT1.9			***				
PHO2						***	
PAP10			***			***	
PAP12		***	***				
SPX1/2			***				
SPX 3			***				











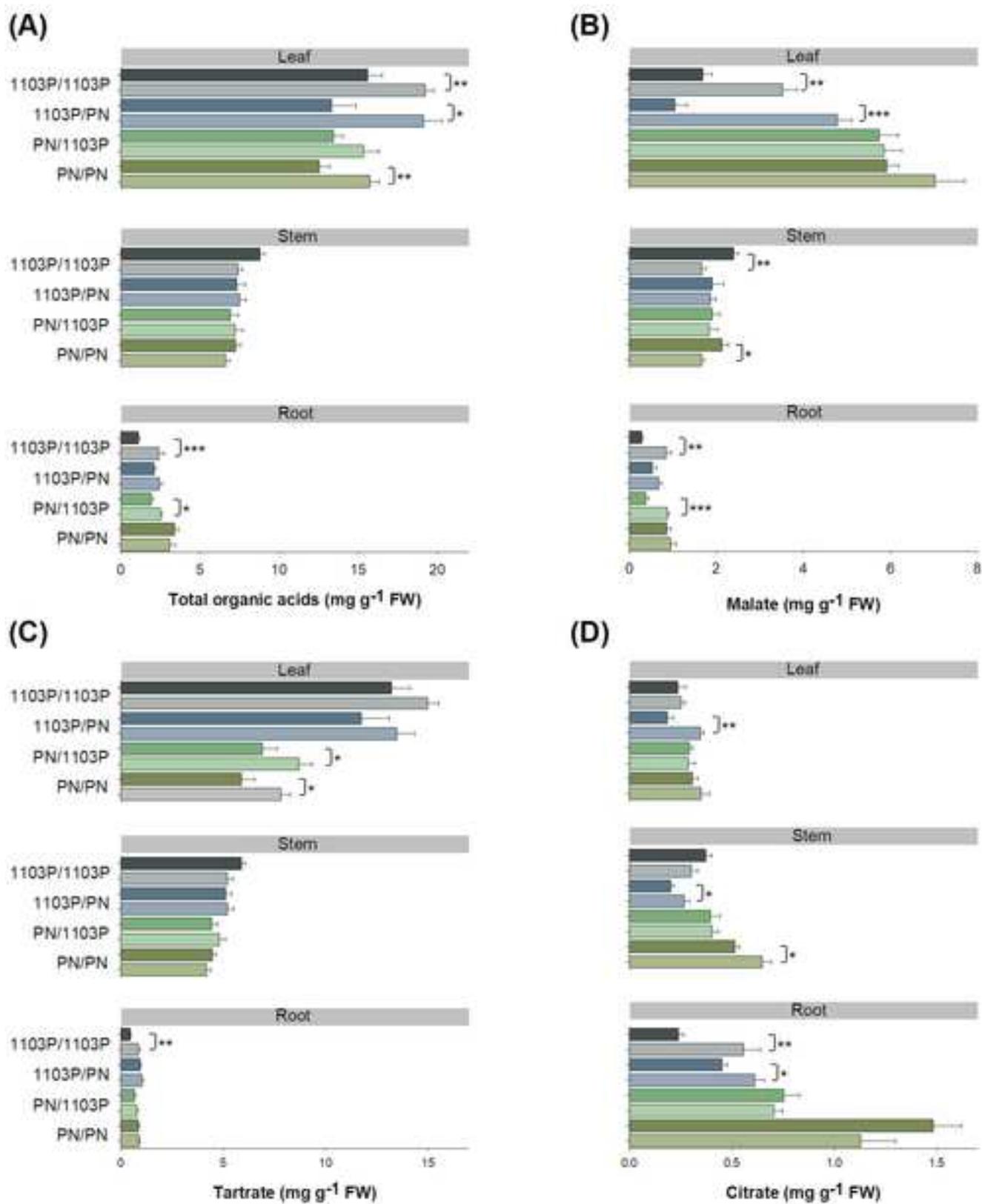
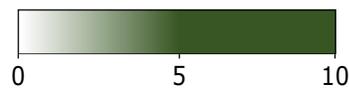
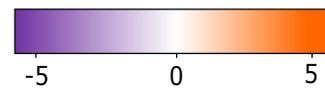
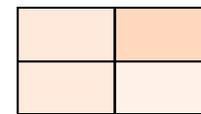
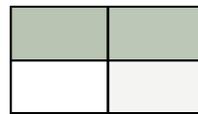


Figure 7

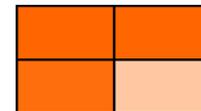
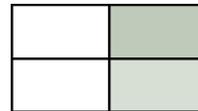
1103P	PN
1103P	1103P
1103P	PN
PN	PN

Expression level (log<sub>2</sub>)Fold change (log<sub>2</sub>)

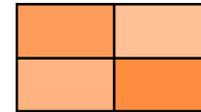
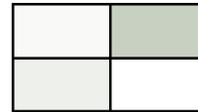
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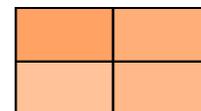
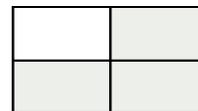
PHT1.3b  
VIT\_16s0050g02380



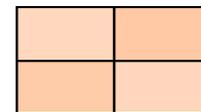
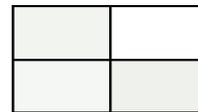
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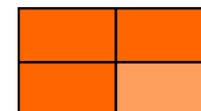
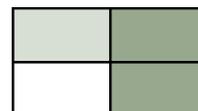
PHT1.4b  
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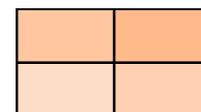
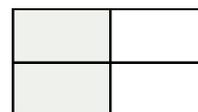
PHT1.4c  
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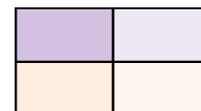
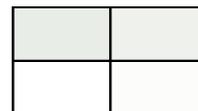
PHT1.4d  
VIT\_16s0050g02370



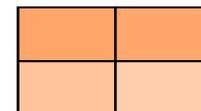
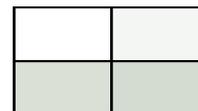
PHT1.9  
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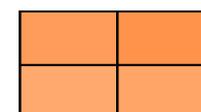
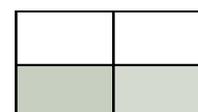
PHO2  
VIT\_00s0265g00070



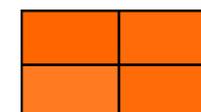
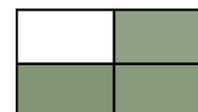
PAP10  
VIT\_03s0038g00230



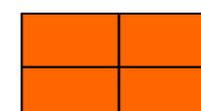
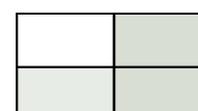
PAP12  
VIT\_18s0001g13340

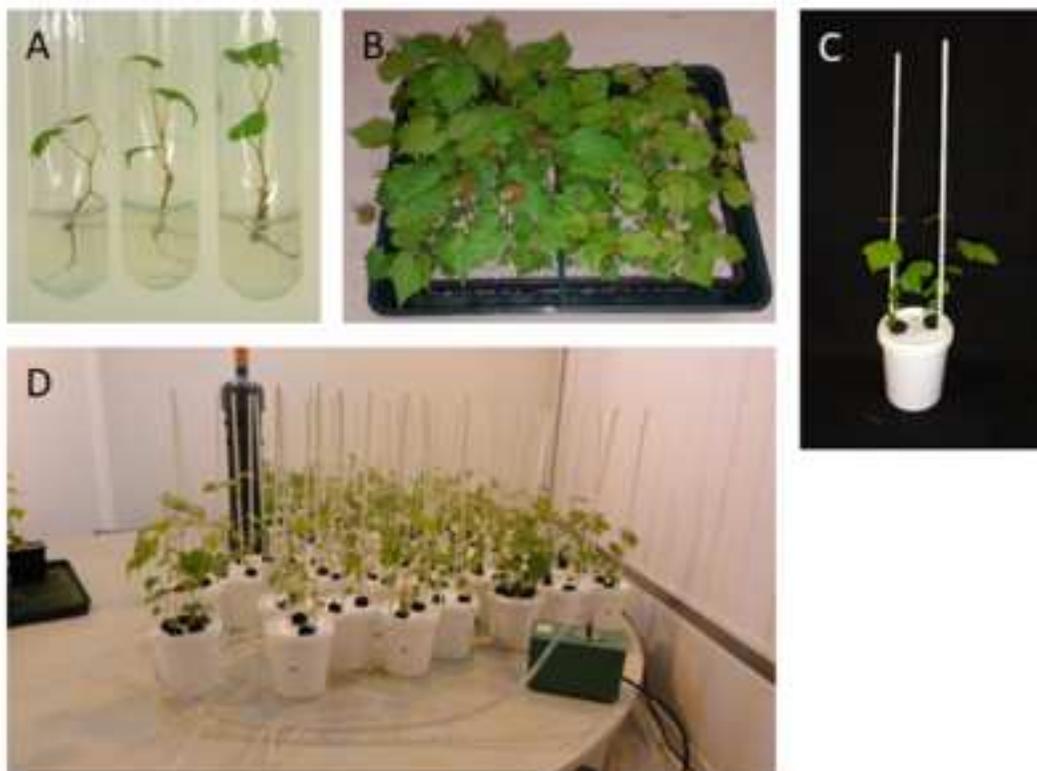


SPX1/2  
VIT\_11s0016g05330



SPX3  
VIT\_15s0048g00190





Supplementary Figure 1. Photographs of the plants used in the manuscript at different stages A. in vitro grafted grapevines, B. acclimation in perlite, C. and D. plants growing in hydroponic culture.