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

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Running head: Reciprocal grafts under different P supplies

## 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.

## Introduction

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

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

In addition to modifying the rhizosphere to increase Pi availability, under low Pi supply plants can also modify biomass allocation, root system architecture and morphology, gene expression and Pi transport to increase root foraging capacity (Lan *et al.*, 2015; Lynch, 2011; Morcuende *et al.*, 2007; Pant *et al.*, 2015). This is often mediated by the preferential allocation of biomass to the roots rather than the shoots, by the increase in the degree of lateral root branching, and by the increase in the number and length of root hairs (Lynch, 2011). Low Pi supply induces the expression of many PSI genes, for example, genes coding for PHT1s, which can increase root Pi transport capacity (Lan *et al.*, 2015). As low Pi reduces plant growth, metabolites such as starch, sugars and many amino acids increase in concentration, while phosphorylated metabolites such as hexose phosphates, ATP and

ADP decrease in concentration (Morcuende *et al.*, 2007). Tissue organic acid concentrations often change in response to Pi supply to maintain internal pH balance (Vance et al. 2003).

One method to determine the roles of the shoot and root in regulating plant responses to the environment is by grafting two different genotypes together. Grafting is an old horticultural technique used for the cultivation of perennial fruit crops (*e.g.* grape, apple, citrus, etc.) and annual vegetables and fruits (*e.g.* tomatoes, cucumber and pepper) (Mudge et al. 2009; Bie et al. 2017). In horticulture, grafting allows us to combine desirable traits of the shoot and the root of two different genotypes in a single plant. Rootstocks have been selected for various agronomical traits such as controlling scion growth (i.e. inducing dwarfing and modifying scion architecture), improving water use efficiency/drought tolerance, altering mineral element status, improving yield and fruit quality, and providing resistance to root pathogens (Nawaz et al. 2016; Warschefsky et al. 2016). Since the 19$^{th}$ century, the Eurasian grapevine species (*Vitis vinifera*) is cultivated grafted using North American *Vitis spp.* as rootstocks because of their tolerance to the American aphid pest Phylloxera. In addition to providing Phylloxera resistance, grapevine rootstocks are also chosen based on their known adaptation to soil conditions and ability to control scion vigour (Ollat *et al.*, 2016). Grapevine rootstocks are known to modify the mineral element composition in the scion (Cordeau 1998; Bavaresco et al. 2003) and these changes are linked to the genetic background of the rootstock (Gautier *et al.*, 2020b). Grapevine rootstocks modify plant responses to nutrient supply, such as nitrogen (Lecourt et al. 2015; Cochetel et al. 2017), potassium (Ruhl 1989, 1991), phosphorus (Grant and Matthews 1996b, a; Gautier et al. 2018) and iron (Jiménez et al. 2007; Covarrubias and Rombolà 2015; Covarrubias et al. 2016).

As grapevine rootstocks are known to modify scion phosphorus content and plant responses to Pi supply, we used reciprocal grafting of two widely used grapevine genotypes to explore the roles of both the shoot and the root in regulating Pi content and plant responses to Pi supply in grafted grapevine. The genotypes chosen were a *V. vinifera* scion obtained from the sexual reproduction from self-pollination of Pinot Noir, named 40024 (PN), which grows well in *in vitro* culture and grafts easily, and a rootstock genotype of American origin, *V. berlandieri* x *V. rupestris* cv. 1103 Paulsen clone 198 (1103P), which confers a high scion vigour and petiole phosphorus concentration in the vineyard (Gautier *et al.*, 2020b). These two genotypes were also chosen because they differ in their phosphorus acquisition efficiency in the first 21 d of development when grown as woody cuttings (Gautier et al., 2018). Firstly, we aimed to determine if the scion and/or rootstock alters plant growth, biomass allocation, Pi content and phosphate acquisition efficiency (PAE) in non-limited Pi supply. Secondly, we assessed the growth response of grafted grapevines to low Pi supply and quantified the expression of different *PHT1* genes. In addition, because Pi acquisition interacts with

both sulphate and nitrate uptake, both sulphate and nitrate acquisition efficiencies were calculated. Thirdly we asked does the scion/rootstock combination affect the exudation of APases and the abundance of *PAP* transcripts. And finally we asked whether the scion/rootstock combinations differed in tissue organic acid concentration and whether organic acid concentrations respond to Pi supply. This was achieved by comparing all possible scion/rootstock combinations of two grapevine genotypes grown under two contrasted Pi supplies.

## Materials and methods

*Plant material and growing conditions*

All four possible scion/rootstock combinations of 1103P and PN were micro-grafted using the cleft grafting system, i.e. 1103P/1103P, 1103P/PN, PN/1103P and PN/PN and grown as previously described (Gautier *et al.*, 2020a). Both genotypes were introduced into *in vitro* culture after surface sterilisation. Plants were cultivated *in vitro* on McCown Woody Plant Medium (Duchefa) supplemented with 30 g $L^{-1}$ sucrose, 0.27 µM 1-naphthalene acetic acid and 0.4 % agar in a growth chamber at 22 °C and with a photoperiod of 16 h light/8 h dark at a photon flux density of 55 µmol $m^{-2}$ $s^{-1}$ (Supplementary Fig. 1A). Six-week-old plantlets were then acclimated for four weeks to perlite-filled pots, irrigated with water, in a growth chamber at 26 °C and with a photoperiod of 16 h light/8 h dark at a photon flux density of 145 µmol $m^{-2}$ $s^{-1}$ at plant level (Supplementary Fig. 1B). Plants were then transferred into hydroponic culture with an aerated solution; each pot contained 2 plants of the same scion/rootstock combination with 700 mL of high P solution (HP, 0.6 mM P). Four days later, half the pots continued receiving the HP solution and the other half were subjected to a low P (LP, 0.001 mM P) treatment (Supplementary Fig. 1C and D). The macronutrient composition was 2.45 mM $KNO_3$, 0.69 mM $MgSO_4$ and 1.27 mM $CaCl_2$ for both the HP and LP solutions; HP solution also contained 0.6 mM $KH_2PO_4$ and 0.6 mM $CaSO_4$, whereas the LP solution contained 0.3 mM $K_2SO_4$ and 0.3 mM $CaSO_4$. Micronutrients were supplied as 46 µmol $H_3BO_3$, 9.1 µmol $MnCl_2$, 2.4 µmol $ZnSO_4$, 0.5 µmol $CuSO_4$ and 14 nmol $(NH_4)_6Mo_7O_{24}$, and iron was supplied as 8.5 mg $L^{-1}$ Sequestrene 138 (i.e. 31.3 µmol ethylenediamine-N,N'-bis (2-hydroxyphenylacetic acid) NaFe, Syngenta Agro S.A.S.). The solutions were changed once a week for 28 d.

*Plant growth measurements*

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

*Root system morphology analysis*

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

*Activity of APases released by the roots*

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

*Metabolite profile of plant tissues*

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

*Quantification of transcript abundance*

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

The transcript abundance of *PAP10* and *PAP12* was analysed in root tips after 28 d of LP or HP (Fig. 7). The transcript abundance of *PAP10* was not affected by the scion or rootstock genotype under HP, but was higher in plants grown in LP than HP. The increase of abundance of *PAP10* transcripts

was higher in scion/rootstock combinations with an 1103P than a PN rootstock (Fig. 7). The abundance of *PAP12* transcripts was higher in plants with a PN than an 1103P rootstock under HP and was higher in plants grown under LP than HP all scion/rootstock combinations.

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

## Discussion

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

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

In summary, the scion genotype exerted control over whole plant development in the reciprocal grafts of grapevine studied, which is largely in agreement with the literature.

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

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

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

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

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

The genotypes studied in this work are known to differ in their PAE, when 1103P is cultivated as un-grafted, woody cuttings, it has a higher PAE than PN (Gautier et al. 2018). However, in this study on herbaceous micro-grafts, PAE was higher for PN/PN homo-grafts compared to 1103P/1103P homo-grafts, with intermediate values for the two hetero-grafts PN/1103P and 1103P/PN. The methods used in these studies were not the same; in Gautier et al. (2018) PAE was defined by slope of linear

regression between $^{32}P$ uptake of the shoot and biomass root FW, whereas in this work PAE was estimated by total plant Pi divided by root FW (assuming that Pi content at the beginning of the treatment was negligible). This could suggest that plant growth conditions and/or plant developmental stage alters PAE in grapevine and/or that the method used to calculate PAE affects the results.

The abundance of seven *PHT1* transcripts in the root was quantified under both HP and LP. Under HP, several transcripts showed scion and/or rootstock specific accumulation patterns, suggesting an intrinsic difference in *PHT1* transcript abundance between the two rootstock genotypes studied and that it is altered by shoot-derived signals. All transcripts were strongly up-regulated under LP supply, suggesting a higher capacity to take up Pi from the soil under LP, but given only 0.001 mM Pi was provided to the plants PAE could not be quantified. *PHOSPHATE2* abundance was affected by LP in a rootstock dependent manner, suggesting differences in post-transcriptional regulation and potentially in the quantity of PHT1 protein in the different rootstock genotypes.

Under HP, the different scion/rootstock combinations differed in SAE, which was highest in 1103P/1103P and lowest in PN/PN with the hetero-grafts having intermediate values, suggesting that under HP both the scion and rootstock genotype regulate SAE. Genotypic differences in sulphur accumulation have been reported in different scion/rootstock combinations of grapevine and 1103P has been shown to confer higher sulphur contents than other rootstock genotypes (Gautier *et al.*, 2020b; Lecourt *et al.*, 2015). Under HP, there was an inverse relationship between PAE and SAE; there are well-described interactions between Pi and sulphate nutrition in plants with phospholipids being replaced by sulpholipids in times of Pi limitation and vice versa, although the molecular mechanisms are poorly understood (Bouain *et al.*, 2019; Rouached *et al.*, 2011). In grafted plants with an 1103P scion, SAE was lower under LP than HP (whereas SAE was not affected by Pi supply when the scion was PN); this suggests that the scion genotype can modify SAE in response to LP in grafted grapevine. The magnitude of differences in SAE in this study suggest that the differences reported here are due to more than subtle differences in the composition of lipids and may be related to the accumulation of other sulphur containing molecules such as glutathione.

Nitrate acquisition efficiency was affected by the scion genotype, it was higher with a PN scion (largely due to higher shoot N concentration), and its response to LP was scion genotype dependent (remaining unchanged with a PN scion but lower with an 1103P scion). In summary, this suggests that in response to LP, the 1103P scion reduces NAE and SAE, whereas the PN scion does not.

***The release of APases by roots in response to LP is associated with the increase in abundance of PAP10 and PAP12***

The activity of APases released into the media was approximatively two-fold higher for each scion/rootstock combination grown under LP compared to HP. Related to this increase of APase activity, the relative abundances of *PAP10*, and *PAP12* transcripts were higher in plants grown under LP supply. *PURPLE ACID PHOSPHATASE10* and *PAP12* encode secreted APases, which are known to be increased in response to Pi starvation in species such as Arabidopsis, tomato or rice (Tian et al. 2012). These results suggest that grapevine is also able to use organic phosphorus from the soil. The activity of APases released into the media was affected by the scion and rootstock genotype and their interaction, whereas only the rootstock genotype affected the abundance of *PAP12* and only the interaction between the rootstock genotype and Pi supply affected the abundance of *PAP10* suggesting that other APase genes may be underlying the activity of APases released by the roots.

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

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

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

phosphorus in the soil (Vance et al. 2003). In grapevine, differences in organic acid concentration in root tips were previously observed under iron deficiency and in response to different nitrogen sources (Jiménez et al. 2007; Covarrubias and Rombolà 2015). In response to LP, scion/rootstock combinations with an 1103P rootstock showed an increase of total organic acid concentration in root, which was particularly associated with an increase in malate concentration. Recently, the capacity of grapevine to release organic acids into the rhizosphere in responses to alkalinity were demonstrated (Xiang et al. 2019). Unfortunately, due to technical difficulties we were unable to measure root organic acid exudation in these experiments.

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

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

## Conclusion

The objective of this study was to determine the roles of both the shoot and the root in employing strategies to cope with LP supply in grapevine. Under HP, the homograft PN/PN has higher tissue Pi concentrations and higher PAE than 1103P, which was associated with a higher constitutive expression of a number of *PHT1* genes, a lower SAE, and reduced growth. Reciprocal grafting showed that growth was regulated by the scion genotype, presumably via its capacity of assimilation and/or translocation of carbon. Whereas the heterografts, PN/1103P and 1103P/PN, had intermediate phenotypes in terms of tissue Pi concentrations, PAE and SAE suggesting that these parameters were regulated by both the scion and rootstock. In response to LP, all scion/rootstock combinations altered growth to favour root foraging, increase the expression of PSI genes and increased the exudation of APases (with the concomitant increase in the abundance of *PAP10* and *PAP12* transcripts). The organic acid concentration in the different tissues was affected by the scion/rootstock combination and the response to LP supply showed an effect on scion and rootstock

genotype. Many signals are known to move between the shoot and the root (and vice versa) to regulate plant responses to P supply (Zhang *et al.*, 2014), by grafting different genotypes together we have shown that genetic variation in the shoot exerts long-distance control over a number of these responses.

**Data and Materials availability**

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

**Supplementary Data**

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.

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

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.

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**Authors' contributions**

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

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List of Figures

1103 Paulsen (1103P) and V. vinifera cv. Pinot noir (PN) grown under high (dark colours) or low (ligh 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 | TGGTCCTCCCCAGAGAGTTG | 96.5 | 106 |
| PHT 1.3b | VIT_16s0050g02380 | CCCCACTAAAGATAAGCCTGGG | TCCTACTAGGGCAACCCCAA | 103.2 | 73 |
| PHT 1.4a | VIT_05s0049g00920 | TTGTGCTCGGGGTAGTCAAC | CCAGTCCTGGTAGAAGGGGA | 90 | 148 |
| PHT 1.4b | VIT_05s0049g00930 | CCCACCTGGTATTGGAATGAGA | TAGTAGGAGGCTGCATGTCCA | 85 | 166 |
| PHT 1.4c | VIT_05s0049g00940 | CCCGAGTCCAAGGGAAAGTC | GGAACAGTCCTAGCCTGCTG | 99.1 | 98 |
| PHT 1.4d | VIT_16s0050g02370 | TTGGTGACGGAGACCAAAGG | GTGTGCTAGGCATCTGGGTT | 94.5 | 90 |
| PHT 1.9 | VIT_18s0122g00780 | CCCGTGAAACCAAGGGAAGA | GAATCCGCGATGAGTTTCGC | 95.8 | 167 |
| PHO2 | VIT_00s0265g00070 | TTGTGCTGTGGAAGCAGGAT | GTGCAGGCAAACTCAAACCA | 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 | TGGGGATGGGAGAGTGGAAT | 91.5 | 140 |
| ACTIN | VIT_04s0044g00580 | CTTGCATCCCTCAGCACCTT | TCCTGTGGACAATGGATGGA | 93.6 | 82 |
| GAPDH | VIT_17s0000g10430 | CCACAGACTTCATCGGTGACA | TTCTCGTTGAGGGCTATTCCA | 91 | 70 |
| SAND3' | VIT_06s0004g02820 | TGCTGGGTTACCCCGGAGTTTGA | 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 |

**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 | | | *** | | | | |

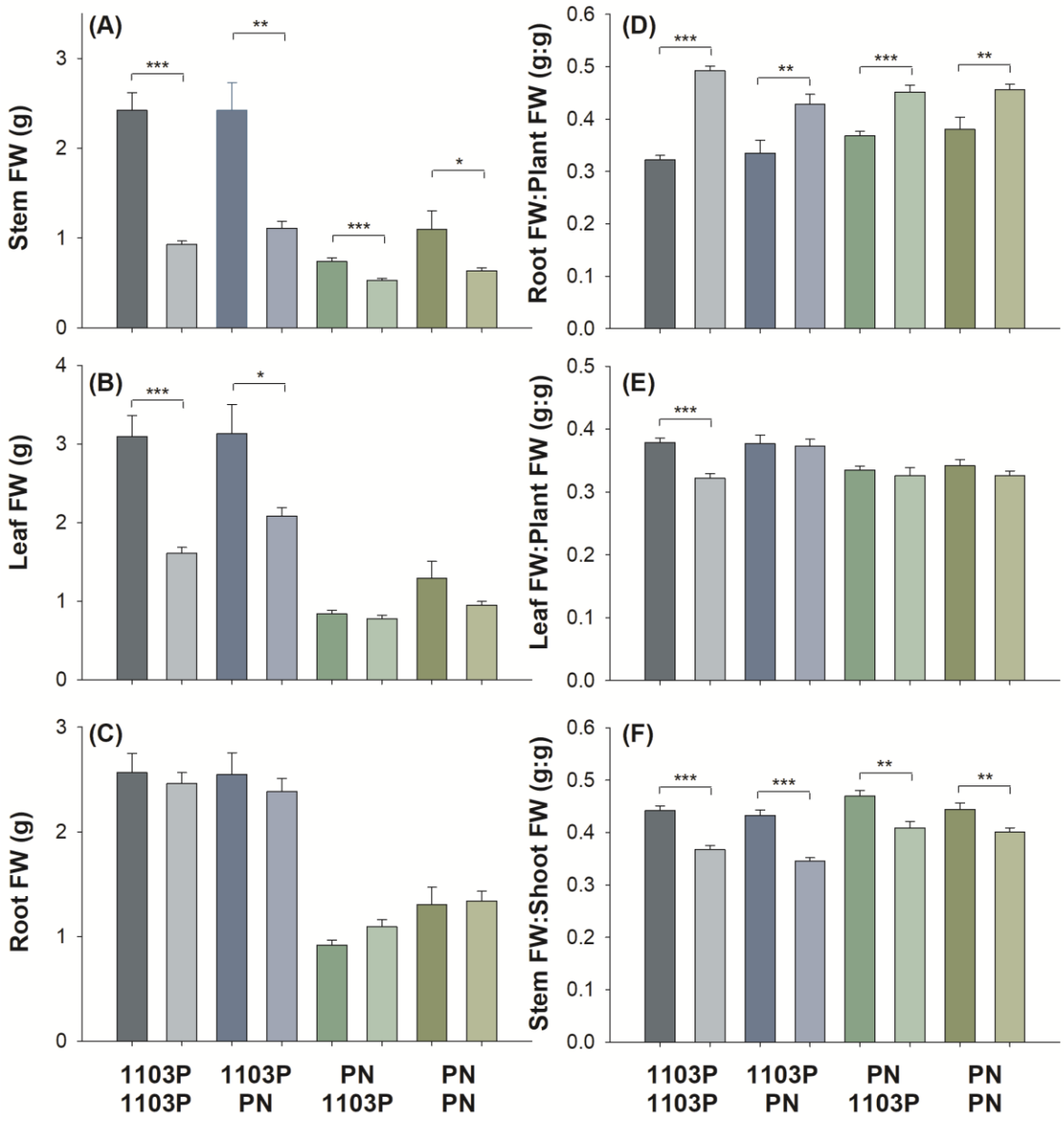
(A)
Stem FW (g)
(B)
Leaf FW (g)
(C)
Root FW (g)
(D)
Root FW:Plant FW (g:g)
(E)
Leaf FW:Plant FW (g:g)
(F)
Stem FW:Shoot FW (g:g)
1103P 1103P
1103P PN
PN 1103P
PN PN

Figure 1.

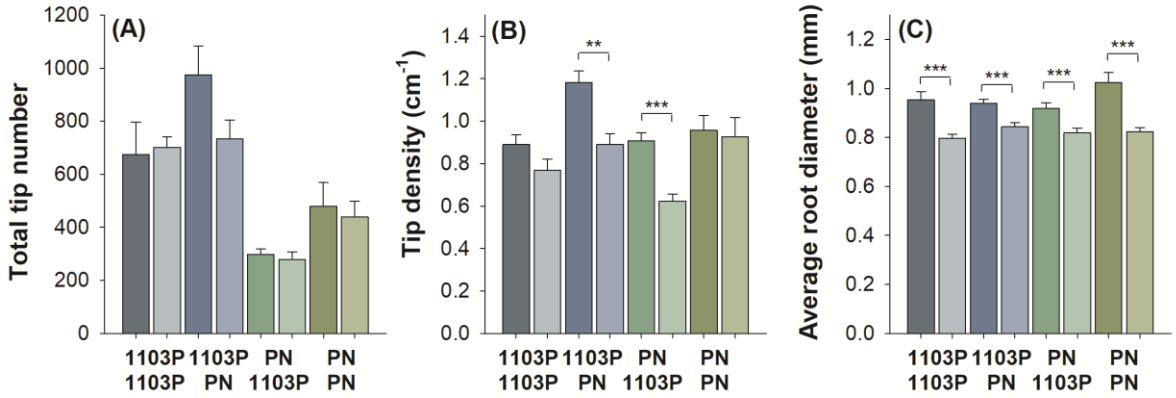
(A)
Total tip number
1200
1000
800
600
400
200
0
1103P 1103P
1103P PN
PN 1103P
PN PN
(B)
Tip density (cm-1)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
**
***
1103P 1103P
1103P PN
PN 1103P
PN PN
(C)
Average root diameter (mm)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
***
***
***
***
1103P 1103P
1103P PN
PN 1103P
PN PN

Figure 2

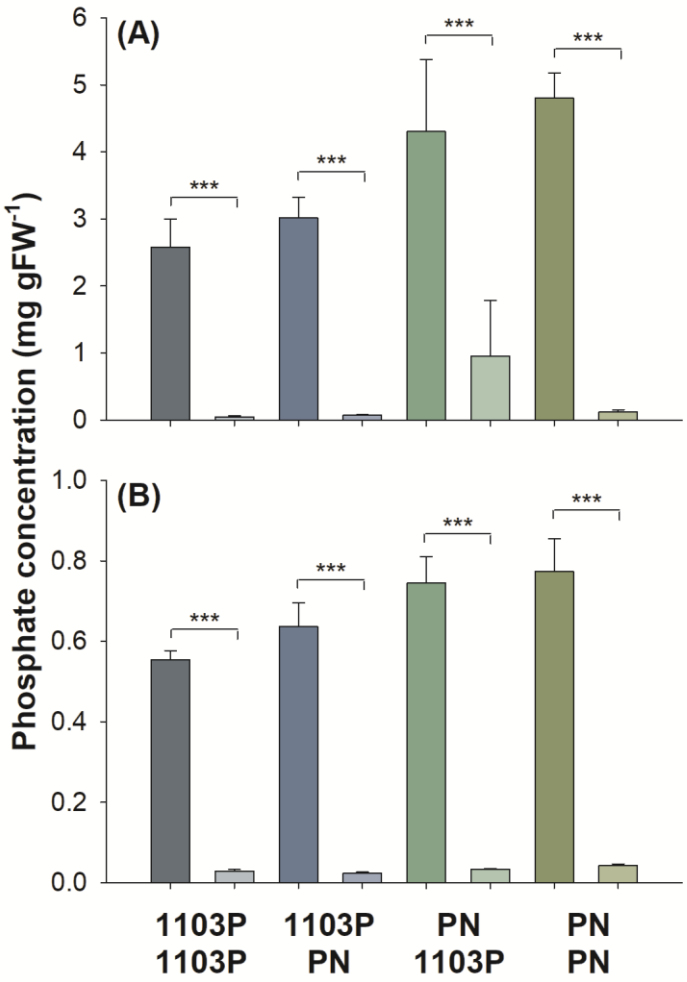
(A)
(B)
Phosphate concentration (mg gFW-1)
***
1103P 1103P
1103P PN
PN 1103P
PN PN

Figure 3.

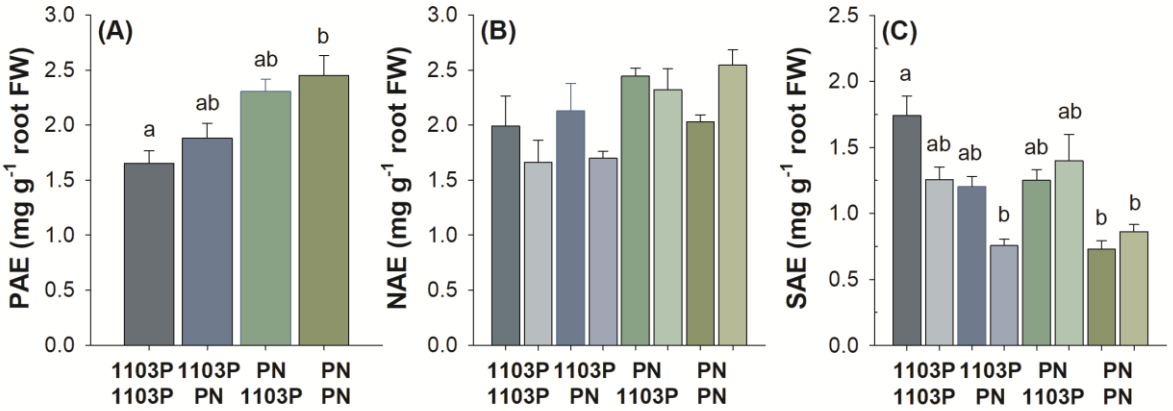


Figure 4.

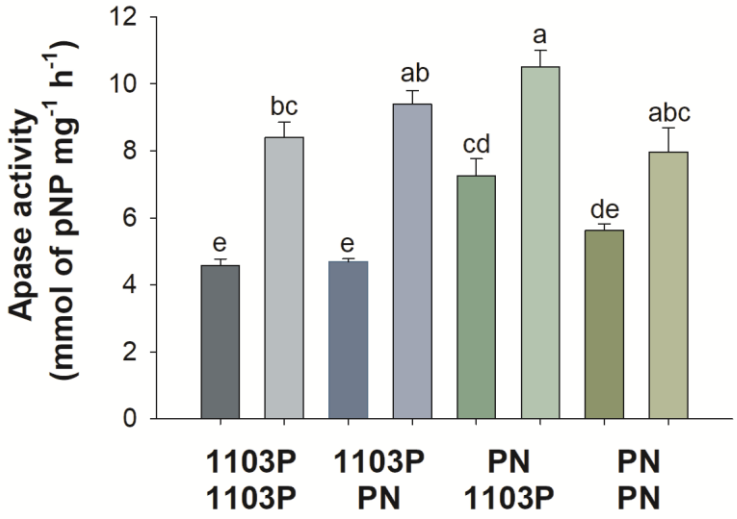


Figure 5.

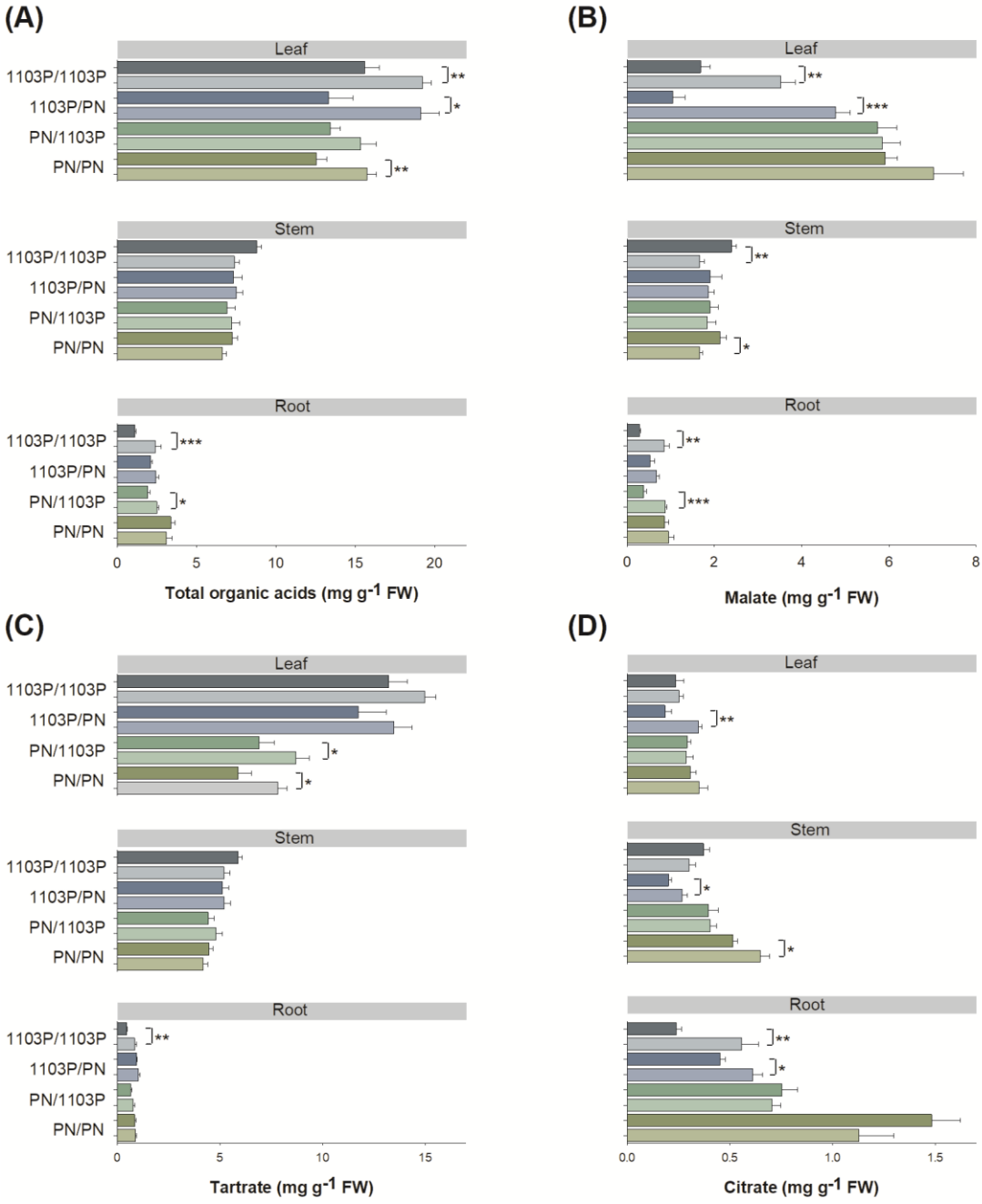
(A)
Leaf
Stem
Root
1103P/1103P
1103P/PN
PN/1103P
PN/PN
**
*
***
0
5
10
15
20
Total organic acids (mg g-1 FW)
(B)
0
2
4
6
8
Malate (mg g-1 FW)
(C)
0
5
10
15
Tartrate (mg g-1 FW)
(D)
0.0
0.5
1.0
1.5
Citrate (mg g-1 FW)

Figure 6.