



HAL
open science

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

Antoine Gautier, Isabelle Merlin, Patrick Doumas, Noé Cochetel, Alain Mollier, Philippe Vivin, Virginie Lauvergeat, Benjamin Péret, Sarah Cookson

► To cite this version:

Antoine Gautier, Isabelle Merlin, Patrick Doumas, Noé Cochetel, Alain Mollier, et al.. Identifying roles of the scion and the rootstock in regulating plant development and functioning under different phosphorus supplies in grapevine. *Environmental and Experimental Botany*, 2021, 185, pp.104405. 10.1016/j.envexpbot.2021.104405 . hal-03150882

HAL Id: hal-03150882

<https://hal.science/hal-03150882>

Submitted on 4 Mar 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Identifying roles of the scion and the rootstock in regulating plant**
2
3 **development and functioning under different phosphorus supplies in**
4
5 **grapevine**
6

7
8 **Authors:** Antoine T. Gautier^{1,2}, Isabelle Merlin¹, Patrick Doumas³, Noé Cochetel⁴, Alain Mollier⁵,
9
10 Philippe Vivin¹, Virginie Lauvergeat¹, Benjamin Péret³ and Sarah J. Cookson^{1,6}

11
12 ¹EGFV, Univ. Bordeaux, Bordeaux Sciences Agro, INRAE, ISVV, F-33882, Villenave d'Ornon, France

13
14 ²Crop Production and Biostimulation Laboratory, Université Libre de Bruxelles, Campus Plaine, B-
15
16 1050 Brussels, Belgium

17
18 ³BPMP, Univ. Montpellier, CNRS, INRAE, Institut Agro, Montpellier, France

19
20 ⁴Department of Biochemistry and Molecular Biology, University of Nevada, Reno, Reno, NV 89557,
21
22 USA

23
24 ⁵ISPA, Bordeaux Sciences Agro, INRAE, 33140 Villenave d'Ornon, France

25
26 ⁶Corresponding author: sarah.cookson@inrae.fr, Tel: +33 5 57 57 59 23

27
28 **Keywords:** *Vitis spp.*, Root system architecture, Rhizosphere, Root exudation, Gene Expression, RT-
29
30 qPCR

31
32
33 Running head: Reciprocal grafts under different P supplies
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Abstract

1
2
3
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.
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Introduction

1
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
34

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).

3
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 19th 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).

23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
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
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

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.
7
8
9

10 **Materials and methods**

11 *Plant material and growing conditions*

12
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⁻¹ 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⁻² s⁻¹ (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⁻² s⁻¹ 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 KNO₃, 0.69 mM MgSO₄ and 1.27 mM CaCl₂ for both the HP and LP solutions; HP
27 solution also contained 0.6 mM KH₂PO₄ and 0.6 mM CaSO₄, whereas the LP solution contained 0.3
28 mM K₂SO₄ and 0.3 mM CaSO₄. Micronutrients were supplied as 46 μmol H₃BO₃, 9.1 μmol MnCl₂, 2.4
29 μmol ZnSO₄, 0.5 μmol CuSO₄ and 14 nmol (NH₄)₆Mo₇O₂₄, and iron was supplied as 8.5 mg L⁻¹
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.
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

50 *Plant growth measurements*

51
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).
56
57
58
59
60
61
62
63
64
65

Root system morphology analysis

1
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.
5
6

Activity of APases released by the roots

7
8
9
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.
15
16
17
18

Metabolite profile of plant tissues

19
20
21
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).
35
36
37
38
39
40
41
42
43
44

Quantification of transcript abundance

45
46
47
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
56
57
58
59
60
61
62
63
64
65

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

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

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***

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

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

35 In leaves, total organic acid concentration (i.e. the sum of malate, tartrate and citrate concentration)
36 was not affected by the scion or rootstock genotype (Fig. 6.A); however, the proportion of malate
37 and tartrate was affected by the scion genotype (Fig. 6.B; Fig. 6.C). Grafts with an 1103P scion had
38 approximately 10-fold more tartrate compared to malate, while combinations with a PN scion had
39 approximately equal malate and tartrate concentrations. Leaf citrate concentrations were higher in
40 PN than 1103P scions (Fig. 6.D). In stems, tartrate was the main organic acid in all scion/rootstock
41 combinations (approximately 70 %, Fig. 6.B) and was higher in grafts with an 1103P scion (Fig. 6.B,
42 Supplementary Table 3). However, grafts with a PN scion had higher citrate concentrations in the
43 leaves and the stems (Fig. 6.D, Supplementary Table 3). The rootstock did not affect scion organic
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 acid concentrations although the interaction between scion and rootstock genotype affected stem
2 citrate concentrations (Supplementary Table 3).

3
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.

34
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
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

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.
12
13
14
15
16
17

18 Discussion

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

20
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 m² of soil) and root distribution in the soil (Ferlito *et al.*, 2020).
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
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***

5
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***

33
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
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 regression between ³²P 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.
6
7

8
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.
17
18

19
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.
34
35

36
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.
41
42

43
44
45
46
47
48
49
50
51
52
53
54
55
56
57 ***The release of APases by roots in response to LP is associated with the increase in abundance of***
58 ***PAP10 and PAP12***
59
60
61
62
63
64
65

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

12 ***Organic acid profile of grapevine tissues varies between genotypes and can be modified by grafting*** 13 ***and phosphate supply*** 14

15 In many species, the major organic acids are citrate and malate (Neumann and Römheld 1999),
16 whereas tartrate is an organic acid restricted to a small number of species, including the *Vitaceae*
17 (Bennet-Clark 1933). In the present study, organic acids were mainly found in the highest
18 concentrations in the leaves without differences in total concentration between the four
19 scion/rootstock combinations although the concentration of individual organic acids varied. Tartrate
20 and malate concentrations have been shown to differ between different *V. vinifera* varieties,
21 different leaf ages and during the growing season (Attia *et al.*, 2007). The rootstock did not affect
22 organic acid concentration in scion in our study although the rootstock has been shown to alter
23 organic acid concentration in 5-year old grapevines grown in an inert substrate and irrigated with a
24 nutrient solution (Attia *et al.*, 2007). The stem organic concentrations were lower than those of the
25 leaves and were different between the different scion genotypes. Root total organic acid
26 concentration was different between the different rootstock genotypes studied as has been shown in
27 other work (Covarrubias *et al.*, 2016) and was affected by the scion genotype. This could suggest that
28 the shoots provide roots with some organic acids, in particularly malate which is the major organic
29 acid in the phloem (Keller, 2010). We have previously shown that grafting an 1103P rootstock with a
30 PN scion (i.e. PN/1103P) reduces the expression of *NADP-MALIC ENZYME2/Vitvi04g00009* (a key
31 enzyme regulating malate metabolism) in comparison to the 1103P/1103P homograft control further
32 suggesting that scion could provide malate to the rootstock in grapevine.
33

34 Tissue organic acid concentrations often change in response to Pi supply due to a number of reasons,
35 such as, under HP supply, the co-transport of Pi with protons can lead to metabolic adjustments
36 (such as a reduction in organic acid concentration) to maintain internal pH, whereas under LP supply,
37 organic acids may be synthesized to be released by the roots to increase the availability of
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

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.
9

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.
27
28
29
30
31
32
33
34
35
36

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
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

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.
5

6 **Data and Materials availability**

7
8
9 Experimental data and materials available to third party academic researchers upon reasonable
10 request.
11

12 **Supplementary Data**

13
14
15
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.
18

19
20
21
22 Supplementary Table 1. List of primers used for RT-qPCR experiments.

23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000

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).

Conflict of interest
None declared.

Funding

This study has been carried out with financial support from the French National Research Agency
(ANR) in the frame of the Investments for the future Program, within the Cluster of Excellence COTE
(ANR-10-LABX-45).

Acknowledgements

We thank Cyril Hévin for his technical assistance.

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.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

References

- 1
2
3 **Attia F, Garcia F, Garcia M, Besnard E, Lamaze T.** 2007. Effect of rootstock on organic acids in leaves
4 and berries and on must and wine acidity of two red wine grape cultivars 'Malbec' and 'Négrette'
5 (*Vitis vinifera* L.) grown hydroponically. *Acta Horticulturae* **754**, 473-482.
6
- 7 **Bari R, Pant BD, Stitt M, Scheible WR.** 2006. PHO2, microRNA399, and PHR1 define a phosphate-
8 signaling pathway in plants. *Plant Physiology* **141**, 988-999.
9
- 10 **Bolan NS, Naidu R, Mahimairaja S, Baskaran S.** 1994. Influence of low-molecular-weight organic-
11 acids on the solubilization of phosphates. *Biology and Fertility of Soils* **18**, 311-319.
12
- 13 **Bouain N, Krouk G, Lacombe B, Rouached H.** 2019. Getting to the Root of Plant Mineral Nutrition:
14 Combinatorial Nutrient Stresses Reveal Emergent Properties. *Trends in Plant Science* **24**, 542-552.
15
- 16 **Cookson SJ, Clemente Moreno MJ, Hevin C, Nyamba Mendome LZ, Delrot S, Trossat-Magnin C,**
17 **Ollat N.** 2013. Graft union formation in grapevine induces transcriptional changes related to cell wall
18 modification, wounding, hormone signalling, and secondary metabolism. *J Exp Bot* **64**, 2997-3008.
19
- 20 **Covarrubias JI, Retamales C, Donnini S, Rombolà AD, Pastenes C.** 2016. Contrasting physiological
21 responses to iron deficiency in Cabernet Sauvignon grapevines grafted on two rootstocks. *Scientia*
22 *Horticulturae* **199**, 1-8.
23
- 24 **Duan K, Yi KK, Dang L, Huang HJ, Wu W, Wu P.** 2008. Characterization of a sub-family of Arabidopsis
25 genes with the SPX domain reveals their diverse functions in plant tolerance to phosphorus
26 starvation. *Plant Journal* **54**, 965-975.
27
- 28 **Dumont C, Cochetel N, Lauvergeat V, Cookson SJ, Ollat N, Vivin P.** 2016. Screening root morphology
29 in grafted grapevine using 2D digital images from rhizotrons. In: Gaiotti F, Battista F, Tomasi D, eds. *I*
30 *International Symposium on Grapevine Roots*, Vol. 1136, 213-219.
31
- 32 **Ferlito F, Distefano G, Gentile A, Allegra M, Lakso AN, Nicolosi E.** 2020. Scion-rootstock interactions
33 influence the growth and behaviour of the grapevine root system in a heavy clay soil. *Australian*
34 *Journal of Grape and Wine Research* **26**, 68-78.
35
- 36 **Gahoonia TS, Asmar F, Giese H, Gissel-Nielsen G, Erik Nielsen N.** 2000. Root-released organic acids
37 and phosphorus uptake of two barley cultivars in laboratory and field experiments. *European Journal*
38 *of Agronomy* **12**, 281-289.
39
- 40 **Gautier AT, Cochetel N, Merlin I, Hevin C, Lauvergeat V, Vivin P, Mollier A, Ollat N, Cookson S.**
41 2020a. Scion genotypes exert long distance control over rootstock transcriptome responses to low
42 phosphate in grafted grapevine. *Bmc Plant Biology* **20**, 367.
43
- 44 **Gautier AT, Cookson SJ, Lagalle L, Ollat N, Marguerit E.** 2020b. Influence of the three main genetic
45 backgrounds of grapevine rootstocks on petiolar nutrient concentrations of the scion, with a focus on
46 phosphorus. *OENO One* **54**, 1-13.
47
- 48 **Harrison N, Barber-Perez N, Pennington B, Cascant-Lopez E, Gregory PJ.** 2016. Root system
49 architecture in reciprocal grafts of apple rootstock-scion combinations. *International Society for*
50 *Horticultural Science (ISHS)*, Leuven, Belgium, 409-414.
51
- 52 **Hinsinger P.** 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced
53 chemical changes: a review. *Plant and Soil* **237**, 173-195.
54
55
56
57
58
59
60
61
62
63
64
65

1 **Hinsinger P, Plassard C, Tang CX, Jaillard B.** 2003. Origins of root-mediated pH changes in the
2 rhizosphere and their responses to environmental constraints: A review. *Plant and Soil* **248**, 43-59.

3 **Jones DL.** 1998. Organic acids in the rhizosphere - a critical review. *Plant and Soil* **205**, 25-44.

4 **Keller M.** 2010. *The Science of Grapevines Anatomy and Physiology.*: Academic Press.

5 **Lan P, Li WF, Schmidt W.** 2015. 'Omics' approaches towards understanding plant phosphorus
6 acquisition and use. In: Plaxton WC, Lambers H, eds. *Phosphorus Metabolism in Plants*, Vol. 48, 65-
7 97.

8 **Lecourt J, Lauvergeat V, Ollat N, Vivin P, Cookson SJ.** 2015. Shoot and root ionome responses to
9 nitrate supply in grafted grapevines are rootstock genotype dependent. *Australian Journal of Grape
10 and Wine Research* **21**, 311-318.

11 **Lefort PL, Legisle N.** 1977. Quantitative stock-scion relationships in vine <p>Preliminary
12 investigations by the analysis of reciprocal graftings. *Vitis* **16**, 149-161.

13 **Liu HT, White PJ, Li CJ.** 2016. Biomass partitioning and rhizosphere responses of maize and faba bean
14 to phosphorus deficiency. *Crop & Pasture Science* **67**, 847-856.

15 **Lu LH, Qiu WM, Gao WW, Tyerman SD, Shou HX, Wang C.** 2016. OsPAP10c, a novel secreted acid
16 phosphatase in rice, plays an important role in the utilization of external organic phosphorus. *Plant
17 Cell and Environment* **39**, 2247-2259.

18 **Lynch JP.** 2011. Root Phenes for Enhanced Soil Exploration and Phosphorus Acquisition: Tools for
19 Future Crops. **156**, 1041-1049.

20 **Marschner H.** 1995. *Mineral nutrition of higher plants. 2nd:* Acedemic Press.

21 **Morcuende R, Bari R, Gibon Y, Zheng WM, Pant BD, Blasing O, Usadel B, Czechowski T, Udvardi MK,
22 Stitt M, Scheible WR.** 2007. Genome-wide reprogramming of metabolism and regulatory networks of
23 Arabidopsis in response to phosphorus. *Plant Cell and Environment* **30**, 85-112.

24 **Nussaume L, Kanno S, Javot H, Marin E, Nakanishi TM, Thibaud M-C.** 2011. Phosphate Import in
25 Plants: Focus on the PHT1 Transporters. *Frontiers in Plant Science* **2**.

26 **Ollat N, Peccoux A, Papura D, Esmenjaud D, Marguerit E, Tandonnet JP, Bordenave L, Cookson SJ,
27 Barrieu F, Rossdeutsch L, Lecourt J, Lauvergeat V, Vivin P, Bert PF, Delrot S.** 2016. Rootstocks as a
28 component of adaptation to environment. In: Geros H, Chaves MM, Gil HM, Delrot S, eds. *Grapevine
29 in a Changing Environment: A Molecular and Ecophysiological Perspective*, 68-108.

30 **Oslobeanu M.** 1978. Quelques aspects de l'interaction entre le greffon et le porte-greffe à la vigne.
31 *Symposium International 'Ecologie de la Vigne'*. Constanta-Roumanie: Office International de la Vigne
32 et du Vin, 227-236.

33 **Pant BD, Pant P, Erban A, Huhman D, Kopka J, Scheible WR.** 2015. Identification of primary and
34 secondary metabolites with phosphorus status-dependent abundance in Arabidopsis, and of the
35 transcription factor PHR1 as a major regulator of metabolic changes during phosphorus limitation.
36 **38**, 172-187.

37 **R Core Team.** 2018. R: A language and environment for statistical computing. [http://www.R-
38 project.org](http://www.R-project.org). Vienna, Austria. : R Foundation for Statistical Computing.

1 **Rives M.** 1971. Principes d'une étude du déterminisme de la vigueur en vue de la création de variétés
2 nouvelles de porte-greffes de vigueur donnée. *Ann Amélioration Plantes* **21**, 5-13.

3 **Robinson WD, Park J, Tran HT, Del Vecchio HA, Ying S, Zins JL, Patel K, McKnight TD, Plaxton WC.**
4 2012. The secreted purple acid phosphatase isozymes AtPAP12 and AtPAP26 play a pivotal role in
5 extracellular phosphate-scavenging by *Arabidopsis thaliana*. *Journal of Experimental Botany* **63**,
6 6531-6542.

7
8
9 **Rouached H, Secco D, Arpat B, Poirier Y.** 2011. The transcription factor PHR1 plays a key role in the
10 regulation of sulfate shoot-to-root flux upon phosphate starvation in *Arabidopsis*. *Bmc Plant Biology*
11 **11**, 19.

12
13 **Rubio V, Linhares F, Solano R, Martin AC, Iglesias J, Leyva A, Paz-Ares J.** 2001. A conserved MYB
14 transcription factor involved in phosphate starvation signaling both in vascular plants and in
15 unicellular algae. *Genes & Development* **15**, 2122-2133.

16
17
18 **Smart DR, Schwass E, Lakso A, Morano L.** 2006. Grapevine rooting patterns: A comprehensive
19 analysis and a review. *American Journal of Enology and Viticulture* **57**, 89-104.

20
21
22 **Southey JM.** 1992. Root Distribution of Different Grapevine Rootstocks on a Relatively Saline Soil.
23 2017 **13**, 9.

24
25 **Tandonnet JP, Cookson S, Vivin P, Ollat N.** 2010. Scion genotype controls biomass allocation and
26 root development in grafted grapevine. *Australian Journal of Grape and Wine Research* **16**, 290-300.

27
28 **Zhang ZL, Liao H, Lucas WJ.** 2014. Molecular mechanisms underlying phosphate sensing, signaling,
29 and adaptation in plants. *Journal of Integrative Plant Biology* **56**, 192-220.

30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

List of Figures

1
2 Figure 1. (A) Stem, (B) leaf and (C) root fresh weight (FW), and (D) root:whole plant FW, (E)
3 leaf:whole plant FW and (F) stem:whole shoot FW ratios of reciprocal grafts of *Vitis berlandieri* x *V.*
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
6 rootstock genotype. Means and standard errors shown (n = 15). For each combination, stars indicate
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).

9
10
11
12
13
14
15 Figure 2. (A) Total number of root tips, (B) tip density and (C) average diameter of roots of reciprocal
16 grafts of *Vitis berlandieri* x *V. rupestris* cv. 1103 Paulsen (1103P) and *V. vinifera* cv. Pinot noir (PN)
17 after 28 d of either high (dark colours) or low phosphate (light colours) supply. The scion genotype is
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).

21
22
23
24
25
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).

31
32
33
34
35
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.

42
43
44
45
46
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.

53
54
55
56
57
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.

60
61
62
63
64
65

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

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).

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

16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

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 ⁻¹ FW) | Scion | | | Rootstock | | | Scion x Rootstock Interaction |
|--|--------------|--------------|--------------|--------------|--------------|--------------|-------------------------------|
| | 1103P | PN | Significance | 1103P | PN | Significance | Significance |
| Shoot | | | | | | | |
| 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 |
| Stem | | | | | | | |
| 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 | *** |
| Root | | | | | | | |
| 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 | | | *** | | | | |

1
2
3
4
5
6
7
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^{1,2}, Isabelle Merlin¹, Patrick Doumas³, Noé Cochetel⁴, Alain Mollier⁵,
12 Philippe Vivin¹, Virginie Lauvergeat¹, Benjamin Péret³ and Sarah J. Cookson^{1,6}

13 ¹EGFV, Univ. Bordeaux, Bordeaux Sciences Agro, INRAE, ISVV, F-33882, Villenave d'Ornon, France

14 ²Crop Production and Biostimulation Laboratory, Université Libre de Bruxelles, Campus Plaine, B-
15 1050 Brussels, Belgium

16 ³BPMP, Univ. Montpellier, CNRS, INRAE, Institut Agro, Montpellier, France

17 ⁴Department of Biochemistry and Molecular Biology, University of Nevada, Reno, Reno, NV 89557,
18 USA

19 ⁵ISPA, Bordeaux Sciences Agro, INRAE, 33140 Villenave d'Ornon, France

20 ⁶Corresponding author: sarah.cookson@inrae.fr, Tel: +33 5 57 57 59 23

21 **Keywords:** *Vitis spp.*, Root system architecture, Rhizosphere, Root exudation, Gene Expression, RT-
22 qPCR

23 Running head: Reciprocal grafts under different P supplies
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

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.

Formatted: Font: Italic
Formatted: Font: Italic
Formatted: Font: Italic

1
2
3
4
5
6
7 **Introduction**
8

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).
19
20
21
22

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).
36
37
38
39
40

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
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4
5
6
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).
9

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 19th 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).
29
30
31
32
33
34
35

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
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4
5
6
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.
13
14

15 **Materials and methods**

16 *Plant material and growing conditions*

17
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⁻¹ 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⁻² s⁻¹ (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⁻² s⁻¹ 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 KNO₃, 0.69 mM MgSO₄ and 1.27 mM CaCl₂ for both the HP and LP solutions; HP
32 solution also contained 0.6 mM KH₂PO₄ and 0.6 mM CaSO₄, whereas the LP solution contained 0.3
33 mM K₂SO₄ and 0.3 mM CaSO₄. Micronutrients were supplied as 46 µmol H₃BO₃, 9.1 µmol MnCl₂, 2.4
34 µmol ZnSO₄, 0.5 µmol CuSO₄ and 14 nmol (NH₄)₆Mo₇O₂₄, and iron was supplied as 8.5 mg L⁻¹
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.
37
38
39
40
41
42
43
44

45 *Plant growth measurements*

46
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).
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4
5
6
7 *Root system morphology analysis*

8
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.
12

13 *Activity of APases released by the roots*

14
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.
20
21

22 *Metabolite profile of plant tissues*

23
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).
37
38
39
40

41 *Quantification of transcript abundance*

42
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
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4
5
6
7 SAND3' for normalization (Hellemans et al. 2007). Primer sequences are listed in Supplementary
8 Table 1.
9

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/>).
17
18
19

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.
32
33
34
35
36

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
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4
5
6
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).
13

14
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).
21

22
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).
32

33
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).
38

39 ***Homo-grafted grapevines have differences in phosphate and sulphate, but not nitrate acquisition*** 40 ***efficiencies*** 41

42
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
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4
5
6
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).
16
17
18

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

20
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.
27
28
29

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

32
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.
38
39

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
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4
5
6
7 acid concentrations although the interaction between scion and rootstock genotype affected stem
8 citrate concentrations (Supplementary Table 3).

9
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***

22
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***

35
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.

46
47
48
49 The transcript abundance of *PAP10* and *PAP12* was analysed in root tips after 28 d of LP or HP (Fig.
50 7). The transcript abundance of *PAP10* was not affected by the scion or rootstock genotype under
51 HP, but was higher in plants grown in LP than HP. The increase of abundance of *PAP10* transcripts
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4
5
6
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.

10
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.
18
19
20

21 Discussion

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

23
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 m² of soil) and root distribution in the soil (Ferlito *et al.*, 2020).
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4
5
6
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.
9

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).
24
25
26
27
28
29

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.
38
39
40

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.
43

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
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4
5
6
7 regression between ^{32}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.
12
13

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.
22
23
24

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.
39
40
41
42
43

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.
48
49

50 ***The release of APases by roots in response to LP is associated with the increase in abundance of***
51 ***PAP10 and PAP12***
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4
5
6
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.
17
18
19
20

21 ***Organic acid profile of grapevine tissues varies between genotypes and can be modified by grafting***
22 ***and phosphate supply***
23

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.
42
43
44
45
46
47

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
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4
5
6
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.
15
16
17

18 **The role of long-distance signalling in regulating responses to P supply in grafted grapevine**

19
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.
31
32
33
34

35 **Conclusion**

36
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
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4
5
6
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.
11

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.](#)

19
20
21
22
23 Supplementary Table 1. List of primers used for RT-qPCR experiments.

24
25
26
27
28
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.

33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000

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.

Funding

This study has been carried out with financial support from the French National Research Agency
(ANR) in the frame of the Investments for the future Program, within the Cluster of Excellence COTE
(ANR-10-LABX-45).

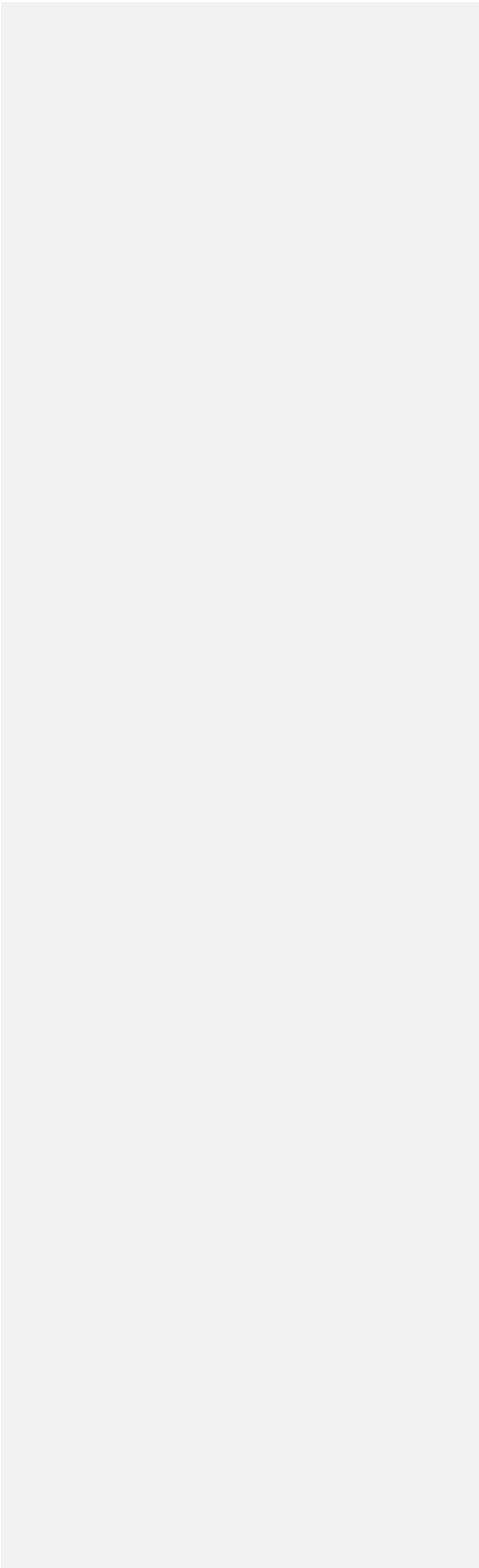
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Acknowledgements

We thank Cyril Hévin for his technical assistance.

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.



1
2
3
4
5
6
7 **References**
8
9

- 10 **Attia F, Garcia F, Garcia M, Besnard E, Lamaze T.** 2007. Effect of rootstock on organic acids in leaves
11 and berries and on must and wine acidity of two red wine grape cultivars 'Malbec' and 'Négrette'
12 (*Vitis vinifera* L.) grown hydroponically. *Acta Horticulturae* **754**, 473-482.
- 13 **Bari R, Pant BD, Stitt M, Scheible WR.** 2006. PHO2, microRNA399, and PHR1 define a phosphate-
14 signaling pathway in plants. *Plant Physiology* **141**, 988-999.
- 15 **Bolan NS, Naidu R, Mahimairaja S, Baskaran S.** 1994. Influence of low-molecular-weight organic-
16 acids on the solubilization of phosphates. *Biology and Fertility of Soils* **18**, 311-319.
- 17
18 **Bouain N, Krouk G, Lacombe B, Rouached H.** 2019. Getting to the Root of Plant Mineral Nutrition:
19 Combinatorial Nutrient Stresses Reveal Emergent Properties. *Trends in Plant Science* **24**, 542-552.
- 20
21 **Cookson SJ, Clemente Moreno MJ, Hevin C, Nyamba Mendome LZ, Delrot S, Trossat-Magnin C,**
22 **Ollat N.** 2013. Graft union formation in grapevine induces transcriptional changes related to cell wall
23 modification, wounding, hormone signalling, and secondary metabolism. *J Exp Bot* **64**, 2997-3008.
- 24 **Covarrubias JI, Retamales C, Donnini S, Rombolà AD, Pastenes C.** 2016. Contrasting physiological
25 responses to iron deficiency in Cabernet Sauvignon grapevines grafted on two rootstocks. *Scientia*
26 *Horticulturae* **199**, 1-8.
- 27
28 **Duan K, Yi KK, Dang L, Huang HJ, Wu W, Wu P.** 2008. Characterization of a sub-family of Arabidopsis
29 genes with the SPX domain reveals their diverse functions in plant tolerance to phosphorus
30 starvation. *Plant Journal* **54**, 965-975.
- 31 **Dumont C, Cochetel N, Lauvergeat V, Cookson SJ, Ollat N, Vivin P.** 2016. Screening root morphology
32 in grafted grapevine using 2D digital images from rhizotrons. In: Gaiotti F, Battista F, Tomasi D, eds. *I*
33 *International Symposium on Grapevine Roots*, Vol. 1136, 213-219.
- 34
35 **Ferlito F, Distefano G, Gentile A, Allegra M, Lakso AN, Nicolosi E.** 2020. Scion-rootstock interactions
36 influence the growth and behaviour of the grapevine root system in a heavy clay soil. *Australian*
37 *Journal of Grape and Wine Research* **26**, 68-78.
- 38 **Gahoonia TS, Asmar F, Giese H, Gissel-Nielsen G, Erik Nielsen N.** 2000. Root-released organic acids
39 and phosphorus uptake of two barley cultivars in laboratory and field experiments. *European Journal*
40 *of Agronomy* **12**, 281-289.
- 41
42 **Gautier AT, Cochetel N, Merlin I, Hevin C, Lauvergeat V, Vivin P, Mollier A, Ollat N, Cookson S.**
43 2020a. Scion genotypes exert long distance control over rootstock transcriptome responses to low
44 phosphate in grafted grapevine. *Bmc Plant Biology* **20**, 367.
- 45 **Gautier AT, Cookson SJ, Lagalle L, Ollat N, Marguerit E.** 2020b. Influence of the three main genetic
46 backgrounds of grapevine rootstocks on petiolar nutrient concentrations of the scion, with a focus on
47 phosphorus. *OENO One* **54**, 1-13.
- 48
49 **Harrison N, Barber-Perez N, Pennington B, Cascant-Lopez E, Gregory PJ.** 2016. Root system
50 architecture in reciprocal grafts of apple rootstock-scion combinations. *International Society for*
51 *Horticultural Science (ISHS)*, Leuven, Belgium, 409-414.
- 52 **Hinsinger P.** 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced
53 chemical changes: a review. *Plant and Soil* **237**, 173-195.
- 54
55
56
57
58
59
60
61
62
63
64
65

- 1
2
3
4
5
6
7 **Hinsinger P, Plassard C, Tang CX, Jaillard B.** 2003. Origins of root-mediated pH changes in the
8 rhizosphere and their responses to environmental constraints: A review. *Plant and Soil* **248**, 43-59.
9
- 10 **Jones DL.** 1998. Organic acids in the rhizosphere - a critical review. *Plant and Soil* **205**, 25-44.
11
- 12 **Keller M.** 2010. *The Science of Grapevines Anatomy and Physiology.*: Academic Press.
13
- 14 **Lan P, Li WF, Schmidt W.** 2015. 'Omics' approaches towards understanding plant phosphorus
15 acquisition and use. In: Plaxton WC, Lambers H, eds. *Phosphorus Metabolism in Plants*, Vol. 48, 65-
16 97.
17
- 18 **Lecourt J, Lauvergeat V, Ollat N, Vivin P, Cookson SJ.** 2015. Shoot and root ionome responses to
19 nitrate supply in grafted grapevines are rootstock genotype dependent. *Australian Journal of Grape
20 and Wine Research* **21**, 311-318.
21
- 22 **Lefort PL, Legisle N.** 1977. Quantitative stock-scion relationships in vine <p>Preliminary
23 investigations by the analysis of reciprocal graftings. *Vitis* **16**, 149-161.
24
- 25 **Liu HT, White PJ, Li CJ.** 2016. Biomass partitioning and rhizosphere responses of maize and faba bean
26 to phosphorus deficiency. *Crop & Pasture Science* **67**, 847-856.
27
- 28 **Lu LH, Qiu WM, Gao WW, Tyerman SD, Shou HX, Wang C.** 2016. OsPAP10c, a novel secreted acid
29 phosphatase in rice, plays an important role in the utilization of external organic phosphorus. *Plant
30 Cell and Environment* **39**, 2247-2259.
31
- 32 **Lynch JP.** 2011. Root Phenes for Enhanced Soil Exploration and Phosphorus Acquisition: Tools for
33 Future Crops. **156**, 1041-1049.
34
- 35 **Marschner H.** 1995. *Mineral nutrition of higher plants. 2nd:* Acedemic Press.
36
- 37 **Morcuende R, Bari R, Gibon Y, Zheng WM, Pant BD, Blasing O, Usadel B, Czechowski T, Udvardi MK,
38 Stitt M, Scheible WR.** 2007. Genome-wide reprogramming of metabolism and regulatory networks of
39 Arabidopsis in response to phosphorus. *Plant Cell and Environment* **30**, 85-112.
40
- 41 **Nussaume L, Kanno S, Javot H, Marin E, Nakanishi TM, Thibaud M-C.** 2011. Phosphate Import in
42 Plants: Focus on the PHT1 Transporters. *Frontiers in Plant Science* **2**.
43
- 44 **Ollat N, Peccoux A, Papura D, Esmenjaud D, Marguerit E, Tandonnet JP, Bordenave L, Cookson SJ,
45 Barrieu F, Rossdeutsch L, Lecourt J, Lauvergeat V, Vivin P, Bert PF, Delrot S.** 2016. Rootstocks as a
46 component of adaptation to environment. In: Geros H, Chaves MM, Gil HM, Delrot S, eds. *Grapevine
47 in a Changing Environment: A Molecular and Ecophysiological Perspective*, 68-108.
48
- 49 **Oslobeanu M.** 1978. Quelques aspects de l'interaction entre le greffon et le porte-greffe à la vigne.
50 *Symposium International 'Ecologie de la Vigne'*. Constanta-Roumanie: Office International de la Vigne
51 et du Vin, 227-236.
52
- 53 **Pant BD, Pant P, Erban A, Huhman D, Kopka J, Scheible WR.** 2015. Identification of primary and
54 secondary metabolites with phosphorus status-dependent abundance in Arabidopsis, and of the
55 transcription factor PHR1 as a major regulator of metabolic changes during phosphorus limitation.
56 **38**, 172-187.
57
- 58 **R Core Team.** 2018. R: A language and environment for statistical computing. [http://www.R-](http://www.R-project.org)
59 [project.org](http://www.R-project.org). Vienna, Austria. : R Foundation for Statistical Computing.
60
61
62
63
64
65

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Rives M. 1971. Principes d'une étude du déterminisme de la vigueur en vue de la création de variétés nouvelles de porte-greffes de vigueur donnée. *Ann Amélioration Plantes* **21**, 5-13.

Robinson WD, Park J, Tran HT, Del Vecchio HA, Ying S, Zins JL, Patel K, McKnight TD, Plaxton WC. 2012. The secreted purple acid phosphatase isozymes AtPAP12 and AtPAP26 play a pivotal role in extracellular phosphate-scavenging by *Arabidopsis thaliana*. *Journal of Experimental Botany* **63**, 6531-6542.

Rouached H, Secco D, Arpat B, Poirier Y. 2011. The transcription factor PHR1 plays a key role in the regulation of sulfate shoot-to-root flux upon phosphate starvation in *Arabidopsis*. *Bmc Plant Biology* **11**, 19.

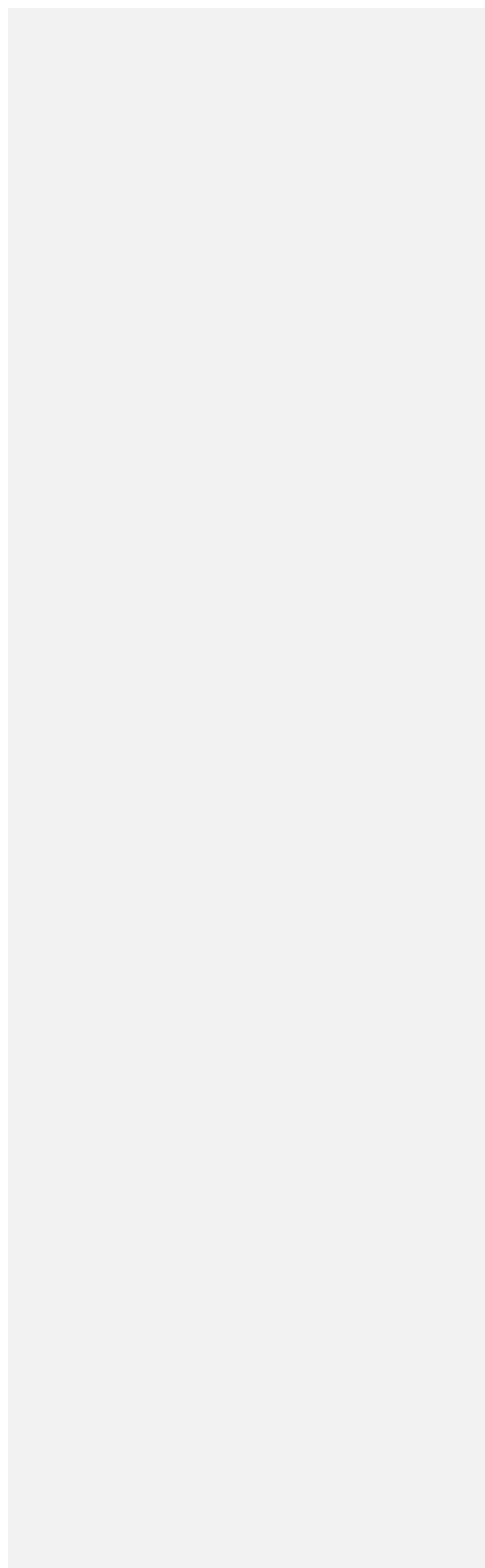
Rubio V, Linhares F, Solano R, Martin AC, Iglesias J, Leyva A, Paz-Ares J. 2001. A conserved MYB transcription factor involved in phosphate starvation signaling both in vascular plants and in unicellular algae. *Genes & Development* **15**, 2122-2133.

Smart DR, Schwass E, Lakso A, Morano L. 2006. Grapevine rooting patterns: A comprehensive analysis and a review. *American Journal of Enology and Viticulture* **57**, 89-104.

Southey JM. 1992. Root Distribution of Different Grapevine Rootstocks on a Relatively Saline Soil. 2017 **13**, 9.

Tandonnet JP, Cookson S, Vivin P, Ollat N. 2010. Scion genotype controls biomass allocation and root development in grafted grapevine. *Australian Journal of Grape and Wine Research* **16**, 290-300.

Zhang ZL, Liao H, Lucas WJ. 2014. Molecular mechanisms underlying phosphate sensing, signaling, and adaptation in plants. *Journal of Integrative Plant Biology* **56**, 192-220.



1
2
3
4
5
6
7 List of Figures
8

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).
16
17

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).
24
25
26

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).
32
33

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.
40
41

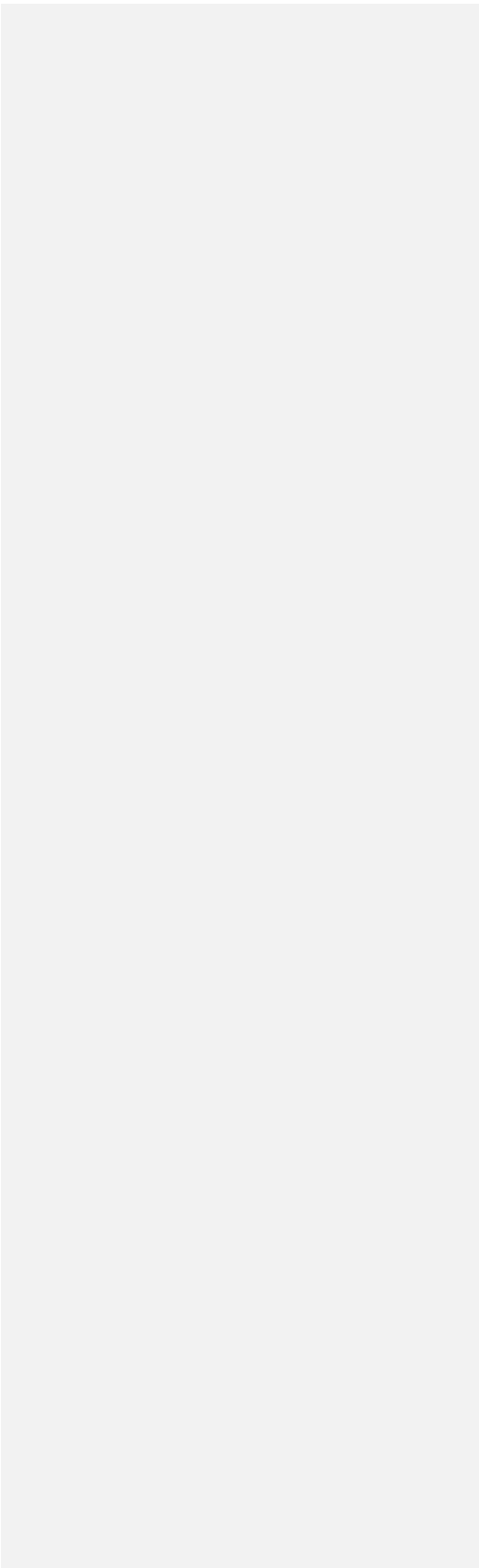
42 Figure 5. Acid phosphatase (APase) activity measured in root exudates of reciprocal grafts of *Vitis*
43 *berlandieri* x *V. rupestris* cv. 1103 Paulsen (1103P) and *V. vinifera* cv. Pinot noir (PN) after 28 d of
44 either high (dark colours) or low phosphate supply (light colours). The scion genotype 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.
48
49
50

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.
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

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).



16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
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 | TGGTCCCTCCCAGAGAGTTG | 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 | AGCCAGTTTTTGACACGCTA | GTGGGTCCACAAACCTTCCA | 96.9 | 136 |
| PAP12 | VIT_18s0001g13340 | GGACCTCTTCAGAAAAATCCTCT | 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 |
| Leaf FW (g) | 3.11 ± 0.22 | 1.07 ± 0.12 | *** | 2.01 ± 0.25 | 2.17 ± 0.27 | NS |
| Stem FW (g) | 2.42 ± 0.17 | 0.91 ± 0.10 | *** | 1.58 ± 0.18 | 1.73 ± 0.21 | NS |
| Root FW (g) | 2.56 ± 0.13 | 1.10 ± 0.08 | *** | 1.74 ± 0.17 | 1.90 ± 0.18 | NS |
| Root:plant FW ratio | 0.33 ± 0.01 | 0.37 ± 0.01 | NS | 0.34 ± 0.01 | 0.34 ± 0.02 | NS |
| Leaf:plant FW ratio | 0.38 ± 0.01 | 0.34 ± 0.01 | *** | 0.36 ± 0.01 | 0.36 ± 0.02 | NS |
| Stem:shoot FW ratio | 0.79 ± 0.02 | 0.85 ± 0.03 | ** | 0.85 ± 0.03 | 0.79 ± 0.02 | ** |
| Number of tips | 823 ± 92 | 389 ± 52 | *** | 485 ± 85 | 726 ± 105 | NS |
| Tip density (cm⁻¹) | 1.03 ± 0.05 | 0.93 ± 0.03 | NS | 0.90 ± 0.02 | 1.07 ± 0.05 | ** |
| Average root diameter (mm) | 0.95 ± 0.02 | 0.97 ± 0.03 | NS | 0.94 ± 0.01 | 0.98 ± 0.02 | NS |

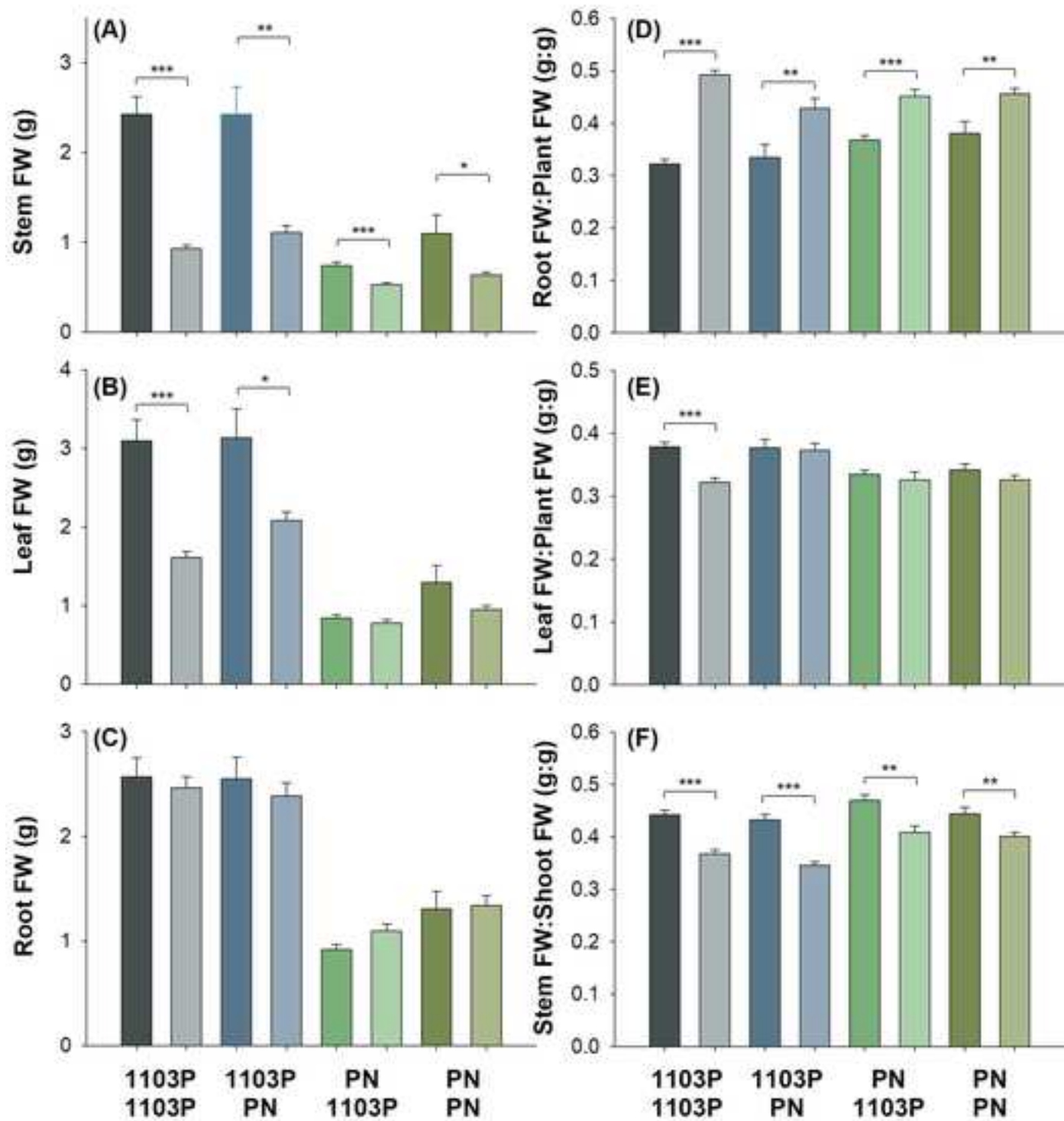
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

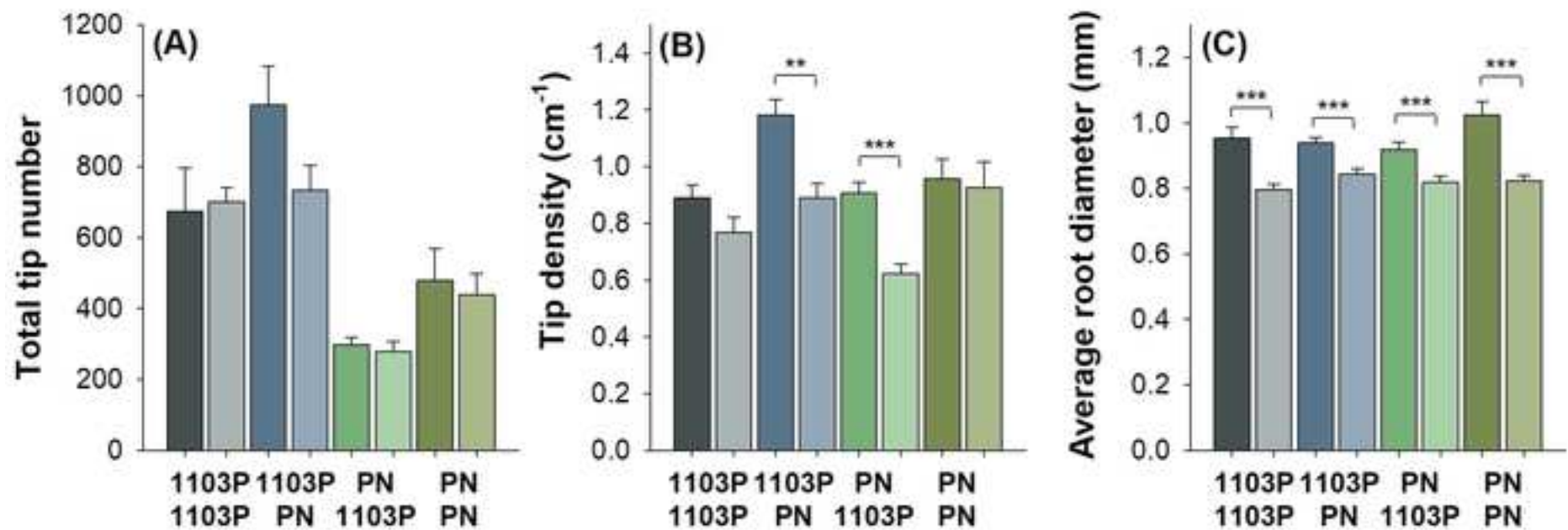
Supplementary Table 3. Statistical analysis of malate, tartrate and citrate concentrations and the sum of these three organic acids in the leaves, stems and roots of reciprocal grafts of *Vitis berlandieri* x *V. rupestris* cv. 1103 Paulsen (1103P) and *V. vinifera* cv. Pinot noir (PN) grown under high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

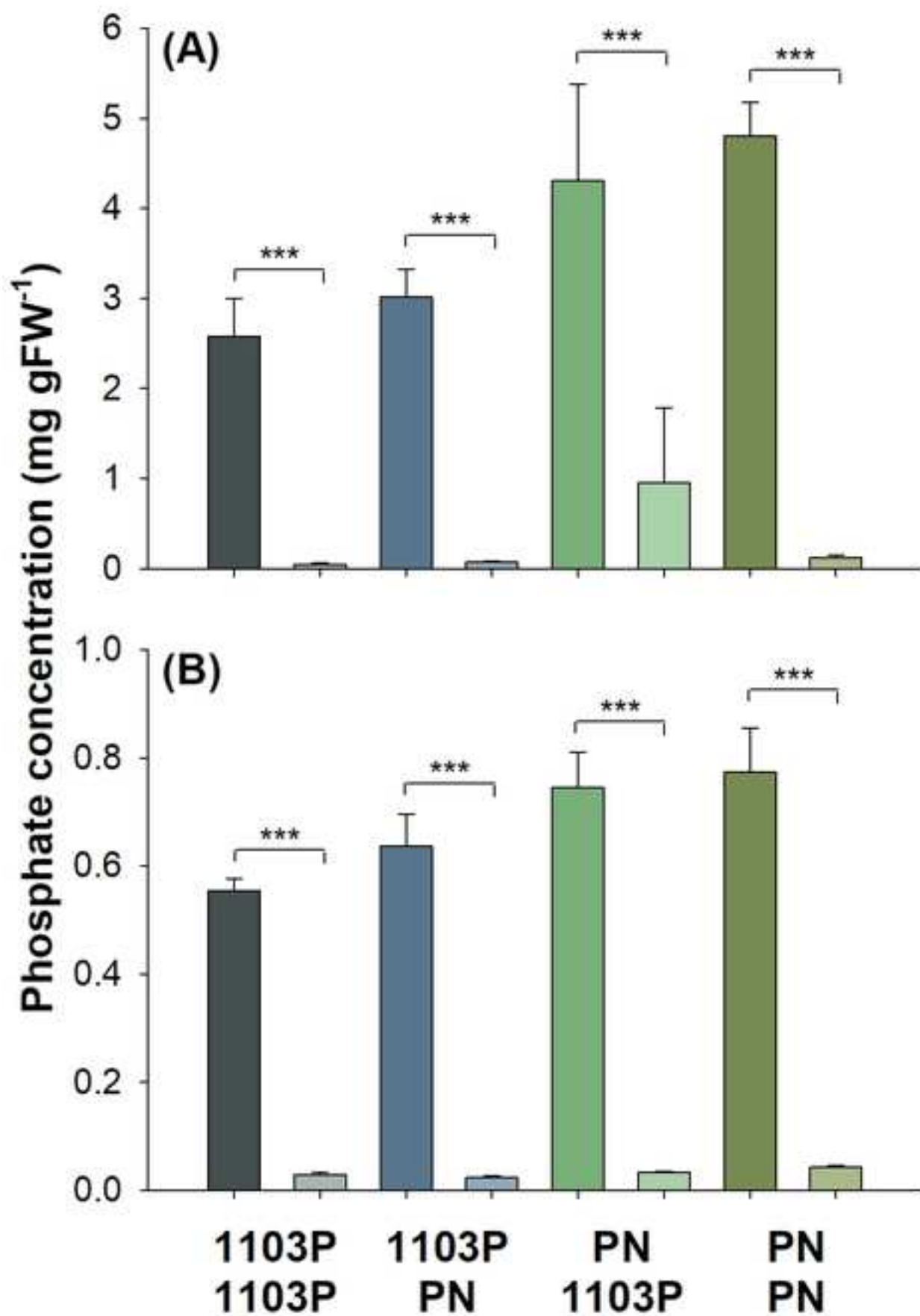
| Organic acid concentration (mg g ⁻¹ FW) | Scion | | | Rootstock | | | Scion x Rootstock Interaction |
|--|--------------|--------------|--------------|--------------|--------------|--------------|-------------------------------|
| | 1103P | PN | Significance | 1103P | PN | Significance | Significance |
| Shoot | | | | | | | |
| 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 |
| Stem | | | | | | | |
| 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 | *** |
| Root | | | | | | | |
| 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 | *** | ** |

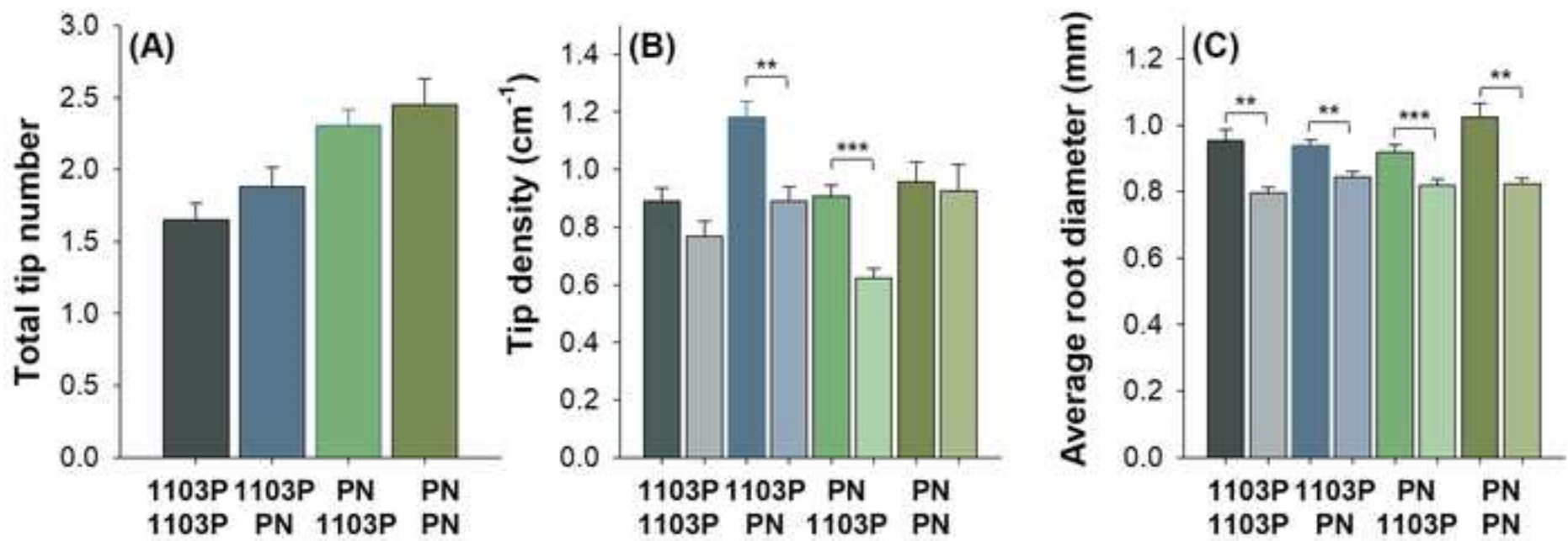
Supplementary Table 4. Results of a 3-way analysis of variance of the abundance of transcripts encoding various *PHOSPHATE TRANSPORTER1 (PHT1)*, *PURPLE ACID PHOSPHATASE (PAP)*, *PHOSPHATE2 (PHO2)* and *SPX DOMAIN CONTAINING PROTEINS (SPX)* genes in the roots of reciprocal grafts of *Vitis berlandieri* 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.

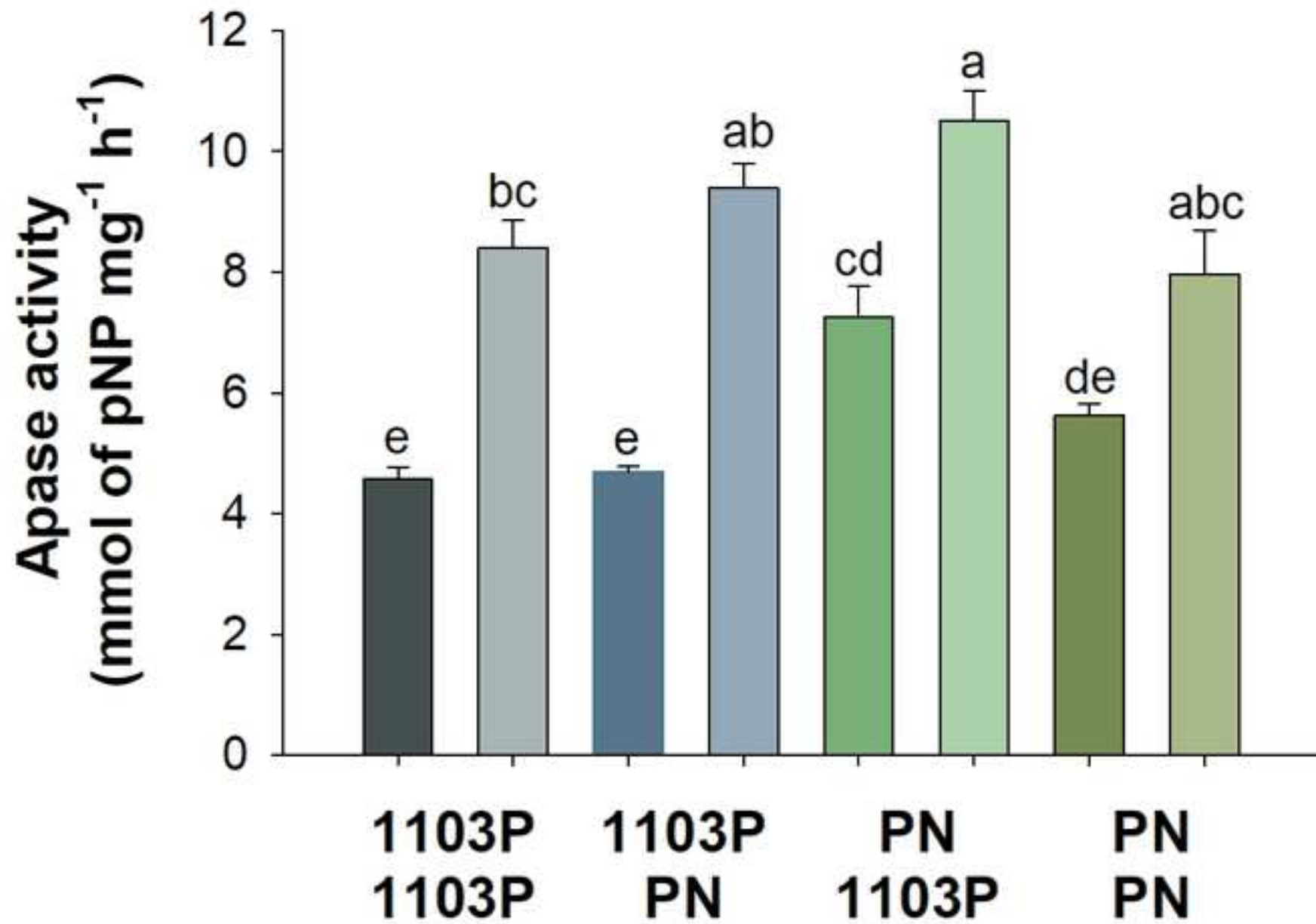
| | Scion | Rootstock | Pi supply | Scion x Rootstock | Scion x Pi supply | Rootstock x Pi supply | Scion x Rootstock x Pi supply |
|---------|-------|-----------|-----------|----------------------|-------------------------|--------------------------|-------------------------------------|
| PHT1.3a |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











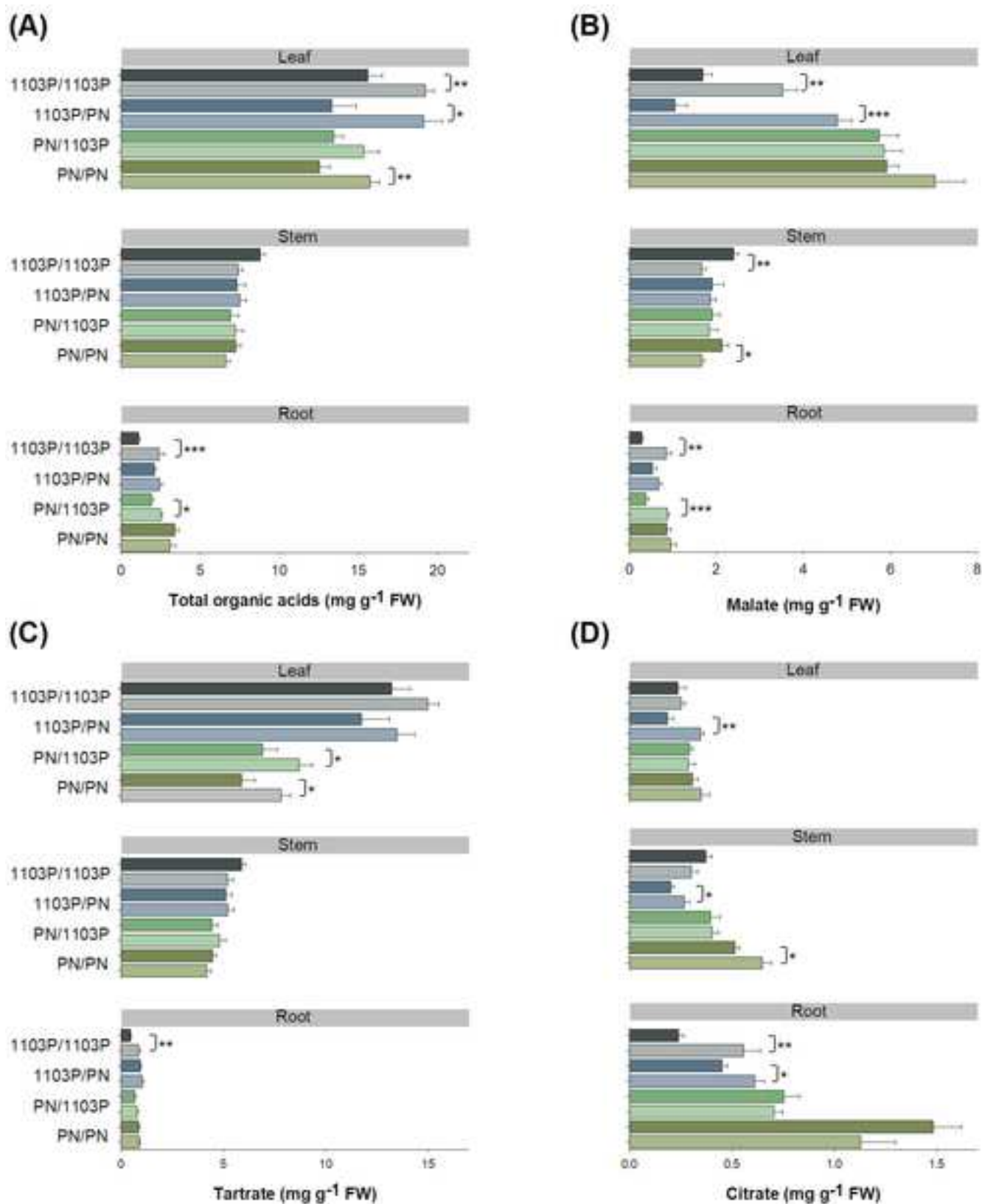
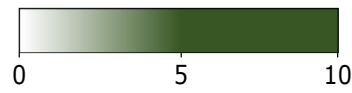
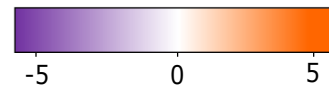
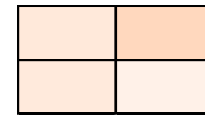
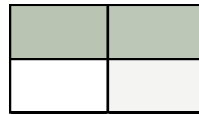


Figure 7

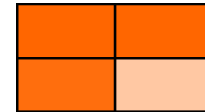
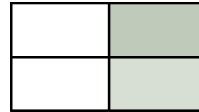
| | |
|-------|-------|
| 1103P | PN |
| 1103P | 1103P |
| 1103P | PN |
| PN | PN |

Expression level (log₂)Fold change (log₂)

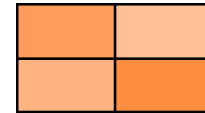
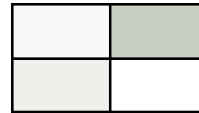
PHT1.3a
VIT_13s0067g03280



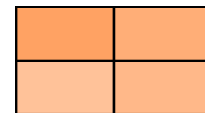
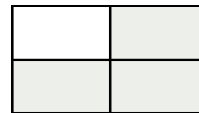
PHT1.3b
VIT_16s0050g02380



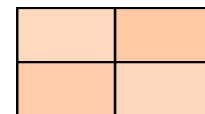
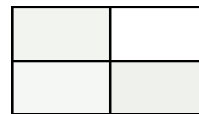
PHT1.4a
VIT_05s0049g00920



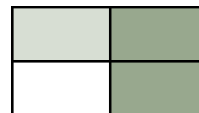
PHT1.4b
VIT_05s0049g00930



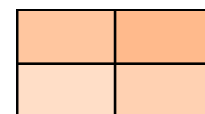
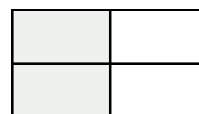
PHT1.4c
VIT_05s0049g00940



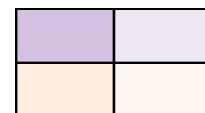
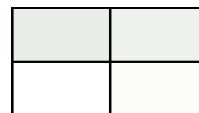
PHT1.4d
VIT_16s0050g02370



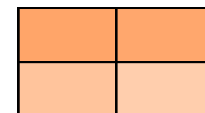
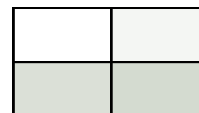
PHT1.9
VIT_18s0122g00780



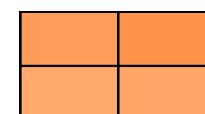
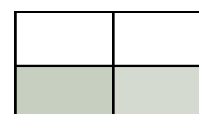
PHO2
VIT_00s0265g00070



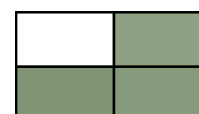
PAP10
VIT_03s0038g00230



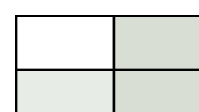
PAP12
VIT_18s0001g13340

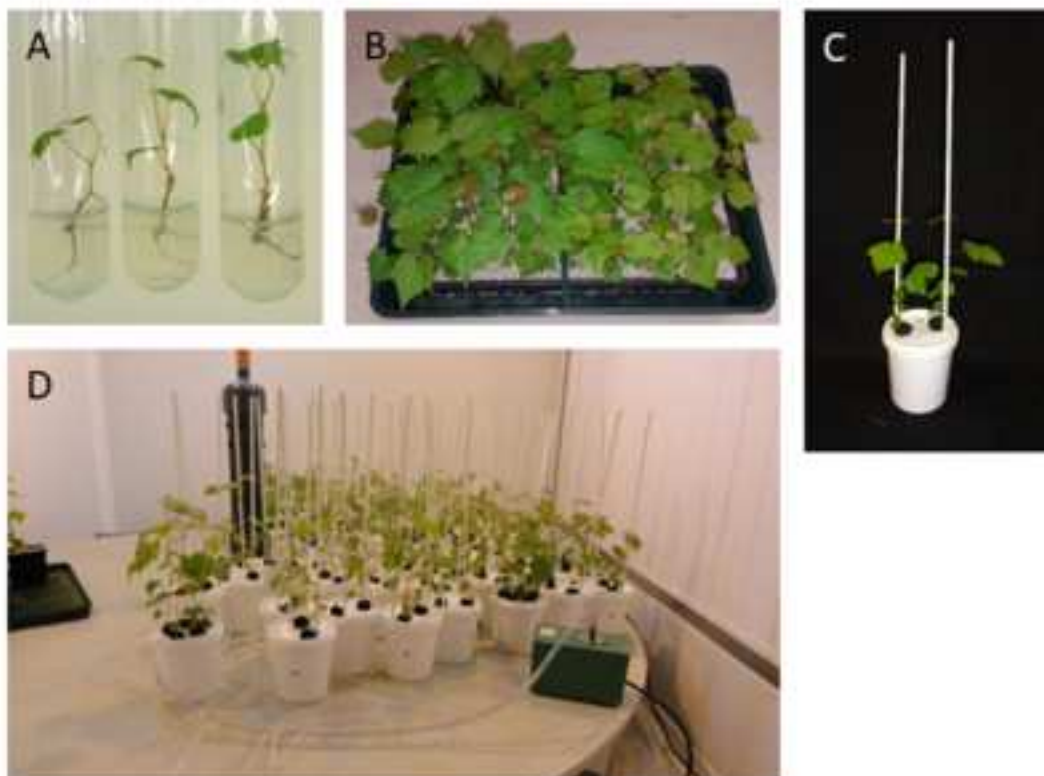


SPX1/2
VIT_11s0016g05330



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.