Rôles du porte-greffe et du greffon dans la réponse à la disponibilité en phosphore chez la Vigne

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Rôles du porte-greffe et du greffon dans la réponse à la disponibilité en phosphore chez la Vigne

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Rôles du porte-greffe et du greffon dans la réponse à la disponibilité en phosphore chez la Vigne

La Vigne est cultivée en système greffé, combinant les qualités de production fruitière des Vignes Européennes (*Vitis vinifera*) et la tolérance phylloxérique des Vignes Américaines (*Vitis spp.*). Cependant l'utilisation de porte-greffes américains modifie le développement, la physiologie, et l'alimentation hydrique et minérale du greffon. Plus particulièrement, le fond génétique des porte-greffes de la Vigne semble impliqué dans la régulation de la nutrition en phosphore (P) du greffon. Le phosphore est un élément nutritif essentiel pour la croissance des plantes, impliqué dans la composition de nombreux composants cellulaires, ainsi que dans le contrôle des voies métaboliques via son apport énergétique et la régulation de l'activité enzymatique. Malgré son importance, P un est des éléments minéraux les plus limitants pour la croissance des plantes en raison de sa faible disponibilité assimilable dans le sol. L'objectif de ce travail est de déterminer les mécanismes impliqués dans le contrôle de la nutrition en P de la Vigne, en comparant deux porte-greffes *V. riparia cv.* Riparia Gloire de Montpellier (RGM) et *V. rupestris* x *V. berlandieri* cv. 1103 Paulsen (1103P) connus pour conférer respectivement de faibles ou fortes concentrations en P à leur greffon. Les résultats montrent que 1103P est plus adapté à acquérir le P que RGM, en partie grâce à un système racinaire plus développé permettant une meilleure exploration du sol ainsi qu'une meilleure efficience d'acquisition du P disponible. Ce porte-greffe montre également meilleure utilisation de ses réserves en P dans les parties pérennes afin d'optimiser la croissance des parties aériennes. En revanche la capacité des génotypes à augmenter le P assimilable dans la rhizosphère ne semble pas être différente. Enfin l'effet du greffage et plus particulièrement du greffon a été étudié, révélant le rôle de *V. vinifera* sur le développement et le fonctionnement racinaire du porte-greffe. Ces résultats contribuent à la compréhension des mécanismes régulant l'alimentation minérale de la Vigne, mettant ainsi en évidence le rôle du fond génétique du porte-greffe sélectionné, ainsi que la régulation de ce dernier par son greffon.

Mots clés : *Vitis spp.*, Phosphore, Développement racinaire, Rhizosphere, Porte-greffe, Greffon

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Roles of the rootstock and the scion in the response to phosphorus availability in grapevine

Grapevine is grown in a grafted system, combining the fruit production qualities of the European species (*Vitis vinifera*) and the phylloxera tolerance of American species (*Vitis spp.*). However, the use of American rootstocks affects the development, the physiology, and the water and mineral status of the scion. Particularly, the genetic background of grapevine rootstocks appears to be involved in the regulation of phosphorus (P) content of the scion. Phosphorus is an essential nutrient for plant growth, involved in the composition of many cellular components, as well as in the control of metabolic pathways via its role in energy transfer and the regulation of enzymatic activity. Despite its importance, P is one of the most limiting mineral elements for plant growth because of its poor availability in the soil. The objective of this work is to determine mechanisms involved in the control of P nutrition in grapevine, by comparing two rootstocks *V. riparia cv.* Riparia Gloire de Montpellier (RGM) and *V. rupestris x V. berlandieri* cv. 1103 Paulsen (1103P) known to confer low and high concentrations of P to their scion respectively. The results show that 1103P is more efficient at acquiring P than RGM, with a higher developed root system allowing greater soil exploration as well as a higher efficiency of P acquisition. In addition, this rootstock shows better use of its reserves of P in perennial parts to optimize the growth of the shoot. However, the ability of genotypes to increase the assimilable P in the rhizosphere does not seem to be different. Finally, the effect of grafting, and more particularly of the scion genotype, has been studied, demonstrating the capacity of *V. vinifera* to alter the development and root functioning of the rootstock. These results contribute to our understanding of the mechanisms regulating the mineral nutrition in grapevine and highlight the role of the genetic background of the rootstock, as well as its regulation by the scion.

Keywords: *Vitis spp.*, Phosphorus, Root development, Rhizosphere, Rootstock, Scion

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A mon père, parti trop tôt

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1103P	*V. rupestris* x *V. berlandieri* cv. 1103 Paulsen
APase	Acid phosphatase
cv.	Cultivar
DEG	Differentially Expressed Gene
DOT	Days Of treatment
DW	Dry Weight
FW	Fresh Weight
HP	High Phosphorus
LP	Low Phosphorus
PAE	Phosphorus Acquisition Efficiency
PN	*V. vinifera* cv. Pinot noir
PUE	Phosphorus Use Efficiency
RGM	*V . riparia* cv. Riparia Gloire de Montpellier
RGR	Relative Growth Rate
***V.* spp.**	*Vitis* spp.

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PREFACE

Depuis plusieurs millénaires, la Vigne (*Vitis spp.*) est cultivée à travers le monde entier en majeure partie pour la production de vin. Les caractéristiques du vin sont étroitement liées au terroir viticole dont il est issu, défini par l'interaction du sol, du climat et du travail de l'Homme. La viticulture est d'une grande importance économique et culturelle. Cependant elle est en perpétuelle évolution, devant faire face aux changements climatiques, pédologiques ou encore sociétaux comme les attentes des consommateurs ou les réglementations.

Afin de sauver le vignoble Européen face à l'invasion phylloxérique, la Vigne est cultivée en système greffé depuis le XIX^{e} siècle, utilisant des espèces d'origines américaines dans le rôle de porte-greffe, sélectionnées au préalable pour leur tolérance naturelle au ravageur. Le greffage induit des modifications dans le développement, la physiologie et la production fructifère du greffon, ainsi que dans la perception et l'adaptation aux conditions environnementales. Dorénavant, le porte-greffe n'est plus uniquement sélectionné pour sa tolérance phylloxérique mais pour de nombreux traits agronomiques visant à adapter la culture de la Vigne au terroir et aux objectifs du producteur. En effet, la filière viticole a pris conscience que les porte-greffes font partie intégrante du vignoble et jouent un rôle dans la typicité des vins.

Les porte-greffes de la Vigne sont connus pour réguler la composition minérale de leur greffon. Cependant les aptitudes des porte-greffes à acquérir les éléments minéraux du sol et à les transférer au greffon sont encore mal connues. En effet, elles dépendent du génotype de porte-greffe, de son affinité avec le greffon et de sa capacité à s'adapter à l'environnement et plus particulièrement à la disponibilité en minéraux.

Ce travail de thèse s'intègre dans la compréhension des caractéristiques des génotypes de porte-greffes de la Vigne et leur influence sur le développement du greffon. Plus précisément, il se focalise sur la nutrition minérale de la Vigne et sa régulation par le porte-greffe. Tout d'abord, ce travail vise à identifier l'ampleur des variations de la composition minérale induites par une gamme de porte-greffes venant de fonds génétiques différents, cultivés dans les mêmes conditions environnementales. Mettant en évidence la forte influence du porte-greffe sur la composition en phosphore du greffon, ce travail est ensuite focalisé sur la compréhension des mécanismes régulant l'acquisition et l'utilisation du phosphore en réponse à différents niveaux de disponibilités dans le milieu.

CONTEXTE

La Vigne

La Vigne Eurasienne, *Vitis vinifera* L., est cultivée depuis 8 000 à 10 000 ans (Galet and Grisard, 2015). Aujourd'hui, *V. vinifera* est cultivée dans le monde entier à travers 6 000 à 10 000 génotypes différents, appelés variétés, cépages ou cultivars (cv.) Cela représente sur la planète plus de 7,5 millions d'hectares cultivés en 2016 (2017 World Vitiviniculture Situation ; http://www.oiv.int/). La production de raisins est dédiée à la production de vin (48%), de raisins de table (36%), de raisins secs (8%) et de jus de fruit (5%). Approximativement 270 millions hectolitres de vin sont produits par an, faisant de la Vigne la culture la plus rentable au monde (http://faostat.fao.org/). Cependant, la vaste variabilité de cépages existante est faiblement utilisée. En effet, 50 % des surfaces cultivées au niveau mondial pour la production de vin utilisent seulement 10 variétés (Figure I.1A) ; et 30 % des surfaces destinées à la production de raisins de table utilisent 3 cultivars (Figure I.1B) (Distribution of the world's grapevine varieties 2017 ; http://www.oiv.int/).

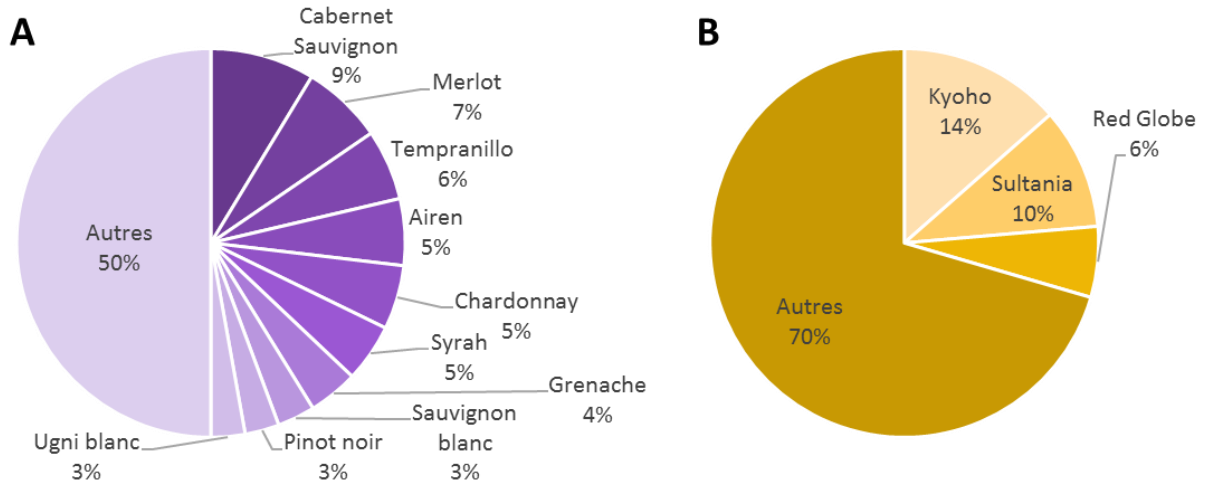

Figure I.1. Surfaces cultivées des variétés de Vigne au niveau mondial (en pourcentage) pour la production de vin (A) et de raisins de table (B) en 2017, à partir de « Distribution of the world's grapevine varieties 2017 » (http://www.oiv.int/).

La qualité du vin est directement associée à la composition du raisin (e.g. les sucres, les acides organiques, les anthocyanes, les composés aromatiques). Le producteur cherche donc à produire des raisins de qualité en respectant un équilibre entre ces différents composés qui participent à la saveur et à la structure du vin. La synthèse et la concentration de ces composés chimiques dépendent du cultivar utilisé (variabilité génétique, longueur du cycle reproductif), mais sont également fortement affectées par les conditions climatiques (températures et précipitations) et par les caractéristiques du sol (e.g. composition minérale et/ou hydrique) (Keller, 2015).

En raison de l'importance économique et sociale du vin, le changement climatique est un challenge majeur pour les producteurs et la filière vini-viticole pour préserver la production de vins typiques de leur région et répondre à la demande des consommateurs. Comprendre la physiologie de la Vigne pour pratiquer une viticulture de précision est cruciale afin d'obtenir des raisins de qualité nécessaires pour la vinification.

La crise phylloxérique

Durant la seconde moitié du XIX^{e} siècle, l'introduction accidentelle en Europe, en provenance du continent Nord-Américain, du puceron *Daktulosphaira vitifoliae* (Phylloxera), causa une catastrophe agronomique sans précédent. Détecté pour la première fois en France en 1863, le Phylloxera de la Vigne provoque des nodosités puis des nécroses au niveau des racines, induisant le dépérissement du plant en seulement 3 ans. Après seulement quelques années, la totalité du vignoble français fût attaqué, avec 1,66 millions d'hectares touchés ou détruits sur les 2,44 millions répertoriés, diminuant considérablement la production de vin de 55 à 25 millions hectolitres (Legros and Argelès, 1994). Afin de faire face à la propagation fulgurante du Phylloxera, une commission d'étude fut désignée, constituée de 3 membres : Gaston Bazile, Jules Emile Planchon and Félix Sahut. Après avoir testé différents moyens de lutte plus ou moins efficaces et difficiles à mettre en place, tels que la désinfection ou l'immersion des sols, les agronomes français réalisèrent que les racines des Vignes provenant d'Amérique étaient tolérantes au Phylloxera. Pour maintenir la production de vin en Europe et pour conserver les variétés traditionnelles utilisées jusqu'à présent, *V. vinifera* fut greffée sur des Vignes d'origine américaine, afin de combiner la qualité du fruit à la tolérance phylloxérique (Cordeau, 1998; Pouget, 2015).

L'utilisation des *Vitis* américaines

Il existe environ 30 espèces de *Vitis spp.* originaires du continent américain. Après la crise phylloxérique, le premier critère de sélection était bien entendu la résistance au puceron. En conséquence, *Vitis riparia* et *Vitis rupestris* furent les premières espèces américaines utilisées comme porte-greffe. Leurs principales caractéristiques sont disponibles en Table I.1 (Cordeau, 1998; Galet, 1988; Pongracz, 1983). Rapidement, les variétés de *V. vinifera* greffées sur ces porte-greffes exprimèrent en sol calcaire des symptômes de chlorose, ce qui conduit à l'introduction d'une troisième espèce américaine, *Vitis berlandieri* (Table I.1). *V. berlandieri* est originaire du sud du Nouveau-Mexique, de la région nord du Mexique ainsi que du Texas, où les sols sont connus pour être calcaires (McLean, 1973) ce qui peut expliquer les propriétés de cette espèce (Table I.1 - Figure I.2).

	V. riparia	***V. rupestris***	***V. berlandieri***
Tolérance au Phylloxera	18/20	18/20	17/20
Enracinement	90-95%	80%	15%
Réussite au greffage	Très bonne	Bonne	Faible
Tolérance au calcaire (% de calcaire actif)	6%	10-14%	40%
Vigueur conférée	Faible	Forte	Forte
Tolérance à la sécheresse	Susceptible	Susceptible	Tolérant

Table I.1. Principales caractéristiques des espèces américaines *V. riparia*, *V. rupestris* and *V. berlandieri*, adapté de (Galet, 1988; Galet and Smith, 1998).

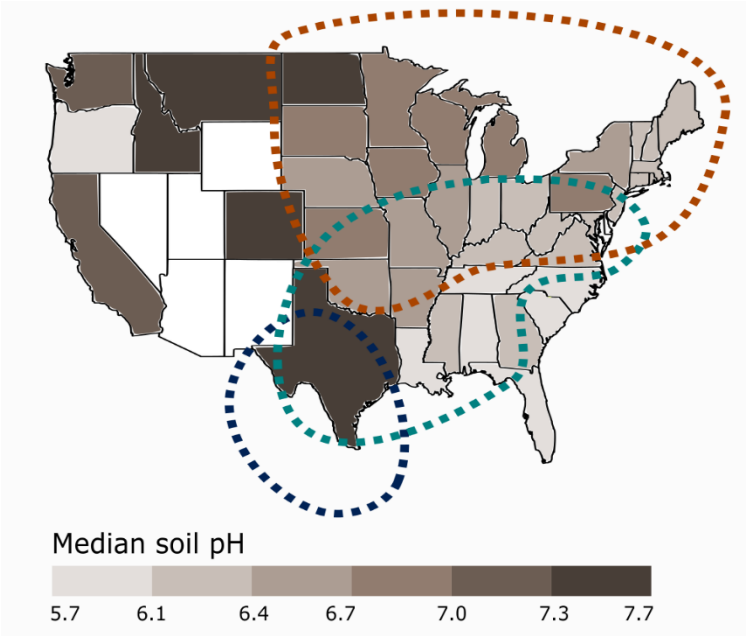

Figure I.2. Valeurs moyennes du pH des sols d'Amérique du Nord, calculés sur 4,3 millions d'échantillons en 2010 (http://www.cropnutrition.com/efu-soil-ph) ; et les origines géographiques des principales espèces de Vigne utilisées en porte-greffes, *V. riparia* (marron), *V. rupestris* (turquoise) *et V. berlandieri* (bleu), d'après (Galet (1988); Galet and Smith (1998)).

Cependant, cette espèce montrait de faibles capacités à raciner et de réussite au greffage. Ceci amorça la création et la sélection de porte-greffes hybrides dans le but d'associer les caractéristiques des différentes espèces américaines (Galet, 1988; Galet and Smith, 1998). D'après la base de données Vitis International Variety Catalogue (http://www.eu-vitis.de), la majorité des porte-greffes utilisés actuellement en Europe, sont le résultat de croisements interspécifiques entre ces trois espèces de *Vitis spp.* avec quelques espèces dont *V. vinifera* (47% dont le font génétique est issue de *V. berlandieri*, 52% de *V. riparia* et 30% de *V. rupestris*). En France, les porte-greffes hybrides issus de croisements interspécifiques n'utilisant uniquement ces trois espèces, représentent 61% des 31 porte-greffes autorisés en (Table I.2).

Porte-greffe	Abréviation	Croisement
101-14 Millardet et de Grasset	101-14MGt	*V. riparia x V. rupestris*
110 Richter	110R	*V. rupestris x V. berlandieri*
1103 Paulsen	1103P	*V. rupestris x V. berlandieri*
140 Ruggeri	140Ru	*V. rupestris x V. berlandieri*
1447 Paulsen	1447P	*V. rupestris x V. berlandieri*
161-49 Couderc	161-49C	*V. riparia x V. berlandieri*
1616 Couderc	1616C	*V. riparia x V. longii*
196-17 Castel	196-17Cl	1203C *(V. vinifera x V. Rupestris) x V. riparia*
216-3 Castel	216-3Cl	1616C (*V. riparia x V. longii) x V. rupestris*
3309 Couderc	3309C	*V. riparia x V. rupestris*
333 Ecole de Montpellier	333EM	*V. vinifera x V. berlandieri*
34 Ecole de Montpellier	34EM	*V. riparia x V. berlandieri*
4010 Castel	4010Cl	*V. riparia x V. rupestris*
41B Millardet et de Grasset	41B	*V. Vinifera x V. berlandieri*
420A Millardet et de Grasset	420A	*V. riparia x V. berlandieri*
44-53 Malègue	44-53M	*V. riparia x* 144M *(V. cordifolia x V. rupestris)*
99 Richter	99R	*V. berlandieri x V. rupestris*
Berlandieri - Colombard 2	BC2	*V. berlandieri x V. vinifera*
Fercal	Fercal	BC1 *(V. berlandieri x V. vinifera) x* 31R *(V. berlandieri x V. longii)*
Gravesac	Gravesac	161-49C *(V. riparia x V. berlandieri) x* 3309C *(V. riparia x V. rupestris)*
Grézot 1	G1	*V. rupestris x V. vinifera*
Kober 125AA	125AA	*V. berlandieri x V. riparia*
Kober 5BB	5BB	*V. berlandieri x V. riparia*
Nemadex Alain Bouquet	Nemadex	*V. vinifera x V. rupestris x V. berlandieri*
Rességuier Birolleau 1	RSB1	*V. berlandieri x V. riparia*
Rupestris du Lot	Lot	*V. rupestris*
Riparia Gloire de Montpellier	RGM	*V. riparia*
Sélection Oppenheim 4	SO4	*V. berlandieri x V. riparia*
Teleki 5C	5C	*V. riparia x V. berlandieri*
Teleki 8B	8B	*V. berlandieri x V. riparia*
Vialla	Vialla	*V. Labrusca x V. riparia*

Table I.2. Liste des porte-greffes inscrit au catalogue français. Les porte-greffes issus uniquement du croisement de *V. riparia*, *V. berlandieri* et *V. rupestris* sont surlignés en marron. (A partir de http://plantgrape.plantnet-project.org/fr/).

Les hybrides conservent en grande partie les caractéristiques de leurs parents :

- Les hybrides *V. riparia* x *V. rupestris* (e.g. 3309C ou 101-14MGt) sont connus pour leur forte tolérance au Phylloxera, leur fort taux d'enracinement et de réussite au greffage, cependant ils sont généralement sensibles à la sécheresse et aux sols calcaires, et sont connus pour conférer une faible vigueur à leur greffon. Ils sont adaptés à la production de vins de grande qualité et diminuent les rendements.

- Les hybrides *V. riparia* x *V. berlandieri* (e.g. SO4 ou 161-49C) sont connus pour leur forte tolérance au Phylloxera, des taux d'enracinement et de réussite au greffage variables, une tolérance moyenne à la sécheresse et aux sols calcaires. Ils confèrent une vigueur moyenne à forte à leur greffon.

- Les hybrides *V. rupestris* x *V. berlandieri* (e.g. 110R ou 1103P) sont connus pour leur forte tolérance à la sécheresse et pour conférer une forte vigueur à leur greffon, cependant ils ont de faibles capacités d'enracinement et de réussite au greffage.

- Les croisements entre *V. berlandieri* et *V. vinifera* (e.g. 41B) permettent d'obtenir des porte-greffes très tolérants aux sols calcaires, conférant une vigueur moyenne à forte à leur greffon. Ils possèdent comme *V. berlandieri* de faibles taux de réussite au greffage et à l'enracinement (Cordeau, 1998; Galet, 1988; Galet and Smith, 1998).

Aujourd'hui, plus de 85% des vignobles à travers le monde sont greffés, utilisant entre 70 et 80 génotypes de porte-greffes (Keller, 2015; Ollat et al., 2016). Cependant, l'importante variabilité génétique au sein des porte-greffes de la Vigne reste faiblement exploitée. D'après la littérature, il est estimé que 90% des Vignes cultivées dans le monde sont greffés sur moins de 10 génotypes de porte-greffes (Galet, 1988; Galet and Smith, 1998; Ollat et al., 2016). En France, sur 31 porte-greffes commercialisés, 3 génotypes (SO4, 110R et 3309C) représentent 50% des utilisations (Figure I.3).

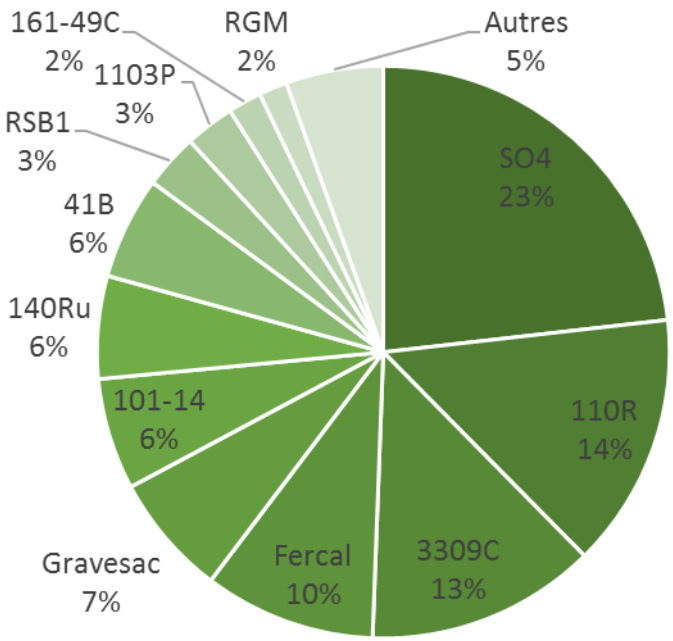

Figure I.3. Utilisation des porte-greffes pour la production de plants de Vigne greffés en France en 2016, à partir des bases de données France Agrimer (http://www.franceagrimer.fr/).

Le greffage

La réussite du greffage chez les plantes est variable et dépend de l'enchainement des processus biologiques complexes suivants (Pina et al., 2017) :

1. la formation d'une ligne de nécrose
2. la dédifférenciation cellulaire suivie d'une prolifération de cellules du cal
3. la fragmentation de la ligne de nécrose et la mise en contact des deux génotypes
4. la différenciation et le développement des tissus vasculaires

Chez la Vigne, plusieurs méthodes de greffage ont été testées. Grâce à sa rapidité de production, sa mécanisation et son fort taux de réussite, la greffe sur table oméga est la plus utilisée devançant la greffe sur pied en 'chip budding method', qui consiste à greffer le greffon sur un porte greffe déjà établi au vignoble (Figure I.4).

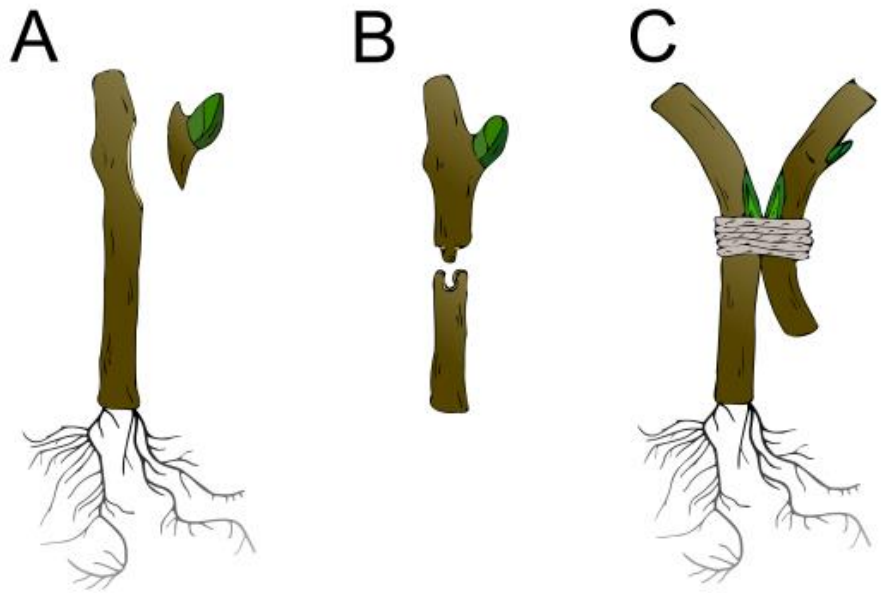

Figure I.4. Représentation de différents types de greffe utilisés sur plantes pérennes ; (A) greffe sur pied 'Chip budding', (B) greffe sur table en oméga et (C) greffe sur pied des tissus ligneux. Gautier et al. 2018 (soumis dans *Journal of Experiment of Botany,* disponible en annexe page 194).

L'influence du porte-greffe en viticulture

Le greffage est une technique horticulturale utilisée depuis des millénaires (Mudge et al., 2009) et toujours pratiquée de nos jours pour la culture des arbres fruitiers (e.g. le pommier, le cerisier et le citronnier), des lianes (e.g. le kiwi et la Vigne) et autres espèces végétales (e.g. le melon, la tomate, etc.). Aujourd'hui, les porte-greffes ne sont pas seulement sélectionnés pour leur tolérance au Phylloxera, mais pour de nombreux traits agronomiques comme chez d'autres espèces fruitières, tels que la résistance à d'autres maladies (e.g. les nématodes), l'adaptation aux caractéristiques du sol (e.g. sols calcaires, acides ou salins), la tolérance à la sécheresse, l'efficience d'acquisition et d'utilisation des nutriments, la compatibilité au greffage ou la vigueur conférée au greffon (Anwar et al., 2002; Cordeau, 1998; Keller, 2015; May, 1994; Warschefsky et al., 2016). En conséquence, le génotype de porte-greffe doit être choisi en accord avec les conditions environnementales et les objectifs de production (Figure I.5).

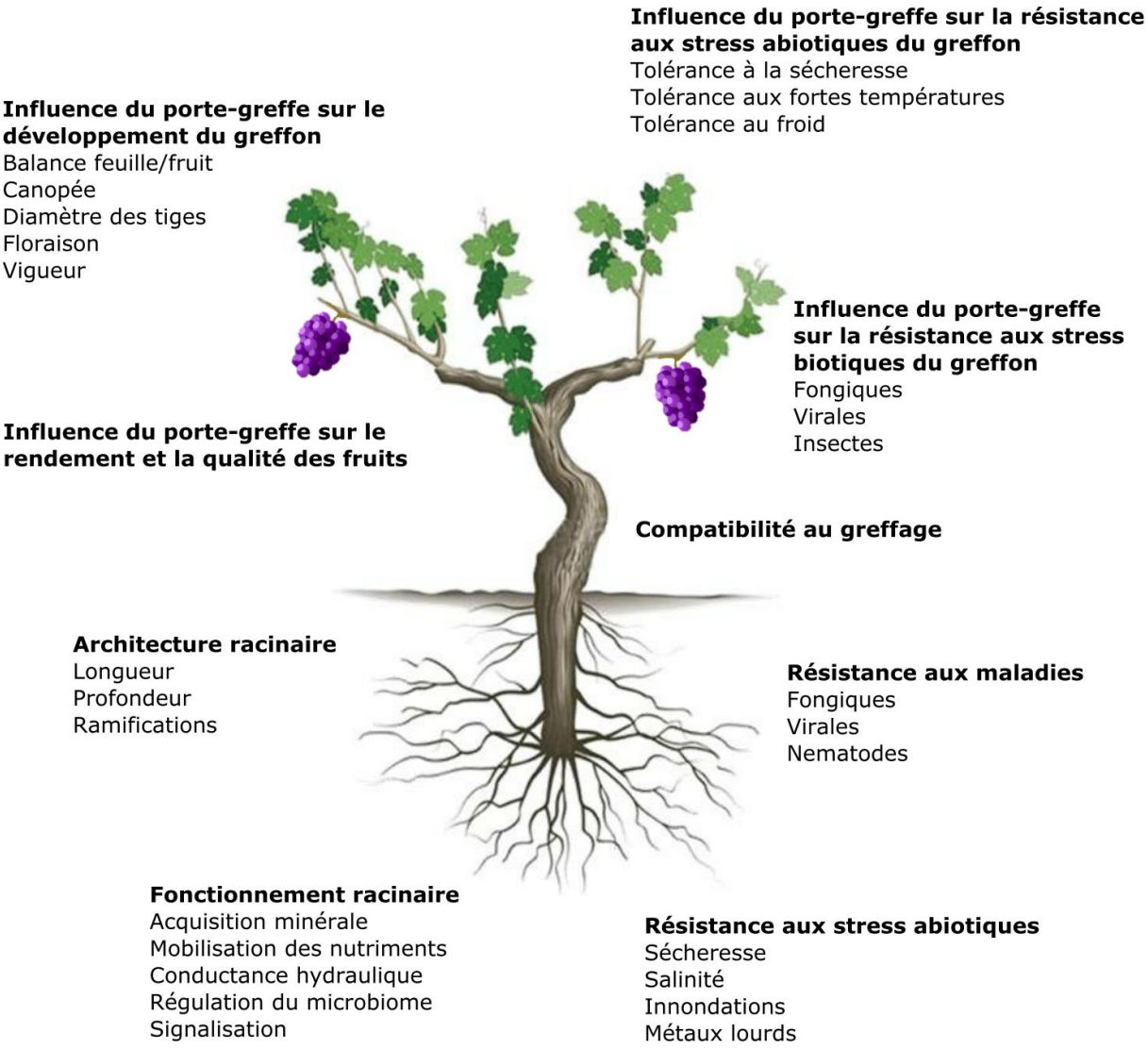

Figure I.5. Influence du porte-greffe sur le greffon en viticulture.

L'influence du porte-greffe sur le phénotype du greffon peut être expliquée par différents mécanismes, décrit par Gautier et al. 2018 (soumis dans *Journal of Experiment of Botany,* disponible en annexe page 194), tels que :

1. Les caractéristiques et le fonctionnement du porte-greffe (e.g. l'architecture du système racinaire, les capacités d'absorption hydrique et minérale, les interactions du système racinaire avec la rhizosphère)
2. L'interface porte-greffe / greffon
3. La régulation par le porte-greffe des signaux entre la partie aérienne et la partie racinaire
4. La perception des signaux environnementaux et climatiques régulant la dormance ou le cycle végétatif par exemple

Chez la Vigne, de nombreuses études montrent l'influence du porte-greffe sur le greffon, en particulier sur la croissance végétative et le cycle reproductif, sur le rendement et la composition des fruits, sur les capacités d'absorption hydrique et minérale. La compréhension de ces mécanismes est nécessaire pour la sélection de génotypes de porte-greffe en fonction du greffon utilisé (compatibilité au greffage), des conditions environnementales et des objectifs de production.

Croissance et développement du greffon

La régulation de la croissance végétative est un facteur crucial pour le viticulteur. En effet un compromis doit être établi entre les développements végétatif et reproducteur de la Vigne, dans le but d'obtenir le rendement souhaité, couplé avec une qualité optimale des baies (Howell, 2001; Jones et al., 2009; Keller, 2015). Chez la Vigne, les porte-greffes sont connus pour modifier la croissance végétative du greffon, évaluée notamment par le poids des bois de taille à la fin du cycle de la plante (Cordeau, 1998; Galet, 1988; Galet and Smith, 1998; Rives, 1971). En considérant l'étude de la vigueur d'un unique greffon, le porte-greffe joue un rôle primordial sur la croissance végétative comme l'ont démontré de nombreuses études. La caractérisation de la croissance aérienne du cultivar *V. vinifera* cv. Colombard greffé sur 25 génotypes différents sur une période de 6 ans en vignoble, montre l'impact du porte-greffe utilisé sur la vigueur du greffon, faisant varier le poids des bois de taille annuel de 1,8 à 0,6 kg (Southey and Jooste, 1991). Des études similaires conduites sur des vignobles âgés (plus de 10 ans) ou sur de jeunes Vignes montrent toutes l'impact du porte-greffe de la Vigne sur les poids de bois de taille du greffon, dans différentes conditions environnementales (Keller et al., 2011; Lecourt et al., 2015; Nikolaou et al., 2000; Ollat et al., 2003b; Ollat et al., 2001; Tandonnet et al., 2011; Wooldridge et al., 2010; Zhang et al., 2016). En général, pour un vignoble de plus de 10 ans, les hybrides *V. riparia*

x *V. rupestris* confèrent une plus faible vigueur que les porte-greffes ayant pour fond génétique *V. berlandieri* (Cordeau, 1998; Galet, 1988; Galet and Smith, 1998).

La croissance aérienne des Vignes dépend de la combinaison des propriétés du greffon et du porte-greffe (Ollat et al., 2003b; Rives, 1971). En conséquence, l'effet propre du porte-greffe sur la vigueur du greffon est difficile à caractériser, celle-ci étant dépendante du génotype du greffon utilisé et de la compatibilité au greffage, donc de l'affinité entre les deux génotypes (Keller et al., 2011; Tandonnet et al., 2008). De plus, l'effet du porte-greffe sur la vigueur du greffon dépend des conditions environnementales et est considéré comme plus important dans des conditions restreintes. De ce fait, l'adaptation du porte-greffe au sol et/ou au climat serait impliquée dans la régulation de la vigueur (Cordeau, 1998). En effet, chez de jeunes Vignes cultivées en conditions contrôlées, les différences de vigueur conférée par le porte-greffe sont faibles, suggérant l'implication d'autres facteurs sont impliqués dans la croissance végétative principalement au cours des premières années, tels que la provenance, la longueur et le diamètre des bois utilisés pour le greffage et donc leurs réserves minérales et carbonées, ainsi que les conditions environnementales (Cochetel et al., 2018; Cookson et al., 2012).

Plus spécifiquement, les porte-greffes n'influencent pas seulement le poids des bois de taille du greffon mais également certains paramètres de l'architecture aérienne de la plante, tels que la surface foliaire ou encore l'émergence de ramifications latérales (Cochetel et al., 2018).

Enfin, l'utilisation de porte-greffes permet la régulation le cycle annuel de Vigne en induisant une précocité ou un retard sur le développement végétatif et reproducteur. Ainsi, la date de véraison de *V. vinifera* cv. Merlot, cultivé dans les mêmes conditions peut varier de plusieurs jours en fonction du porte-greffe utilisé (Ollat et al., 2016). La régulation du cycle de la Vigne est d'un grand intérêt dans le contexte du changement climatique, essentiellement pour l'obtention de raisins de qualité destinés à la vinification.

Rendement et qualité des baies

Au-delà de l'importance du génotype de porte-greffe sur la croissance végétative du greffon, le porte-greffe influence directement ou indirectement le rendement ainsi que la qualité des baies. En effet suivant le génotype choisi, le rendement peut varier jusqu'à 40% (Main et al., 2002). Cet effet sur le rendement est généralement associé à la vigueur végétative, ces deux paramètres étant corrélés positivement chez la Vigne (Jones et al., 2009; Ough et al., 1968; Southey and Jooste, 1991). Le rendement étant directement associé à la qualité des baies, de nombreuses études ont montré l'impact du porte-greffe sur les paramètres de qualité du raisin, notamment sur la taille des baies, la concentration en sucres, en acides organiques, en anthocyanes et sur le pH des moûts (Koblet et al., 1994; Main et al., 2002; Ollat et al., 2001; Walker et al., 1998; Wooldridge et al., 2010). Cependant ces variations ne peuvent être uniquement attribuées au porte-greffe et seraient également influencées par l'environnement et la capacité de la combinaison greffon/porte-greffe à s'y adapter (Jackson and Lombard, 1993), comme l'illustrent les travaux réalisés sur la composition en métabolites primaires et secondaire du raisin en fonction du porte-greffe et de la disponibilité en azote (Habran et al., 2016).

Développement et rôle du système racinaire

Les racines permettent l'ancrage de la plante dans le sol, l'absorption de l'eau et des minéraux et interagissent avec les micro-organismes du sol. La Vigne étant une plante pérenne, le système racinaire est également un organe de réserve pour les éléments carbonés et les nutriments, pouvant être utilisés pour la reprise de la croissance au printemps (Richards, 1983). Chez la Vigne âgée, les racines lignifiées, dont le diamètre est supérieur à 3-4 cm, ont pour rôle d'ancrer la plante dans le sol et de stocker des réserves. Les racines dont le diamètre est inférieur à 1 mm, servent à l'absorption de l'eau et des minéraux. La multiplication végétative de la Vigne par bouturage implique la formation d'un système racinaire complexe, débutant par l'apparition de racines adventives depuis le cambium du bois de la bouture, suivie par l'émergence de racines latérales (Pratt, 1974). Le système racinaire de la Vigne, issus de bouturage ressemble au système racinaire des monocotylédones (Figure I.6A) (Osmont et al., 2007). Cependant, le système racinaire de la Vigne reste atypique car contrairement aux autres espèces, l'initiation des racines latérales ne se limite pas à la zone apicale (Keller, 2015). De plus, la Vigne est une des espèces végétale dont le système racinaire est le plus développé, avec une longueur totale pouvant atteindre 100 km, 100 m² de surface racinaire, afin d'alimenter moins de 10 m² de surface foliaire (Figure I.6B) (Keller, 2015). Le développement et l'architecture du système racinaire sont des paramètres fondamentaux impliqués dans la prospection du sol et donc dans l'acquisition des ressources hydriques et minérales.

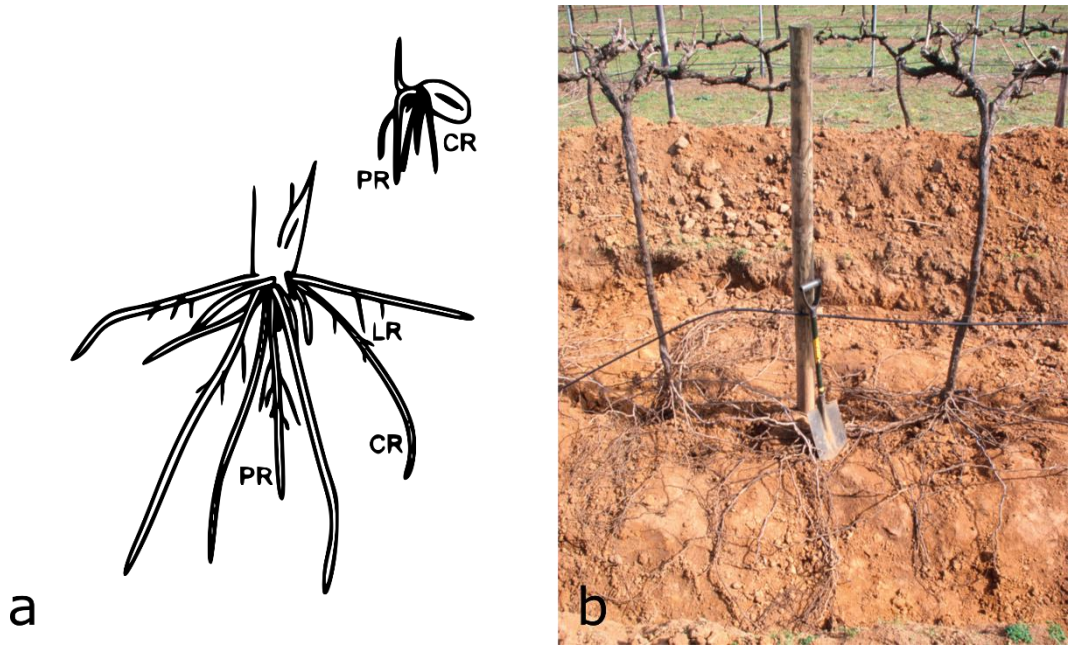

Figure I.6. (a) Représentation d'un système racinaire typique des monocotylédones. PR, racine primaire ; LR, racine latérale ; CR, racine adventive (Osmont et al., 2007). (b) Distribution d'un système racinaire de Vigne au vignoble (Keller, 2015).

Les plantes ont des capacités variables pour prospecter le sol. Ces variations résultent de deux composantes : une première génétique, spécifique au génotype ; une deuxième, environnementale (e.g. structure du sol, disponibilité en eau et/ou en nutriments) modulant la première via la plasticité propre du génotype. Cette dernière semble être plus déterminante sur l'ensemble des caractéristiques du système racinaire (Peret et al., 2011; Rellan-Alvarez et al., 2016). Chez la Vigne, les porte-greffes montrent sur les jeunes plants des différences d'architecture racinaire, principalement en présentant des systèmes racinaires dit « traçant » ou « plongeant ». En effet *V. riparia* présente un système racinaire dont la caractéristique principale est la prospection traçante, contrairement à *V. rupestris* qui lui, montre une stratégie de prospection plutôt plongeante (Smart et al., 2006). Les hybrides semblent hériter des caractéristiques racinaires de leurs parents, comme le montre la Figure I.7 où le 3309C (*V. riparia* x *V. rupestris*, au centre) présente un système racinaire intermédiaire à ses deux parents (Figure I.7).

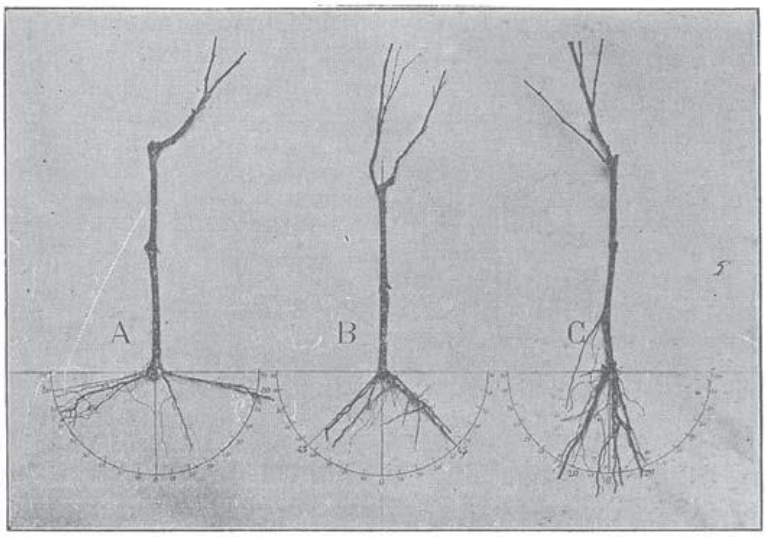

Figure I.7. Angles d'émergence des racines adventives de Vignes, (A) *V. riparia cv.* Riparia Gloire de Montpellier ; (B) *V. riparia x V. rupestris cv.* 3309C ; (C) *V. rupestris cv.* Rupestris du Lot (Guillon, 1905).

Cependant le développement du système racinaire de la Vigne n'est pas seulement lié au génotype de porte-greffe et aux conditions climatiques. En effet, le greffon jouerait un rôle dans le développement racinaire et régulerait la croissance racinaire ainsi que l'allocation de la biomasse à l'échelle de la plante entière (Tandonnet et al., 2010).

Nutrition hydrique

Chez la Vigne, comme chez les autres plantes, l'eau est indispensable aux cycles végétatif et reproducteur, et influence le développement des feuilles, le rendement et la composition de la baie (Keller, 2015). La Vigne est souvent cultivée sans irrigation dans des régions relativement sèches, ce qui peut occasionner des stress hydriques, malgré la forte tolérance de cette plante à la sécheresse (Lovisolo et al., 2010). Cependant, le genre de *Vitis spp.* montre une large variabilité en termes de réponse face au stress hydrique (Chaves et al., 2010), et le porte-greffe est connu pour influencer la tolérance au stress hydrique de la Vigne (Marguerit et al., 2012). Ces réponses pourraient être liées, entre autres, aux voies de la biosynthèse et de signalisation de l'acide abscissique (Ferrandino and Lovisolo, 2014; Hopper et al., 2016). La tolérance au stress hydrique est plus forte chez les porte-greffes issus du croisement *V. berlandieri* x *V. rupestris* (Rossdeutsch et al., 2016).

Nutrition minérale

Comme décrit précédemment, le porte-greffe est responsable de l'acquisition des minéraux dans le sol puis de leur allocation vers le greffon. Agronomiquement, nous savons depuis longtemps que les porte-greffes influencent la composition minérale du greffon et qu'ils possèdent des aptitudes contrastées à assimiler les éléments minéraux (Table I.3) (Bavaresco et al., 2003; Cordeau, 1998). De nombreuses études de l'influence du porte-greffe sur la nutrition minérale du greffon ont été menées. Les concentrations en macro et oligo-éléments dans le greffon varient suivant le porte-greffe utilisé (Brancadoro et al., 1994; Dalbo et al., 2011; Downton, 1977; Ruhl et al., 1988). Les concentrations foliaire et pétiolaire en azote (N), phosphore (P) et potassium (K) de *V. labrusca* cv. Concord sont influencées par le génotype de porte-greffe utilisé (Cook and Lider, 1964). Les concentrations pétiolaires en N, P et K dépendent aussi du greffon mais peuvent varier du double pour N, au triple pour P et K en fonction du porte-greffe et de la combinaison porte-greffe/greffon, (Ibacache G. and Sierra B., 2009).

Porte-greffe	P			K			Mg		
	B	M	F	B	M	F	B	M	F
101-14MGt			*	*				*	
RGM			*			*		*	
SO4	*				*				*
41B	*					*	*		
1103P	*				*		*		
3309C			*			*		*	
5BB	*				*			*	
161-49C		*			*		*		
Fercal	*			*				*	
44-53M		*		*					*
420A		*				*		*	
140Ru		*				*	*		
99R	*			*				*	
110R	*			*					*
Gravesac			*		*			*	
G1	*					*	*		

Table I.3. Aptitudes des porte-greffes de la Vigne à assimiler les différents éléments minéraux (B = bonne, M = moyenne, F = faible). Caractéristiques définies à partir d'une quinzaine de parcelles du vignobles bordelais établies sur différents types de sol, avec pour greffons différents cépages, à partir de diagnostics pétiolaires à la véraison. Reproduit à partir de Cordeau (1998).

De nombreux autres facteurs tels que le greffon, l'âge de la plante, le stade phénologique, le climat et surtout le type sol ainsi que sa disponibilité en nutriments, affectent la nutrition minérale (Bavaresco et al., 2003; Benito et al., 2013; Dominguez et al., 2015; Ibacache G. and Sierra B., 2009; Wooldridge et al., 2010; Zamboni et al., 2016). Ainsi, Lecourt et al. (2015) ont démontré l'influence de la disponibilité en azote sur les concentrations des autres éléments minéraux dans les racines et les feuilles de la Vigne, avec un effet porte-greffe marqué.

Des études suggèrent des capacités d'acquisition différentes suivant les génotypes de porte-greffe étudiés, pour N (Keller et al., 2001; Zerihun and Treeby, 2002), pour K (Avenant et al., 2017; Mpelasoka et al., 2003; Wolpert et al., 2005) et pour P (Grant and Matthews, 1996a), ou des différences dans leur capacité à allouer les nutriments vers la partie aérienne (Lecourt et al., 2015). Les porte-greffes semblent également avoir des réponses contrastées à la disponibilité en nutriments dans le sol. Cela a été démontré pour N (Cochetel et al., 2017; Cochetel et al., 2018; Lecourt et al., 2015), K (Ruhl, 1989; Ruhl, 1991), P (Grant and Matthews, 1996a; Grant and Matthews, 1996b) ou encore Fe (Covarrubias et al., 2016; Covarrubias and Rombolà, 2015).

Le fond génétique du porte-greffe influence la concentration pétiolaire en P du greffon chez la Vigne.

Avant-propos

Bien que l'influence du porte-greffe sur la nutrition minérale du greffon ne soit plus à démontrer, la compréhension des mécanismes régulant ces observations reste faiblement décrite. Face à l'impact des autres composantes (greffon, combinaison, sol, etc.), il est difficile d'établir une tendance générale de l'influence du fond génétique des porte-greffes sur la nutrition minérale du greffon.

Afin de compléter les connaissances scientifiques de l'impact du porte-greffe sur la nutrition minérale du greffon, tout en limitant l'influence du sol et du climat, nous avons étudié la composition minérale d'un même greffon en fonction du porte-greffe sur dispositif expérimental GreffAdapt. Cette parcelle, plantée en 2015, est située sur le domaine de la Grande-Ferrade (Bordeaux). Elle est constituée de 55 génotypes de porte-greffes en combinaison avec 5 variétés de *V. vinifera*. Le dispositif est défini selon 3 blocs (classés par tests de résistivité du sol), comprenant chacun 5 répétitions des combinaisons greffon/porte-greffe, ce qui en fait un dispositif expérimental idéal afin d'étudier et de comparer l'impact du porte-greffe sur le greffon au vignoble.

L'étude suivante porte sur l'analyse minérale de pétioles de *V. vinifera* cv. Cabernet Sauvignon greffé sur 13 génotypes de porte-greffes, sélectionnés en fonction de leur fond génétique, permettant ainsi une représentation des hybrides les plus couramment utilisés en viticulture provenant du croisement des espèces américaines *V. riparia, V. rupestris* et *V. berlandieri*.

Les résultats obtenus ont permis la rédaction d'un article scientifique actuellement soumis à *Australian Journal of Grape and Wine Research,* mettant en évidence l'impact du fond génétique des porte-greffes de la Vigne sur la composition minérale du greffon et plus particulièrement sur la nutrition phosphatée.

Article 1 : Petiole phosphorus concentration is controlled by the rootstock genetic background in grapevine

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Abstract

Aims: Grapevine, *Vitis vinifera*, requires grafting on Phylloxera tolerant rootstocks of American origin in most viticultural areas of the world. The most commonly used species in rootstock creation are *V. berlandieri*, *V. riparia* and *V. rupestris*. Rootstocks not only provide tolerance to Phylloxera but assure the supply of water and mineral nutrients to the scion. The objective of this work was to determine to what extent rootstocks of different parentages alter the mineral composition of petioles of grapevine.

Methods: *Vitis vinifera* cv. Cabernet Sauvignon was grafted onto 13 rootstock genotypes and planted in a vineyard in two blocks. The concentration of 13 mineral elements was determined in the petioles at veraison (berry softening).

Results: Parentage of rootstocks has a significant effect on petiole mineral composition. Rootstocks with at least one *V. riparia* parent reduced the concentration of phosphorus and increased the concentration of manganese and sulphur in the petiole of Cabernet Sauvignon.

Conclusion: Rootstocks with a *V. riparia* parent generally confer low scion vigour and we have shown that they also confer low petiole phosphorus concentration; this could suggest that phosphorus uptake and use is related to rootstock conferred vigour in grapevine.

Keywords: Rootstocks, mineral element, phosphorus, grapevine, *Vitis spp.*, nutrient availability

Introduction

Grafting is a horticultural technique that has been used millennia (Mudge et al., 2009) and still practiced today for the cultivation of perennial fruit crops (*e.g.* citrus, apple, peach, cherry and grape), and annual fruits and vegetables (*e.g.* watermelon, tomatoes, etc). This technique allows us to combine desirable traits of two different genotypes in the same plant. The use of rootstocks affects plant vigour, phenology, resistance to pests, fruit quality, yield and tolerance to deleterious environmental conditions such as water deficit and nutrient limitations (Warschefsky et al., 2016).

The accidental introduction of the American aphid pest, Phylloxera, to Europe in the 19th century caused the near destruction of the European vineyard because the lack of tolerance roots of *Vitis vinifera*, the Eurasian grapevine species. Scientists at the time rapidly realised that naturally resistant *Vitis spp.* of North American origin was the solution to this crisis. To maintain the production of wine in Europe and to cater to consumer taste expectations, *V. vinifera* was grafted onto American *Vitis spp.* to combine the fruit quality with the phylloxera tolerance. There are approximately 30 different American *Vitis spp.*, however only a limited number have been used to breed rootstocks. In fact, most rootstocks used in viticulture are the result of breeding between three different American species of *Vitis spp.*: *V. riparia*, *V. rupestris* and *V. berlandieri* coming from different geographic area (Figure II.1). From the database Vitis International Variety Catalogue (http://www.eu-vitis.de), 47% of the 83 rootstocks used in Europe resulted from interspecific crosses of *V. berlandieri*, *V. riparia* and *V.* rupestris. In addition, all rootstocks used in Europe have in their genetic background at least one of these three *Vitis spp.* (47% with *V. berlandieri*, 52% with *V. riparia* and 30% with *V. rupestris*). Furthermore, the potentially high genetic variability of American *Vitis spp.* is poorly exploited because it is estimated than 90% of *V. vinifera* in the world are grafted onto less of 10 different rootstock genotypes. (Galet, 1988; Huglin and Schneider, 1998; Keller, 2015; Ollat et al., 2016). As such, there is considerable scope to improve current grapevine rootstock breeding programmes, not only to select for tolerance to Phylloxera, but also for a variety of other agronomical traits, *e.g*. adaptability to soil properties and/or environmental conditions, resistance against other soil pathogens and ability to control scion vigour (as defined by the extent of shoot growth) (Keller, 2015; May, 1994). Roots acquire mineral nutrients, anchor plants in the ground, acquire water from the soil and interact with soil organisms. Plants require at least 14 mineral elements for adequate development and these elements are generally taken up by the roots from the soil solution (Marschner, 2011). These elements include the macronutrients nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S), and the micronutrients chlorine (Cl), boron (B), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), nickel (Ni) and molybdenum (Mo). These elements are required in different proportions, but

deficiency or excess (toxicity) in any of these elements reduces plant growth and crop yields. Numerous reviews have highlighted that plant mineral homeostasis is highly regulated and that the metabolism of different elements is interconnected (Amtmann and Armengaud, 2009; Williams and Salt, 2009)).In addition, in perennial species, the root system is an organ of storage of carbohydrates and other nutrients that can be used to supply the resumption of growth in the spring (Richards, 1983).

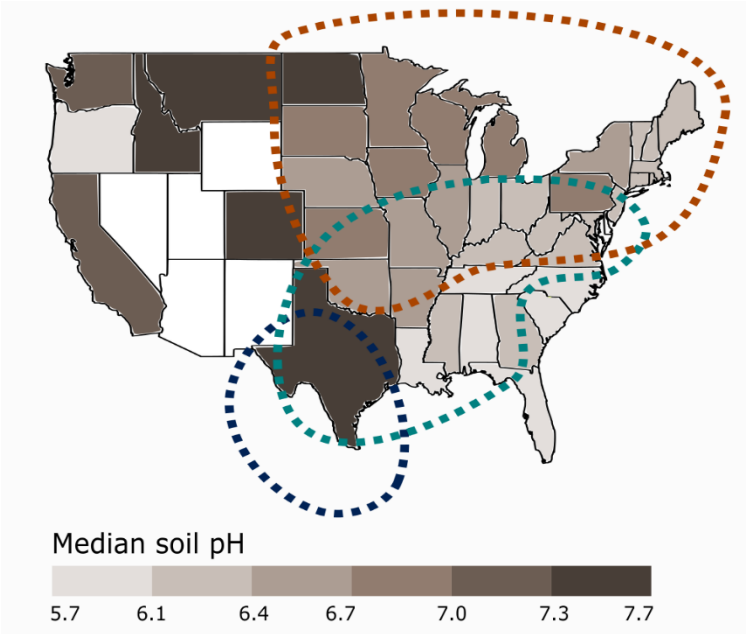

Figure II.1. Median soil pH of USA values calculated in 2010 from 4.3 million samples (http://www.cropnutrition.com/efu-soil-ph); and geographic origin of *V. riparia* (brown), *V. rupestris* (turquoise), and *V. berlandieri* (dark blue) (Galet, 1988; Galet and Smith, 1998).

Differences in plant mineral composition may be due to genotypic factors such as differences in root nutrient and/or water uptake capacities, mobilisation of stored nutrient reserves, allocation within the plant and nutrient use and/or growth (which affect mineral element concentration via dilution). The ability of a root system to take up nutrients and/or water is also related to the development of root system, its distribution in the soil (shallow or deep exploration) and the interaction with the rhizosphere (Dakora and Phillips, 2002; Lambers et al., 2006). However, plant mineral composition is also affected by external factors, mainly by soil structure and composition, nutrient availability and climatic conditions. Plants are known to modify pH of the rhizosphere and to release several compounds whose affect microbial activity and/or nutrient availability in the soil (e.g. hormones,

carboxylates, enzymes…). Consequently, plant mineral composition is related to genotype-environment interactions and the relative plasticity of plant to nutrient availability.

In many grafted crops, the vegetative growth and the mineral composition of the scion are strongly affected by the rootstock (as reviewed recently by Nawaz et al 2016). In grapevine, rootstocks have long been known to modify the mineral element profile of the scion (Bavaresco et al., 2003; Cordeau, 1998). Ibacache and Sierra (2009) studied N, P and K concentration in the petioles of four scion cultivars (*V. vinifera)* grafted onto 10 different rootstocks. They demonstrated an effect of the rootstock on the N, P and K content in the scion. More specifically, several studies shown differences in response of grapevine rootstocks to nutrient supply, such as N (Cochetel et al., 2017; Lecourt et al., 2015), P (Gautier et al., 2018; Grant and Matthews, 1996a; Grant and Matthews, 1996b), K (Ruhl, 1989; Ruhl, 1991) or Fe (Covarrubias et al., 2016; Covarrubias and Rombolà, 2015). These studies have demonstrated that grapevine rootstocks with different genetic background shown different responses to low nutrient supply using few mechanisms such as nutrient acquisition and/or use, nutrient allocation and rhizosphere modification

Frequently in viticulture, the mineral status of grapevines is assessed using petiole elemental composition analyses (Cordeau, 1998; Dalbo et al., 2011; Ibacache G. and Sierra B., 2009). Although there is considerable agronomic knowledge of rootstock adaptability to different soil types and anecdotal evidence that the parentage of the rootstock genotype alters scion mineral concentration, this has not been clearly demonstrated in grapevine. The objective of this work was to determine to what extent rootstocks genetic background alter the elemental composition of petioles of grapevine.

Materials and Methods

Vineyard site and experimental design

Vitis vinifera cv. Cabernet Sauvignon clone 169 was omega grafted onto 13 rootstock genotypes in 2014 and then grown in a nursery for one year. The experimental vineyard was planted in 2015 with two blocks of five grapevines per block for each rootstock genotype. The rootstocks and their parentage are given in Table II.1.

The experiment was undertaken during the 2017 season in an experimental sandy gravelly vineyard located near Bordeaux, France (44°47N, 0°34W, elevation 22 m) with a row spacing of 1.5 x 1 m (6666 Vines/ha). Vines were winter pruned to 3-5 buds per vine on Guyot simple and trained to a vertical trellis system.

Rootstock	Abbreviation	Crossbreeding	Parenting class	Vigour conferred
Riparia Gloire de Montpellier	RGM	*V. riparia*	Riparia	Weak
3309 Couderc	3309C	*V. riparia* tomenteux × *V. rupestris* Martin	Riparia - Rupestris	Weak - medium
101-14 Millardet et de Grasset	101-14MGt	*V. riparia* × *V. rupestris*	Riparia - Rupestris	Medium
420 A	420A	*V. berlandieri* × *V. riparia*	Riparia - Berlandieri	Weak - medium
Teleki n°4 -SO4-	SO4	*V. berlandieri* × *V. riparia*	Riparia - Berlandieri	Weak - medium
44-53 Mallègue	44-53M	*V. riparia* cv. Grand glabre × 144 Malègue (*V. cordifolia* × *V. rupestris*)	Riparia - Other	Medium
Gravesac	Gravesac	161-49 Couderc (*V. riparia* × *V. berlandieri*) × 3309 Couderc	Riparia - Other	Medium - Vigorous
Freedom	Freedom	1 613 Couderc (*V. longii* × Othello) × Dog Ridge	Other	Vigorous
Dog ridge	Dog ridge	*V. rupestris* Scheele × *V. candicans* Engelmann	Other	Higly vigorous
41 B Millardet et de Grasset	41B	*V. vinifera Chasselas* × *V. berlandieri*	Other	Medium - Vigorous
Rupestris du Lot	Rupestris	*V. rupestris*	Rupestris	Vigorous
1 103 Paulsen	1103P	*V. berlandieri* Rességuier n°2 × *V. rupestris* du Lot	Berlandieri - Rupestis	Higly vigorous
110 Richter	110R	*V. berlandieri* Rességuier n°2 × *V. rupestris* Martin	Berlandieri - Rupestis	Vigorous

Table II.1. The rootstocks used in this study. The full name, abbreviation, parentage (Galet, 1988) and vigour conferred to scion (Bettiga, 2003; Cordeau, 1998) for each rootstock are provided.

The canopy management practices included manual shoot thinning before the onset of flowering and shoot hedging and basal leaf removal at veraison. Canopy height and width were on average about 1.6 and 0.4 m respectively. The soil at the site was a gravelly sandy soil. The cation exchange capacity of the soil was on average 3.2 $cmol^+/kg$ and the available water capacity was 0.85 mm/cm. The P content was 0.015%, which is a medium P content often found in vineyards. The soil was not tilled during the experiment; spontaneous weeds between rows and in the vine row were controlled by mowing several times a year, more information on soil profile is given in table II.2.

Soil characteristics	
Density (T/m3)	1.7
Clay % (< 2 µm)	7
Fine silt % (2-20 µm)	7.2
Coarse silt % (20-50 µm)	5.3
Fine sand % (50-200 µm)	12.6
Coarse sand % (200-2000 µm)	67.9
Nutritional statut	
Organic matter (%)	1.765
Total nitrogen (%)	0.079
C/N ratio	12.8
P_2O_5 (g/kg) - Joret Hébert	0.1485
K_2O (g/kg)	0.077
MgO (g/kg)	0.0325
Mn (mg/kg)	3.41
Chemical characteristics	
pH	5.8
pH KCl	4.75
CaO (g/kg)	0.43
Cation Exchange Capacity (cmol+/kg)	3.2

Table II.2. Properties of the vineyard soil used in the experiment.

Growth measurements

Annual growth of each vine was evaluated by quantifying winter cane pruning weight at the end of 2017, after defoliation.

Mineral analysis

Four petioles were harvested from near the clusters from 2 plants for each block (n = 4 per rootstock genotype) at veraison (berry softening, 14/08/2017); and were dried (in an oven at 60°C until they reached a constant mass). The mineral composition of the samples was analysed by Waypoint Analytical (Richmond, VA, USA). Nitrogen content was determined using a Leco FP-528 instrument (LECO, St. Joseph, MI, USA). Other element contents were determined by digesting the plant sample with nitric acid and hydrochloric acid in a CEM Mars5 microwave digester (CEM, Matthews, NC, USA), elemental concentration was determined by reading the solutions on an ICP-OES MS 730-ES (Varian, Palo Alto, CA, USA).

Statistical analysis

Rootstock effects on shoot growth and petiole nutrient concentrations were determined using a one-way analysis of variance (ANOVA $p < 0.05$, with Tukey's Honest Significant Difference test) if the normality of residuals and the homogeneity of variances were respected (cane pruning, petiole N, K, S, Ca, Cu and Al concentrations); or on the other hand, using a Kruskal Wallis test ($p < 0.05$) (petiole P, Mg, Na, B, Zn, Mn and Fe concentrations) , using R statistics environment (R Development Core Team 2005). The effect of *V. riparia*, *V. rupestris* and *V. berlandieri* parents on mean petiole mineral concentrations, were tested with t-tests that 5 % level with a Bonferroni multiple testing correction, using R statistics environment (R Development Core Team 2005). Principal component analysis was performed on Pearson correlations of row data using FactoMineR package on R statistics environment (R Development Core Team 2005).

Results

Differences in petiole mineral element composition induced by rootstocks are not related to rootstock conferred vigour

The petiole elemental composition analysis was done on Cabernet Sauvignon from a 4-year old experimental vineyard, at this developmental stage there were no differences in rootstock conferred vigour, *i.e.* the rootstock genotypes did not affect growth evaluated by cane pruning weight (Figure II.2).

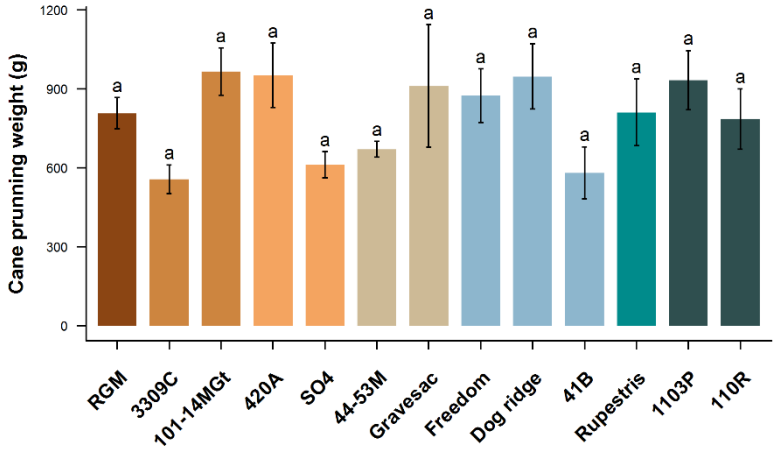

Figure II.2. Winter cane pruning weight from 2017 of *Vitis vinifera* cv. Cabernet Sauvignon grafted onto 13 rootstocks. Full names for each rootstock are given in Table II.1. Bar colour indicates the parentage of the rootstock: brown for *V. riparia*; dark orange for hybrids of *V. riparia* and *V. rupestris,* pale orange for hybrids of *V. riparia* and *V. berlandieri*; sand beige for other hybrids containing *V. riparia*; light blue for hybrids of other *Vitis spp.,* turquoise for *V. rupestris* and dark blue for hybrids of *V. berlandieri* and *V. rupestris*. Means and standard deviations shown (n = 4). Different letters indicate significant differences at $P < 0.05$, tested using one-way ANOVA with rootstock genotype as factor.

However, the concentration of some minerals was affected by the rootstock genotype. Of the three major mineral elements, P was the only major mineral element to vary considerably between the rootstock genotypes (Figure II.3); varying 4-fold between the lowest (RGM) and highest (110 R and Dog Ridge) concentrations. The P concentration in the petiole appeared to be related to the parentage of the rootstock genotype, being lower when the rootstock used had a *V. riparia* parentage and highest in hybrids of *V. berlandieri* and *V. rupestris* (Figure II.3b). Potassium concentration was also affected by rootstock genotypes (Figure II.3c) with a variation 2-fold between the lowest (1103P) and the highest (Dog Ridge). Petiole N concentration was largely unaffected by the rootstock genotype (Figure II.3a). Other macro-nutrients also showed variations between the rootstock genotypes (Figure II.4).

Magnesium was the most affected in concentration across the different rootstock genotypes, varying over 3-fold between the lowest (44-53 M) and highest (Gravesac) concentrations (Figure II.4b). The concentration of S in the petioles of grapevines grafted with the different rootstocks studied varied 2-fold between the highest and lowest concentrations (Figure II.4a). Finally, calcium and sodium concentrations were less affected by rootstocks (Figure II.4c and II.4d).

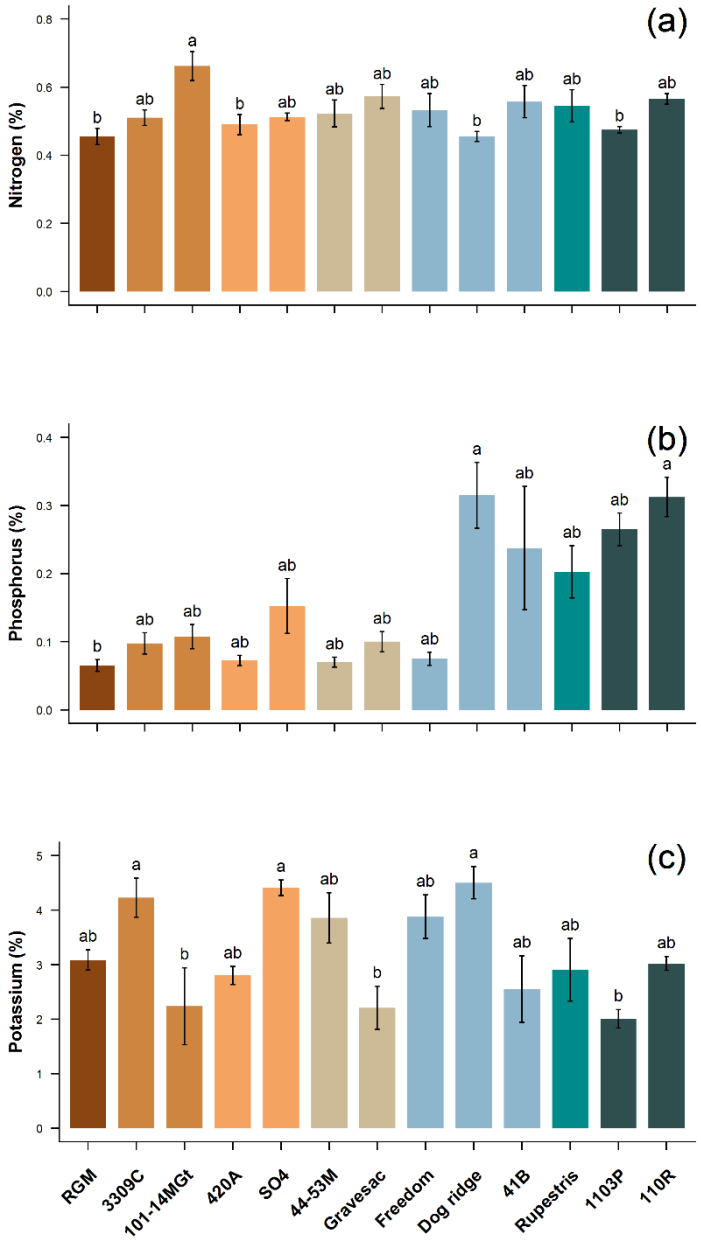

Figure II.3. The concentration of (a) nitrogen, (b) phosphorus and (c) potassium in the petioles of *Vitis vinifera* cv. Cabernet Sauvignon grafted onto 13 rootstocks. Full names for each rootstock are given in Table II.1. Bar colour code is the same as described in Figure II.2. Means and standard deviations shown (n = 4). Different letters indicate significant differences at $P < 0.05$, tested using one-way ANOVA (for N and K) or Kruskal Wallis test (for P); with rootstock genotype as factor.

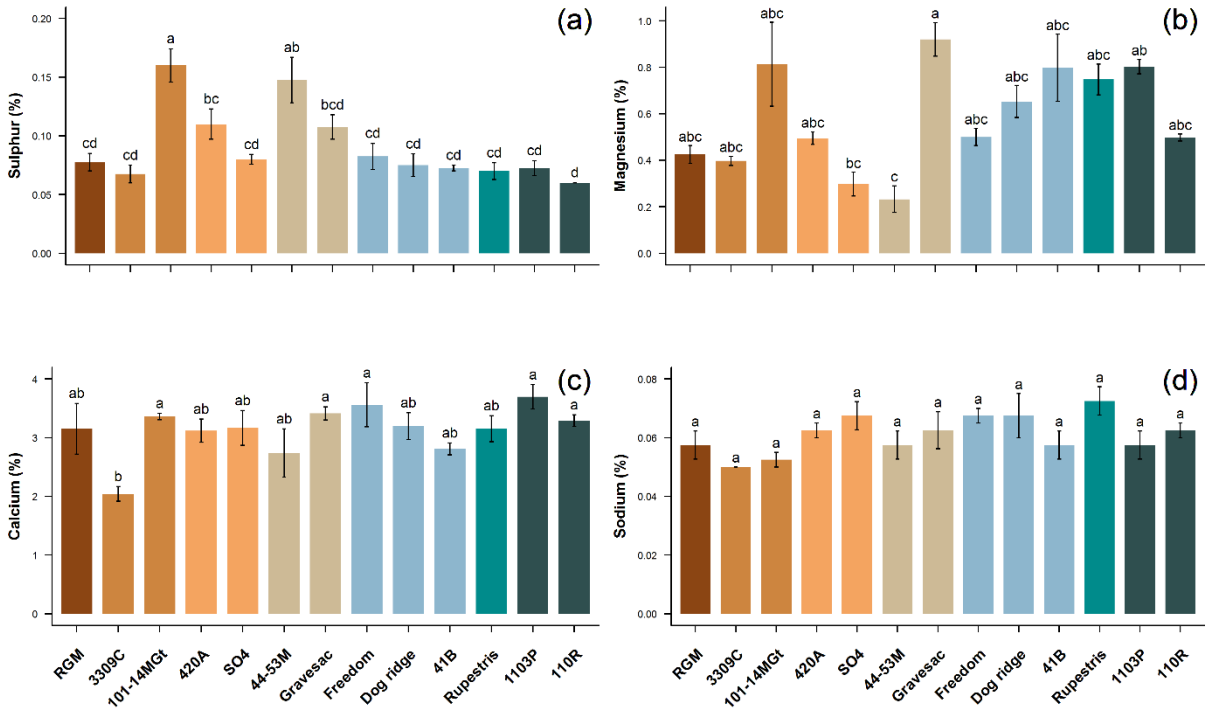

Figure II.4. The concentration of (a) sulphur, (b) magnesium, (c) calcium and (d) sodium in the petioles of *Vitis vinifera* cv. Cabernet Sauvignon grafted onto 13 rootstocks. Full names for each rootstock are given in Table II.1. Bar colour code is the same as described in Figure II.2. Means and standard deviations shown (n = 4). Different letters indicate significant differences at $P < 0.05$, tested using one-way ANOVA (for S and Ca) or Kruskal Wallis test (for Mg and Na); with rootstock genotype as factor.

The concentration of microelements in the petiole was also affected by the rootstock genotype, but there was no clear relationship between the parentage of the rootstock and microelement concentration (Figure II.5). Manganese and iron were the most affected oligo-nutrients by the rootstock genotype, varying over 10-fold and 3-fold between the highest and the lowest values respectively (Figure II.5c and II.5d).

Taken together these results suggest that in young grapevines, differences in petiole mineral content can appear without differences in rootstock conferred vigour.

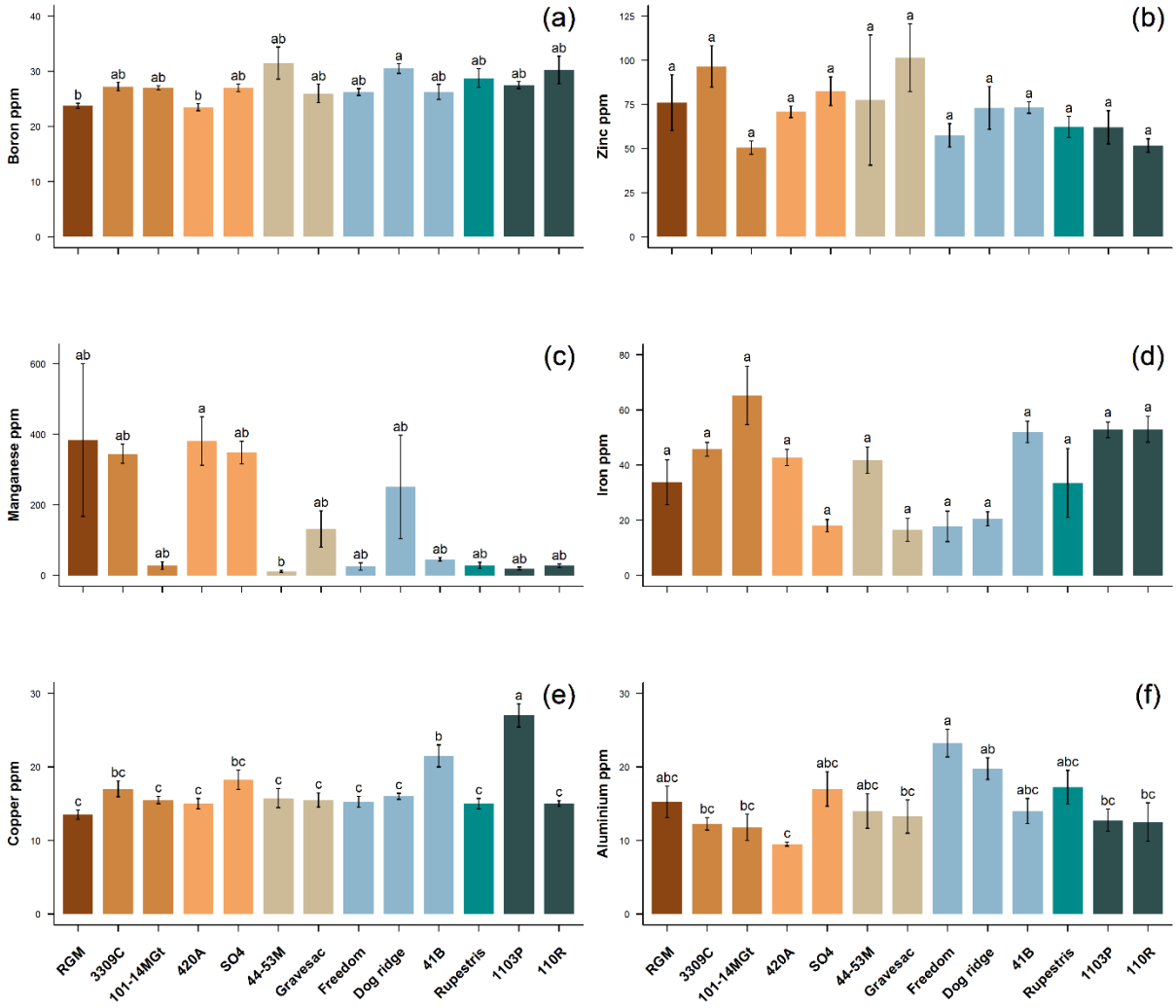

Figure II.5. The concentration of the microelements (a) boron, (b) zinc, (c) manganese, (d) iron, (e) copper and (f) aluminium in the petioles of *Vitis vinifera* cv. Cabernet Sauvignon grafted onto 13 rootstocks. Full names for each rootstock are given in Table II.1. Bar colour code is the same as described in Figure II.2. Means and standard deviations shown (n = 4). Different letters indicate significant differences at $P < 0.05$, tested using one-way ANOVA (for Cu and Al) or Kruskal Wallis test (for B, Zn, Mn and Fe); with rootstock genotype as factor.

Rootstock parentage induces different mineral concentration profile of the scion petiole

Mineral concentration data were studied in a principle component analysis. The first two principle components, PC1 and PC2, explained 20.6 and 16.5 % of total variability respectively (Figure II.6). The concentration of K and Mn was strongly negatively correlated to the PC1, whereas the concentration of Mg was positively correlated to the PC1. Furthermore, the concentration of P was strongly positively and the concentration of S was strongly negatively correlated with PC2. Principle component 2 generally separated the rootstocks based on the presence or absence of a *V. riparia* parent, with rootstocks containing *V. riparia* being on the negative side of PC2 and rootstock without *V. riparia* parent on the positive side. The ellipses of confidence around each rootstock parent also separated the rootstocks based on the presence or absence of a *V. riparia* parent.

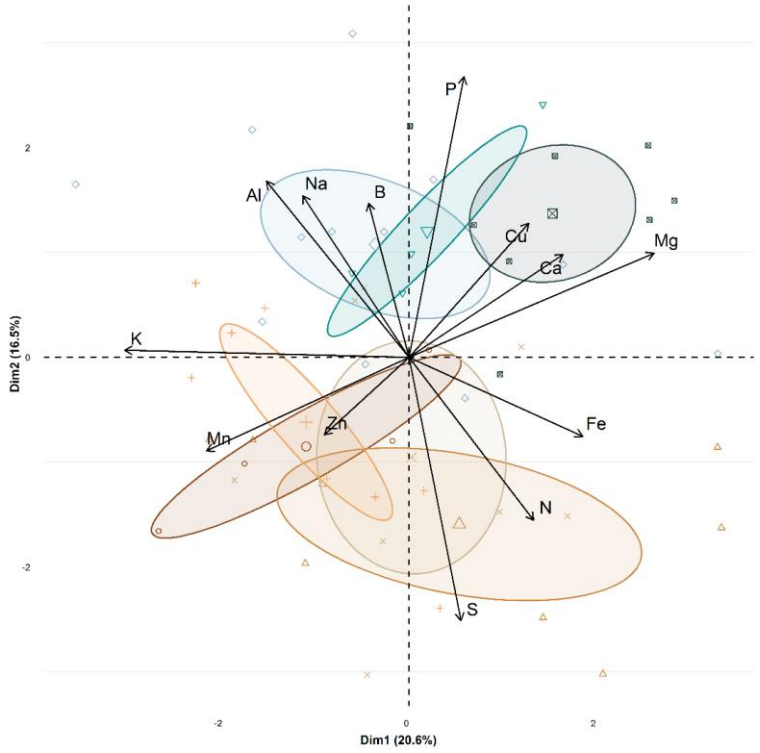

Figure II.6. Principal component (PC) analysis of the concentration of minerals in the petiole of *Vitis vinifera* cv. Cabernet Sauvignon grafted onto 13 rootstocks. The distribution of variables (mineral concentrations given by arrows) and individual observations (symbols) on PC1 and PC2 are given. Symbols and colour indicates the parentage of the rootstock: brown circles for *V. riparia*; dark orange triangles for hybrids of *V. riparia* and *V. rupestris,* pale orange pluses for hybrids of *V. riparia* and *V. berlandieri*; sand beige crosses for other hybrids containing *V. riparia*; light blue diamonds for hybrids of other *Vitis spp.,* turquoise inverted triangles for *V. rupestris* and dark blue filled squares for hybrids of *V. berlandieri* and *V. rupestris*. Ellipses of confidence at the 95 % level are given for each rootstock parentage.

Pair-wise comparisons of the effect of V. riparia, V. rupestris and V. berlandieri parents on petiole mineral concentrations

The effect of the most commonly used species in rootstock creation*, V. riparia, V. rupestris* and *V. berlandieri*, on petiole mineral composition was studied by comparing the concentration of each mineral in rootstocks with or without each of these species (Table II.3). Rootstocks with a *V. riparia* parent conferred a lower concentration of petiole P, but a higher concentration of petiole S and Mn. Rootstocks with a *V. rupestris* parent decreased the concentration of Mn and increased the concentration of B in the petiole, whereas rootstocks with a *V. berlandieri* parent did not alter the concentration of any mineral elements in the petiole.

Discussion

Rootstocks alter petiole mineral concentration

It is well known that in many grafted cultivated species, the mineral nutrition is altered by the rootstock genotype (Nawaz et al., 2016). In our study the mineral composition of Cabernet Sauvignon petioles was effected by the rootstock; this is in agreement with the literature (Cordeau, 1998; Ibacache G. and Sierra B., 2009). The petiole mineral composition could discriminate the different rootstock parentages; this analysis grouped together those rootstocks with and without a *V. riparia* parent. This is the first time that the parentage of rootstock has been linked to its scion mineral content, although similar studies have been done in ungrafted plants for variables associated with drought tolerance (Rossdeutsch et al., 2016) and conferred vigour (Jones et al., 2009).

	N	P	K	S	Mg	Ca	Na	B	Zn	Mn	Fe	Cu	Al
V. riparia (n=28)	0.532	0.095	3.261	0.107	0.511	2.997	0.058	26.57	79.32	232.35	37.67	15.78	13.28
Other (n=24)	0.521	0.234	3.145	0.072	0.660	3.282	0.064	28.25	63.29	65.79	38.25	18.29	16.58
t-test		*		*						*			
V. rupestris (n=36)	0.537	0.171	3.205	0.093	0.618	3.160	0.061	28.33	70.27	96.05	38.52	16.88	15.19
Other (n=16)	0.503	0.131	3.123	0.085	0.503	3.059	0.061	25.12	75.62	289.18	36.62	16.88	13.93
t-test								*		*			
V. berlandieri (n=24)	0.528	0.190	2.833	0.083	0.635	3.247	0.061	26.75	73.62	158.45	39.16	18.70	13.16
Other (n=28)	0.526	0.133	3.529	0.097	0.538	3.027	0.060	27.85	70.46	152.92	36.89	15.42	16.21
t-test													

Table II.3. The effect of *V. riparia*, *V. rupestris* and *V. berlandieri* parents on mean petiole mineral concentrations in the scion, tested using a student-test at the 5 % level with the Bonferroni correction.

Rootstocks with a V. riparia parentage confer reduced petiole P concentration

The presence of a *V. riparia* parent in a rootstock reduced the concentration of P in the petiole of the scion. Agronomic studies have previously shown that *V. riparia* cv. RGM reduces petiole P concentrations (Cordeau, 1998). Experiment in pots have also shown RGM reduces shoot P concentration (except under very high N supply) in comparison to the *V. rupestris* x *V. berlandieri* hybrid cv. 1103P when grafted with Cabernet Sauvignon (Lecourt et al., 2015). In ungrafted cuttings, we have previously shown that RGM is less efficient in P uptake and P remobilisation from perennial woody parts during first stages of grapevine development compared to the 1103P (Gautier et al., 2018). In our present study, we have shown that the presence of only one *V. riparia* parent also confers this low P concentration phenotype. Differences in P acquisition and use between *V. riparia* and others *Vitis spp*. could be explained by their geographic origin. *Vitis riparia* as a large geographic range, being found across most of North America, in comparison, other *Vitis spp.* used as for rootstock breeding such as *V. rupestris*, *V. berlandieri*, *V. cordifolia*, *V. longii* and *V. candicans,* which have a more limited geographic range (Galet, 1988). *Vitis rupestris* and *V. berlandieri* are native from the south of USA (Galet, 1988), in particular from Texas, where soil is calcareous and often deficient in P due to the precipitation of calcium phosphate (McLean, 1973). These species may have acquired efficient mechanisms to increase P acquisition or use in response to the limited P environment.

Petiole P concentration was correlated with known vigour conferred by the rootstocks

Phosphorus is an essential element for plant growth (Bieleski, 1973), involved in many fundamental processes including photosynthesis, biosynthesis and respiration because of its role in energy generation via adenosine triphosphate. In addition, P has structural role for phospholipids and phosphate esters (Marschner, 2011). A reduction of growth of the shoot is a typical response to P starvation in plants (Hermans et al., 2006; Vance et al., 2003). Agronomic studies have previously shown that grapevine rootstocks known to confer high vigour to scion, confer also high P concentration in petiole scion at veraison in a Bordeaux vineyard (Cordeau, 1998). However, these data did not consider the age of plants, soil characteristics or agricultural practises. In our study, no differences in conferred vigour was observed for the reasons described above. However, rootstocks known to confer low vigour to the scion in a mature vineyard (with a *V. riparia* parent) confer also lower P concentration to the scion compared to *V. rupestris* and *V. berlandieri* hybrids.

Rootstocks with a V. riparia parentage have increased of S in the petiole

In addition to reducing petiole P concentration, rootstocks with at least one *V. riparia* parent also increased S concentrations in the petiole. The interaction between P and S nutrition is well known, under P deficiency phospholipids are replaced by sulfolipides, and conversely, under S deficiency sulfolipides are replaced by phospholipids (Essigmann et al., 1998; Härtel et al., 1998; Sugimoto et al., 2007; Yu et al., 2002). We have previously shown the P acquisition efficiency of cuttings of RGM is lower than that of 1103P (Gautier et al., 2018), which could suggest that *V. riparia* rootstocks are less able to take up P from the soil, which could induce a compensatory increase in S uptake and transport to the scion.

The concentration of Mn in petioles increased or decreased with rootstocks of a V. riparia or V. rupestris parentage respectively

Mn and P concentrations were negatively correlated, and rootstock parentage could be linked with Mn concentration. Rootstocks with a *V. riparia* parentage confer increased petiole concentrations of Mn, while rootstocks with a *V. rupestris* parentage decreased it. Concentration of Mn in petiole could be directly related to P status. Despite the medium P concentration for a vineyard in our experimental system (0.15 g/kg), grapevines grafted on a rootstock with a *V. riparia* genetic background had a lower P petiole concentration (0.095% P/DW) than would be expected for a “good nutritional status” of grapevine at berry ripening, which is normally between 0.1 and 0.18% P/DW (Delas, 2010). As described previously, these lower P concentrations could be related to the poor P acquisition efficiency on *V. riparia* (Gautier et al., 2018). In response to low P concentration in shoot, plants can release carboxylates to the rhizosphere (Vance et al., 2003). A study of 13 crop species grown under low P availability showed that species with a low tissue P concentration generally released more carboxylates to the rhizosphere than those with a high tissue P concentration (Pearse et al., 2006). The carboxylates released can mobilise P sorbed onto soil particles with Fe and Al at acid pH, or with Ca at alkaline pH. However, carboxylates do not only affect only P availability and also mobilise some other cations in the rhizosphere such as Fe, Zn and Mn (Lambers et al., 2015b). All these micronutrients could be taken up by root, by a nonspecific transporter, such as an iron-regulated transporter (Lambers et al., 2015b; Xiao et al., 2008). In our case, high petiole Mn concentration conferred by rootstocks with a *V. riparia* background, could be related to the poor P acquisition efficiency and the subsequent induction of carboxylates release. This hypothesis is supported by a higher organic acids concentration of roots of 3309C (a *V. riparia* x *V. rupestris* hybrid) than 110R (a *V. rupestris* x *V. berlandieri* hybrid) under high Fe supply (Covarrubias et al., 2016).

Conclusion

Grapevine rootstocks have long been known to modify the mineral profile of the scion, but mechanisms involved were poorly understood. For the first time, the capacity of different rootstocks to alter scion petiole concentration was associated with the parentage of the rootstock genotype. Phosphorus was the major mineral element differentially accumulated in the petiole in response to the rootstock genotype and the concentration of P in the petiole was reduced by rootstocks with a *V. riparia* parentage. However, rootstocks with a *V. riparia* parent also generally confer a lower level of vigour to the scion. This could suggest that P nutrition is related to rootstock conferred vigour in grapevine. In addition, the poor efficiencies of P uptake from the soil and of P remobilization from perennial woody tissues of *V. riparia* (Gautier et al., 2018) may have indirectly influenced the concentration of other nutrients such S or Mn.

Acknowledgments

We would like to acknowledge all the technical and scientific staff involved in the setting of Greffadapt experimental vineyard in INRA from Bordeaux. This experimental vineyard was set due to the financial support from Council Interprofessional of Wine from Bordeaux (CIVB), and from Burgundy (BIVB) and from France Agrimer.

Funding

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Perspectives

L'article précédent met en évidence l'impact du fond génétique des porte-greffes de la Vigne sur la composition minérale du greffon et particulièrement sur la nutrition phosphatée. Plus précisément, nous montrons que les porte-greffes ayant *V. riparia* comme parent, confèrent des concentrations plus faibles en P à leur greffon, comparés aux hybrides issus de *V. berlandieri* ou *V. rupestris*. Bien que notre étude n'ait pas pu mettre en évidence l'impact du porte-greffe ainsi que du fond génétique sur le développement du greffon, la capacité des porte-greffes de la Vigne à assurer la nutrition phosphatée de leur greffon semble relativement bien corrélée avec les connaissances scientifiques sur la vigueur conférée par ces porte-greffes.

Ces résultats nous amènent donc à étudier de plus près aux mécanismes impliqués dans la nutrition phosphatée de la Vigne. Cependant, peu d'informations sont disponibles sur ce sujet et en particulier sur la capacité des porte-greffes à absorber le P et/ou à assurer son transfert et son utilisation vers le greffon.

Le phosphore

Le phosphore (P) est un élément indispensable à la vie cellulaire, à la croissance et au développement de la plante. En effet, il participe à la structure des membranes cellulaires (phospholipides), des acides nucléiques (acide désoxyribonucléique et acide ribonucléique, respectivement ADN et ARN), ainsi que des sucres phosphatés. De plus, via son rôle de transfert énergétique conféré en liaison avec l'adénosine triphosphate (ATP) et l'adénosine diphosphate (ADP), P est impliqué dans la majorité des voies métaboliques, particulièrement dans la photosynthèse et la respiration cellulaire (Marschner, 2011).

En contradiction avec son rôle primordial, P est un des éléments les plus limitants pour la croissance et le développement des plantes. En effet, P n'existe pas sous forme libre et se combine rapidement avec d'autres éléments comme l'oxygène ou l'hydrogène. Sa forme la plus oxydée, combine quatre atomes d'oxygènes et est appelée orthophosphate (Pi). Pi existe sous quatre formes différentes en fonction du pH (Figure III.1), $H_2PO_4^-$ étant la forme préférentiellement assimilée par les plantes.

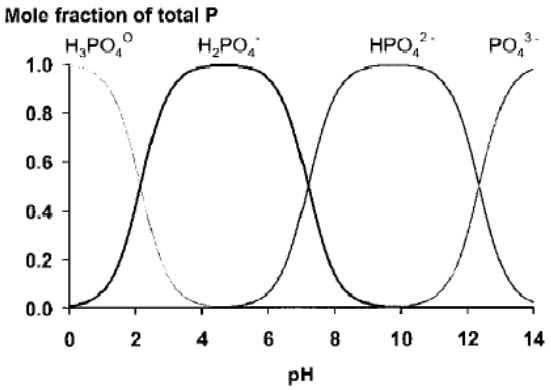

Figure III.1. Formes des ions orthophosphates (Pi) en solution en fonction du pH, exprimées en fraction molaire sur le P total (Hinsinger, 2001).

La fraction de Pi directement assimilable par la plante ne se trouve que sous forme soluble dans la solution du sol. Cependant une grande partie est fortement sorbée aux oxydes et hydroxydes de fer (goethite et ferrihydrite), d'aluminium (gibbsite) et de manganèse (principalement à pH acide) ; ou sorbée au calcium qui produit un complexe insoluble, caractéristique des sols calcaires (Figure III.2) (Barrow, 2016; Schlesinger and Bernhardt, 2013). De plus, on retrouve dans le sol une forte concentration en P organique (Po), composé de molécules telles que des sucres-phosphatées, des nucléotides, des phospholipides ou encore des phytates (Turner et al., 2005; Turner and Engelbrecht,

2010), mais cette fraction de P n'est pas directement assimilable par la plante et nécessite d'être hydrolysée. La fraction directement assimilable par la plante, c'est-à-dire le Pi dans la solution du sol en équilibre avec le Pi de la phase solide du sol (pouvoir tampon), reste très faible. Dès 1927, Pierre et Parker ont mesuré la concentration en Pi dans la solution du sol de 21 sites du Sud et de l'Ouest des Etats unis d'Amérique. Ils ont montré une grande variabilité dans la concentration en Pi, allant de 0.6 à 11 µM, avec une moyenne d'environ 3 µM de Pi dans la solution du sol (Pierre and Parker, 1927). Ces résultats sont très loin des concentrations cellulaires retrouvées dans la plante pour une croissance optimale, qui sont de l'ordre de 5 à 20 mM suivant les espèces (Vance et al., 2003). Face à ce constat, l'analyse des fractions de P du sol non assimilables par la plante devient indispensable pour comprendre l'alimentation minérale en P. Dans la même étude, Pierre et Parker montrent que les concentrations en Po peuvent être largement supérieures aux concentrations en Pi, atteignant 15 µM et donc 5 fois plus élevées (Pierre and Parker, 1927). L'activité microbienne et les interactions plante-rhizosphère (définie comme étant le volume de sol influencé par l'activité racinaire), peuvent augmenter la part de Pi assimilable par la plante .

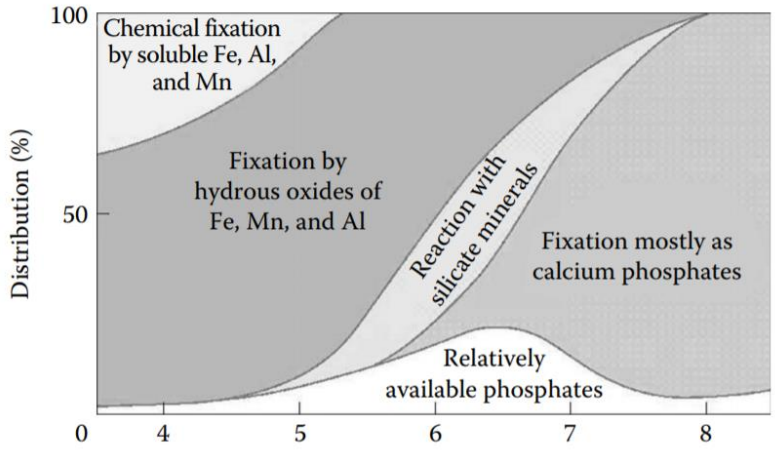

Figure III.2. Solubilité du Pi dans le sol en fonction du pH (Schlesinger and Bernhardt, 2013). La disponibilité en P est limitée par le fer et l'aluminium à pH acide et par le calcium à pH basique.

Enfin, P n'est pas distribué de façon homogène dans le sol. On retrouve généralement de plus fortes concentrations dans les couches supérieures, dues aux dépôts de matières organiques (Laliberté et al., 2012) et l'historique de la fertilisation agricole (Holanda et al., 1998). A cause de sa forte réactivité avec les autres éléments ; la diffusion du P dans le sol est très lente comparée à d'autres éléments minéraux (Bath and Nye, 1973; Bhadoria et al., 1991; Drew and Nye, 1970). Tous ces éléments réunis

expliquent la faible disponibilité en Pi dans le sol pour l'assimilation et montrent son aspect limitant dans le développement et la croissance des plantes.

L'utilisation de la fertilisation phosphatée minérale en agriculture est amenée à être diminuée voire à disparaître. En effet, les roches phosphatées utilisées par la fabrication de fertilisants phosphatés sont non renouvelables et les réserves diminuent considérablement (Duff et al., 1989). De plus, certaines sources de roches phosphatées sont contaminées par des métaux lourds et sont difficiles à extraire (Filippelli, 2002), augmentant le coût des fertilisants. Enfin, les engrais phosphatés ont comme inconvénient de polluer les eaux de ruissellement, et d'occasionner des phénomènes d'eutrophisation. Cette dernière est une forme de pollution des écosystèmes aquatiques. Elle se produit lorsque que le milieu reçoit trop d'éléments nutritifs, induisant une forte prolifération des algues. Les principaux nutriments à l'origine de ce phénomène sont le phosphore et l'azote. L'eutrophisation a pour effets indésirables d'appauvrir la biodiversité et d'entraîner des difficultés de traitement des eaux potables. L'eutrophisation des eaux continentales de surface est importante puisqu'en France près d'un tiers d'entre elles est concerné par ce phénomène (Correll, 1998).

Face à ces problématiques, il est donc indispensable de comprendre les mécanismes impliqués dans la nutrition phosphatée de la plante, ainsi que les stratégies d'adaptation mis en place par la plante en réponse à la faible disponibilité en Pi afin de maintenir son développement.

La nutrition phosphatée chez la plante

La nutrition phosphatée de la plante régule la concentration en P dans ses tissus. Elle dépend principalement de deux paramètres appelés efficience d'utilisation du P (PUE pour « Phosphate Use Efficiency »), et efficience d'acquisition du P (PAE pour « Phosphate Acquisition Efficiency ») (Figure III.3). La PUE est généralement définie comme la capacité de la plante à utiliser le P pour synthétiser de la biomasse et donc assurer sa croissance, mais peut être calculée de différentes façons (Hammond et al., 2009). Une plante ayant une forte PUE est définie comme étant capable de produire autant de biomasse, comparé aux autres, sur un milieu faible en P ; ou de produire une biomasse supérieure à concentration en P égale (Rose et al., 2011). Derrière le concept de PUE (Figure III.3), plusieurs facteurs entrent en jeu, tels que la croissance de la plante en fonction de la teneur en P disponible, la régulation des voies métaboliques, et la remobilisation du P au sein de la plante, depuis les organes de stockage (bois ou racines), les feuilles sénescentes, le Pi libre vacuolaire ou encore depuis les structures cellulaires (*e.g*. les phospholipides ou les acides nucléiques).

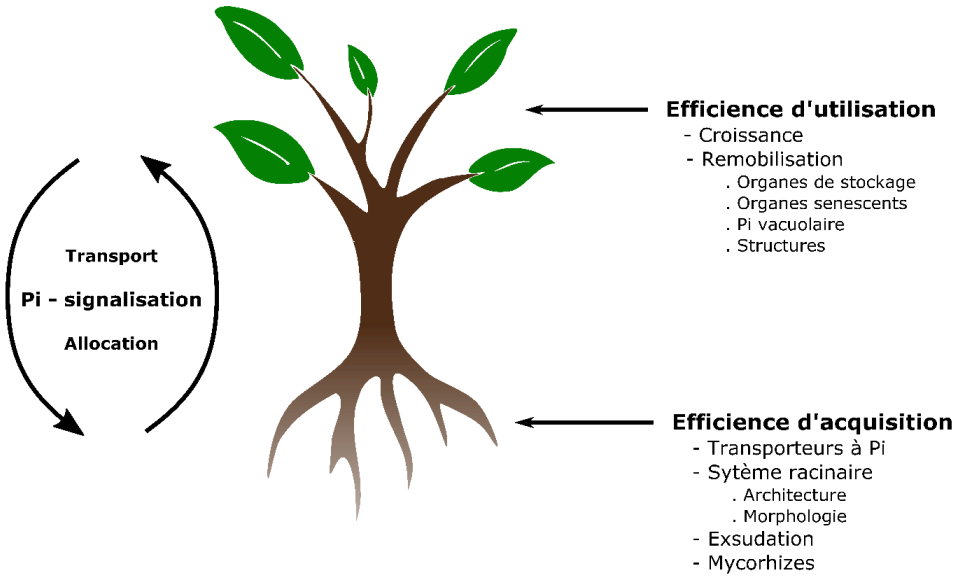

Figure III.3. Facteurs principaux régulant la nutrition phosphatée chez la plante pérenne.

Étroitement liée à la PUE, la PAE est définie comme la capacité de la plante à acquérir le P depuis le sol. Elle inclue de nombreux paramètres permettant l'acquisition du P par la plante (Figure III.3), tels que l'absorption du Pi au travers de la membrane plasmique, l'architecture et la morphologie du système racinaire c'est-à-dire sa capacité à explorer le sol et positionner les racines dans les zones riches en Pi, l'exsudation de composés dans la rhizosphère permettant d'augmenter la disponibilité en Pi assimilable (*e.g.* modification du pH, exsudation d'acides organiques ou d'acides phosphatases), et enfin la capacité de la plante à réaliser des symbioses mycorhiziennes (Vance et al., 2003).

La PUE et la PAE d'une plante sont étroitement liées et dépendent de l'espèce et du génotype étudié. En effet certains favorisent la PUE au détriment de la PAE, ou inversement. De plus, il semblerait que ces concepts n'aient pas la même influence sur la nutrition phosphatée de la plante en fonction du milieu sur lequel elle se trouve. En effet, la PAE semble contribuer plus fortement à l'alimentation en P sur milieux faibles en Pi, alors que la PUE semble plus influencer la nutrition phosphatée sur milieux dont la disponibilité en Pi est définie comme suffisante ou forte (Wang et al., 2010). Ces différences suivant la disponibilité externe en Pi, montrent l'influence des conditions environnementales et de leurs perception par la plante, définie comme la signalisation phosphatée (Figure III.3).

La nutrition phosphatée de la plante dépend donc des caractéristiques (PAE et PUE) de l'espèce, du génotype étudié, des conditions environnementales, et plus particulièrement de la disponibilité en Pi ; et enfin de l'interaction entre ces deux premières composantes, c'est-à-dire de la capacité de la plante à percevoir puis à s'adapter à la disponibilité en Pi.

Efficience d'utilisation du P

Croissance et allocation de biomasse

Comme décrit précédemment, P est impliqué dans la constitution de nombreuses molécules indispensables à la vie cellulaire telles que les acides nucléiques et les phospholipides. Une réduction de la disponibilité en Pi engendre une diminution de la synthèse de nouvelles structures et donc une diminution de la croissance de la plante. En effet, en réponse à la carence en P, de nombreuses études ont montré une réduction de croissance sur la partie aérienne et plus spécifiquement l'impact sur la surface foliaire totale de la plante (Plénet et al., 2000; Rodríguez et al., 1998). Cela est dû à une diminution du nombre de feuille et/ou une diminution de la surface spécifique de chaque feuille (Fredeen et al., 1989). Cette dernière est influencée par deux facteurs principaux, la vitesse de division de cellulaire et l'élongation des cellules. L'impact de la faible disponibilité en Pi a déjà été démontré sur ces deux composantes chez différentes espèces telles que le soja (*Glycine max*) et le maïs (*Zea mays*) pour la division cellulaire (Assuero et al., 2004), et le coton (*Gossypium hirsutum*) pour l'élongation cellulaire (Radin and Eidenbock, 1984). En plus de réduire la surface foliaire, la faible disponibilité en Pi affecte également l'activité photosynthétique et en conséquence l'assimilation de carbone par la plante et donc sa croissance (Brooks, 1986; Warren, 2011).

La réduction de croissance de la plante ne se fait pas de manière égale si on considère les différents organes. En effet, dans la majorité des cas, la croissance racinaire va être moins affectée que la croissance aérienne, ce qui va induire une diminution du rapport biomasse aérienne sur biomasse racinaire. Cette réponse est une adaptation courante de la faible disponibilité en nutriments et permet d'augmenter la partie « source » par rapport à la partie « puits » de la plante au niveau de la demande en éléments nutritifs (Mollier and Pellerin, 1999; Wen et al., 2017).

Remobilisation des réserves

La remobilisation des réserves est un processus très important dans le bon fonctionnement de la plante, essentiellement en réponse à la faible disponibilité en nutriments. En effet, dans le cas du P, la remobilisation des ressources permet de maintenir la croissance de la plante en réponse à la faible disponibilité en Pi externe, d'assurer ses premiers stades de croissance via la remobilisation depuis la graine (Nadeem et al., 2012) ou la reprise végétative chez les plantes pérennes (Netzer et al., 2017; Zambrosi et al., 2012).

Plusieurs sources de P sont disponibles dans la plante pour la remobilisation vers les parties en croissance (Dissanayaka et al., 2018). Parmi elles, on discerne les organes de stockage ou parties pérennes (bois ou racines âgées), les organes en sénescence, plus particulièrement les feuilles, le phosphate libre vacuolaire, et enfin le phosphate utilisé dans les structures cellulaires tels que les phospholipides et les acides nucléiques.

Remobilisation depuis les parties pérennes

Chez les plantes pérennes, le tronc et les racines lignifiées jouent un rôle de stockage en nutriments et autres composés carbonés (e.g. acides aminés et amidon). Ces organes de réserves constituent un pool de métabolites et de minéraux pour assurer la reprise végétative après la dormance et/ou la croissance de la plante en cas de faible disponibilité en nutriments au cours de la saison (Richards, 1983). Généralement, le P présent dans les organes annuels est transmis dans les cellules du bois ou dans les bourgeons vers à la fin du cycle de la plante, destiné à la croissance l'année suivante. En effet, on observe une augmentation de la concentration en P dans ces tissus au détriment des feuilles âgées à la fin de la saison végétative (Netzer et al., 2017; Rennenberg and Herschbach, 2013).

Cependant la contribution de ces réserves à la croissance l'année suivante est controversée. Elle dépendrait du génotype étudié et tous n'auraient pas la même capacité à remobiliser leurs réserves. Un lien entre remobilisation en P depuis les parties pérennes et la PAE a été mis en avant chez *Citrus sinensis* en comparant deux porte-greffes. Le génotype ayant une meilleure PAE, donc capable d'acquérir une plus grande quantité de P est moins dépendant de ses réserves phosphatées (Zambrosi et al., 2012).

La capacité d'un génotype à remobiliser ses réserves en P depuis les parties pérennes serait dépendante de la disponibilité en P. Sur un sol considéré comme riche en P, l'étude menée par Netzer et al. (2018), montre la faible contribution des réserves en P pour la croissance annuelle chez *Populus* x *canescens*. Les auteurs montrent aussi l'absence de mise en réserve du P dans les parties boisées de la plante durant les mois de mise en réserves précédant la dormance de la plante. Cela met en avant la perception d'un environnement riche en P régulant et même réduisant la mise en réserve de P.

Remobilisation depuis les feuilles

Durant son développement, la feuille a pour rôle principal de s'étendre afin de capter l'énergie lumineuse et permettre l'activité photosynthétique optimale pour le bon fonctionnement de la plante. Avec l'âge, la feuille devient de moins en moins rentable en termes de coût énergétique. En effet, l'augmentation des coûts respiratoires et l'ombrage grandissant provoqué par les jeunes feuilles en croissance, diminuent l'efficacité photosynthétique de l'organe. La feuille âgée devient alors plus rentable pour la plante en tant qu'organe « source » en nutriments pour assurer la croissance et la mise en place de nouvelles feuilles (Stigter and Plaxton, 2015; Veneklaas et al., 2012). La feuille entre alors dans sa phase de senescence, primordiale à la réduction du coût énergétique chez la plante. En effet, l'alimentation minérale coûteuse depuis la racine peut alors être supplée par la remobilisation des nutriments contenus dans la feuille sénescente afin d'être réutilisés pour la croissance des organes actifs, tels que les jeunes feuilles ou les fines racines, ou bien pour la mise en réserve avant la dormance (Stigter and Plaxton, 2015).

Toutes les plantes n'ont pas la même capacité à remobiliser le P de leur feuilles sénescentes. Chez *A. thaliana*, la remobilisation du P dans la feuille sénescente représente environ 75% du P total présent dans l'organe durant cette phase de fin de vie (Robinson et al., 2012b). Chez certaines espèces, connues pour s'adapter à des sols pauvres en P, le taux de remobilisation du P depuis la feuille peut atteindre 85 à 95%, respectivement chez *Hakea prostrata* et *Banksia serrata* (de Campos et al., 2013; Lambers et al., 2015a). Contrairement à ces dernières, certaines espèces montrent un taux de remobilisation des réserves en P bien plus faible, par exemple 41 % chez *Acacia truncata* ou encore 25% chez *Glyceria maxima* (de Campos et al., 2013; Ławniczak, 2011). De plus, il semblerait que les espèces capables de remobiliser de grandes quantités de P depuis leurs feuilles sénescentes, soient également capables de s'adapter à des environnements pauvres en P. Le processus de remobilisation en P dans les feuilles sénescentes d'une espèce pourrait également être influencé par le statut phosphaté de la plante et donc la disponibilité en P de l'environnement. En effet, le taux de remobilisation en P peut être divisé par deux dans une feuille dont la concentration en P est augmentée (Vergutz et al., 2012). Enfin, l'analyse transcriptomique d'une feuille en senescence est relativement comparable à celle d'une feuille en carence en P (Breeze et al., 2011; Bustos et al., 2010; Smith et al., 2018; Stigter and Plaxton, 2015). En effet, sur les 1873 gènes sur-exprimés dans la feuille d'*A. thaliana* en carence en P, 711 (soit 38%) le sont également pendant la phase de senescence. A l'inverse, sur les 1795 gènes sous-exprimés en réponse à la faible disponibilité en P, 717 (soit 40%) le sont également chez la feuille en senescence. Cela démontre la similitude des processus de remobilisation mis en place par la plante lors du renouvellement de ses nutriments dans la feuille en senescence et lors de la faible

disponibilité en Pi (Stigter and Plaxton, 2015). Ces études révèlent à quel point ces deux processus très similaires permettent la remobilisation du P déjà acquis par la plante, se trouvant dans les feuilles les moins actives en termes d'activité photosynthétique, vers les parties en croissance de la plante, telles que les jeunes feuilles ou les fines racines afin d'augmenter la rentabilité en production carbonée ou l'acquisition de nouveaux minéraux. La compréhension des mécanismes mis en jeu dans la remobilisation interne du P est primordial pour étudier la nutrition phosphatée de la plante et sa PUE.

La cellule végétale contient de nombreuses molécules contenant du P. Parmi elles, on peut noter tout d'abord des acides nucléiques et des phospholipides en forte abondance, ainsi que d'autres composés tels que les protéines et sucres phosphatés ou encore les pyrophosphates (Veneklaas et al., 2012). Chacun de ces composés est catabolisé chez la feuille sénescente ou en réponse à la faible disponibilité en Pi, afin de libérer le Pi, d'une manière propre à sa structure et fait donc intervenir une multitude de protéines différentes.

Les acides nucléiques contiennent presque la moitié du P total d'une cellule, dont 80% sont sous forme d'ARN ribosomiques (ARNr), ce qui en fait la première source de Pi lors de la remobilisation. Durant la senescence de la feuille les ARNr sont les premiers acides nucléiques à être recyclés, suivis des autres ARN, puis de l'ADN (Breeze et al., 2011). Le recyclage des acides nucléiques est assuré par la famille protéique RNase T2 (Hillwig et al., 2011). En réponse à la faible disponibilité en P, l'expression des gènes codant des RNases T2 est augmentée, montrant le recyclage du P des ARNr pour assurer la croissance des jeunes tissus de la plante (Dodds et al., 1996).

La seconde réserve de P dans la cellule se trouve dans les phospholipides, constituant des membranes cellulaires. Les membranes cellulaires sont normalement sujettes à un renouvellement constant, en effet on estime que 2% des phospholipides sont renouvelés chaque jour (Troncoso-Ponce et al., 2013). Durant la phase de senescence de la feuille, le renouvellement des phospholipides cesse, on observe une augmentation de la dégradation des acides gras de la membrane pouvant atteindre une perte de 80% (Thompson et al., 1998; Troncoso-Ponce et al., 2013; Yang and Ohlrogge, 2009). Les enzymes responsables de la dégradation des phospholipides font partie d'une famille enzymatique appelée phospholipase constituée de 3 groupes : les phospholipases A1 (PLA1), les phospholipases C (PLC) et les phospholipases D (PLD), qui se différencient par leur site de clivage le long du phospholipide, ce qui change la nature du produit formé (Chen et al., 2011a). PLC et PLD sont induites durant la réponse à la faible disponibilité en P montrant le rôle du renouvellement des structures afin d'assurer la croissance la plante (Chen et al., 2011a; Nakamura et al., 2005).

Enfin la cellule contient de nombreuses autres molécules contenant des groupements Pi tels que les pyrophosphates, les protéines et les sucres phosphatés (Veneklaas et al., 2012). Ces composés peuvent être également recyclés afin de libérer le Pi, sous l'action des acides phosphatases (APases ; EC 3.1.3.2) qui catalysent l'hydrolyse des groupes phosphates présents dans les molécules organiques. Ces enzymes sont induites durant la carence en P et/ou la senescence des feuilles (Plaxton and Tran, 2011; Shane et al., 2014). La famille protéique des PAP (Purple Acid Phosphatases) rassemblerait 29 PAP différentes chez *A. thaliana*. Cependant, chacune aurait un rôle précis dans le fonctionnement cellulaire et serait induite de façon différente en réponse aux stress environnementaux (Li et al., 2002).

Remobilisation et homoeostasie intra-cellulaire

La vacuole est un compartiment de stockage du P non utilisé par la cellule pour la synthèse de molécules phosphatées ou ne participant pas aux voies métaboliques pour son rôle énergétique via l'ATP. La concentration en Pi du cytosol est donc soumise à une régulation entre le pool de Pi contenu dans la vacuole et la demande cellulaire. En effet, la distribution du P dans la cellule dépend de la concentration externe en P. Chez l'orge (*Hordeum vulgare*), la proportion de Pi contenu dans la vacuole par rapport au Pi total de la cellule peut varier de 0% en cas de carence en P à environ 80% pour un milieu riche en P (Foyer and Spencer, 1986).

La phosphore assimilé est partagé entre les différents compartiments cellulaires afin d'assurer les fonctions métaboliques dans lesquels il est impliqué. La concentration intra-cellulaire est généralement déterminée de l'ordre de 5 à 20 mM (Vance et al., 2003), cependant la distribution intra-cellulaire n'est pas homogène. En effet, la concentration en Pi du cytosol est estimé à environ 60 µM, alors que l'on retrouve des concentrations de 5 mM dans les organites cellulaires (Pratt et al., 2009). Dans ces derniers, la concentration en Pi n'est pas affectée par la faible disponibilité en Pi, permettant de maintenir la respiration cellulaire et autres voies métaboliques. En revanche, de grandes fluctuations sont observées dans le cytosol, où la concentration en Pi est régulée via les échanges entre cytosol et vacuole (Pratt et al., 2009).

Après avoir utilisé le Pi vacuolaire, on assiste au même renouvellement du Pi au niveau cellulaire que dans le cas de la senescence. En effet les ARN subissent une dégradation et les phospholipides sont progressivement remplacés par des sulfolipides et/ou des galactolipides, permettant la libération du P contenu dans les membranes cellulaires et pouvant ainsi être utilisé dans différentes voies métaboliques. Enfin la régulation des voies métaboliques permet à la plante de limiter l'utilisation de P en favorisant des voies métaboliques secondaires moins consommatrices en énergie et donc en P (Hammond et al., 2004).

Efficience d'acquisition du P

Acquisition du P externe

La concentration en Pi du sol est très inférieure à la concentration dans la plante. En effet, elle est de l'ordre du µM, alors que la concentration en Pi requise chez la plante pour une croissance optimale est de l'ordre de plusieurs mM donc environ 1000 fois supérieure. C'est pour cela que les plantes ne peuvent acquérir le Pi par diffusion, et nécessite l'utilisation de transporteurs afin de l'absorber (Nussaume et al., 2011). Il existe plusieurs familles de transporteurs en Pi chez la plante. Ces familles diffèrent par leur localisation et leur fonction. Les transporteurs de la famille PHT1 sont localisés sur les membranes plasmiques et sont responsables de l'acquisition du Pi externe. Ces transporteurs fonctionnent en symport avec des protons, associés à une pompe à protons ATPase (Figure III.4) (Johri et al., 2015). En revanche, la stœchiométrie des transporteurs à Pi n'est pas encore bien déterminée mais différentes études posent l'hypothèse que l'acquisition d'une fraction de Pi serait couplée à l'absorption de 2 à 4 protons. De plus, l'ensemble des observations montre qu'il existerait plusieurs types de transporteurs que l'on peut classer en deux catégories, des transporteurs à forte affinité, pour des concentrations en Pi comprises entre 3 et 10 µM et des transporteurs à faible affinité, pour des concentrations en Pi comprises entre 50 et 300 µM (Nussaume et al., 2011; Vance et al., 2003).

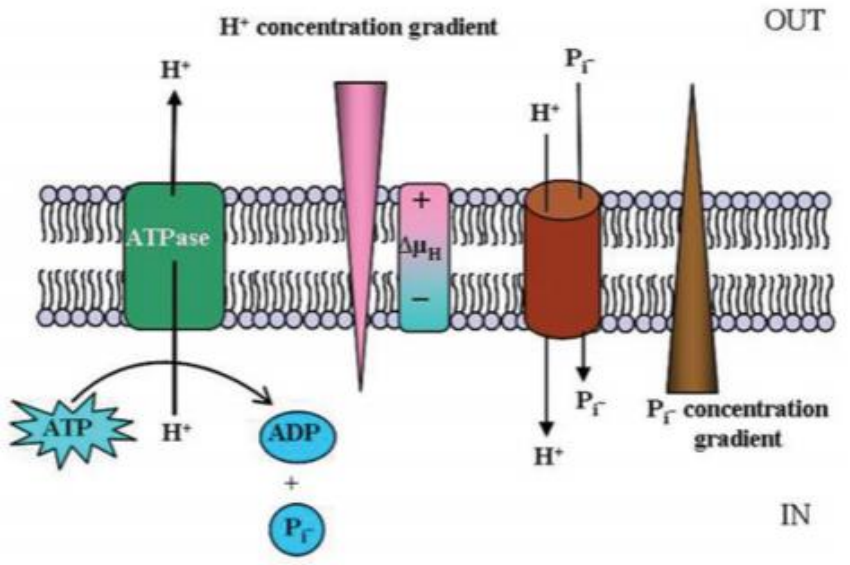

Figure III.4. Absorption du Pi à travers la membrane plasmique. L'acquisition du Pi est réalisée en symport avec des protons, les transporteurs sont couplés avec des pompes à protons (Johri et al., 2015)

Plusieurs transporteurs de la famille PHT1 ont été identifiés chez de nombreuses espèces, par exemple 9 gènes *PHT1* chez *A. thaliana* (*PHT1;1* à *PHT1;9*, (Poirier and Bucher, 2002)) et 12 chez *Populus trichocarpa* (Loth-Pereda et al., 2011). Ces isoformes montrent des profils d'expression différents et sont localisées dans les différents organes (Figure III.5). De plus certaines sont induites dans la réponse à la faible disponibilité en Pi tels que *PHT1;1* et *PHT1;4* (Misson et al., 2004) ou *PHT1;8* et *PHT1;9* chez *A. thaliana* (Remy et al., 2012), augmentant le potentiel d'acquisition du Pi depuis le sol.

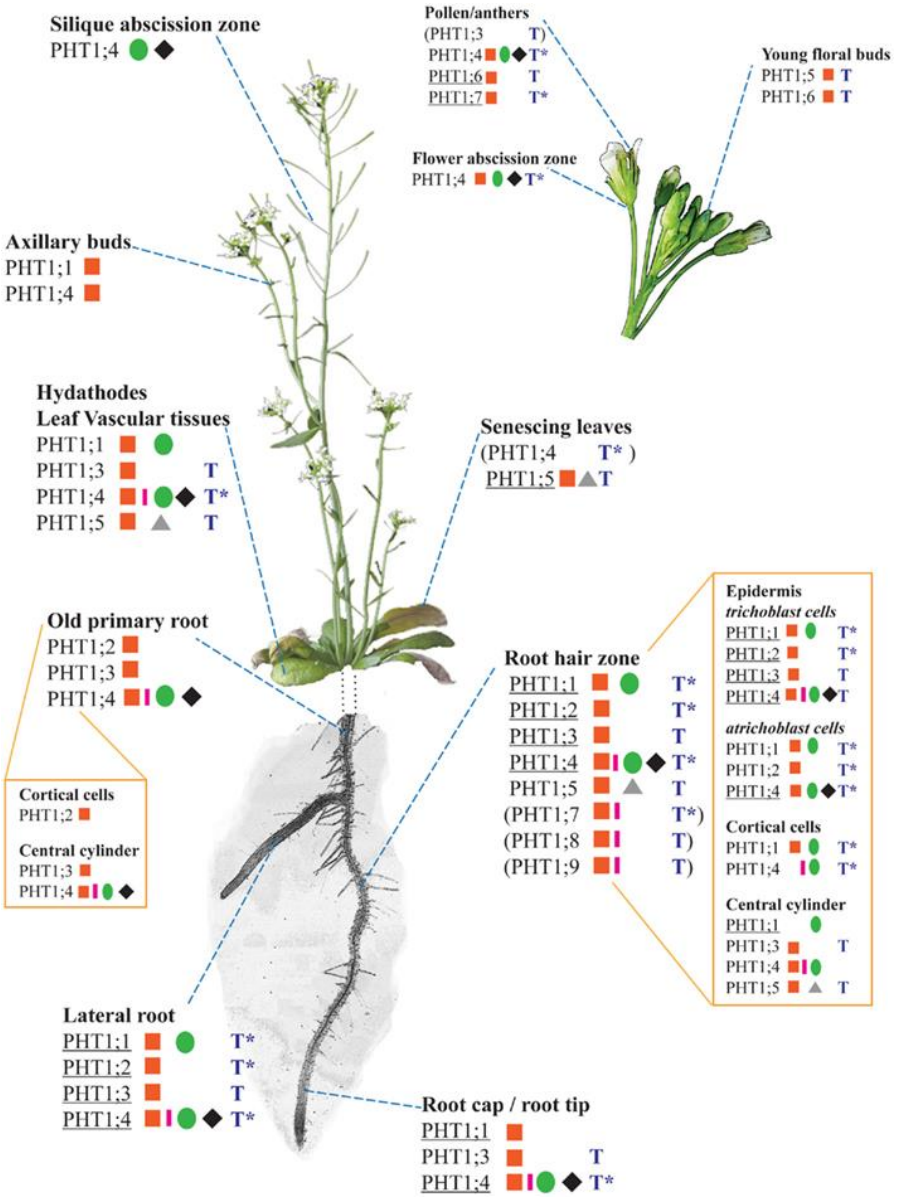

Figure III.5. Localisation et régulation de l'expression des 9 transporteurs de la famille PHT1 chez *A. thaliana* (Nussaume et al., 2011). Ces données regroupent les données transcriptomiques et histologique de plusieurs études (carré rouge (Mudge et al., 2002), cercle vert (Karthikeyan et al., 2002), rectangle rose (Misson et al., 2004), losange noir (Karthikeyan et al., 2002), triangle gris (Nagarajan et al., 2011), T bleu (Winter et al., 2007).

Comme décrit précédemment, P est un élément peu mobile dans le sol et distribué de façon très hétérogène. La capacité de la plante à prospecter le sol et la disposition de son système racinaire suivant les différentes couches jouent donc un rôle primordial dans l'acquisition en P. La variabilité en termes d'architecture racinaire provient de deux composantes : une première génétique, et la deuxième environnementale (e.g. structure du sol, disponibilité en eau et/ou en nutriments) modulant la première via la plasticité propre du génotype. Cette dernière semble être plus déterminante sur l'ensemble des caractéristiques du système racinaire (Peret et al., 2011; Rellan-Alvarez et al., 2016). Afin d'acquérir le Pi, les plantes peuvent mettre en place différentes stratégies basées sur deux composantes essentielles du système racinaire :

- Le développement du système racinaire et la prospection du sol
- Les interactions plante-rhizosphère et la mobilisation du P non assimilable dans le sol

Ces deux composantes sont étroitement liées. En effet la première va influencer la seconde grâce au placement idéal du système racinaire pour une efficacité optimale des interactions plante-rhizosphère dans le but d'augmenter la disponibilité en Pi, via la régulation du pH, l'exsudation de composés carbonés et d'acides phosphatases, et la mise en place de symbioses mycorhiziennes (Lynch, 2011).

Développement et architecture du système racinaire

La croissance racinaire est un facteur déterminant pour l'acquisition en nutriment et en eau chez la plante. La biomasse allouée pour la croissance racinaire au dépend de la croissance aérienne va déterminer le rapport de la partie « source » en minéraux par rapport à la partie « puits ». Généralement, les plantes ayant un ratio biomasse aérienne par biomasse racinaire plus faibles montrent des teneurs en minéraux plus forte dans leur parties aériennes. De plus, la faible disponibilité en minéraux et principalement en Pi affecte moins leur croissance. La régulation de ce ratio est un paramètre important dans la plasticité d'un génotype en réponses à la teneur en Pi dans le sol. Cette adaptation en terme d'allocation de biomasse au sein de la plante suggère que les plantes déficientes en P investissent plus de composés carbonés dans la formation de nouvelles racines (Fernandez and Rubio, 2015). En effet, une diminution du rapport biomasse aérienne par biomasse racinaire est une réponse courante chez les plantes cultivées en faible disponibilité en Pi, ce qui permet d'augmenter la surface de sol explorée et donc l'acquisition en Pi par rapport à la demande aérienne (Hermans et al., 2006; Li et al., 2007; Liu et al., 2016b; Mollier and Pellerin, 1999; Wen et al., 2017; Zhu et al., 2005; Zhu and Lynch, 2004).

Généralement, la concentration en P dans le sol suit un gradient descendant. Les couches les plus superficielles sont relativement plus riches en P, principalement en lien avec la déposition de matière organique (Laliberté et al., 2012). De part cette distribution hétérogène du P dans le sol, l'architecture racinaire est un paramètre crucial pour l'acquisition par la plante d'éléments peu mobiles comme le P. En effet, l'exploration du sol par le système racinaire peut tout d'abord être qualifiée de « traçante » ou « plongeante ». Dans le 1er cas, le système racinaire se développe essentiellement dans les couches superficielles du sol, alors que dans le second les racines se développent en profondeur à la recherche des réserves hydriques. Pour l'assimilation du P, un système racinaire traçant est un avantage. En effet, comme décrit précédemment, les concentrations en Pi ainsi qu'en Po sont plus élevées dans les strates superficielles du sol. Cette configuration du système racinaire permet donc un accès plus aisé des ressources en P (Ge et al., 2000; Lynch, 2011; Lynch and Brown, 2001; Miguel et al., 2015). Le développement du système racinaire en termes de profondeur dépend du gravitropisme (Figure III.6). Ce facteur est un caractère propre à chaque génotype. Il est également régulé par les facteurs environnementaux du sol, tels que la disponibilité en eau ou en éléments minéraux et notamment en P (Bonser et al., 1996). Des simulations de systèmes racinaires chez le haricot (*Phaseolus vulgaris*) montrent l'importance du gravitropisme sur la configuration du système racinaire et en conséquence sur l'acquisition en Pi. Les auteurs montrent que le système racinaire le plus traçant, présente une plus grande efficacité d'acquisition en Pi que le système racinaire plongeant, grâce une meilleure exploration des couches superficielles, ainsi qu'une diminution de la compétition inter-racinaires (Ge et al., 2000; Lynch and Brown, 2001). Enfin l'adaptation du gravitropisme du système racinaire en réponse à la faible disponibilité en Pi, visant à diminuer l'angle des racines primaires par rapport à l'horizontal, dépend du génotype et est souvent associée à une bonne efficience d'acquisition du P (Liao et al., 2001).

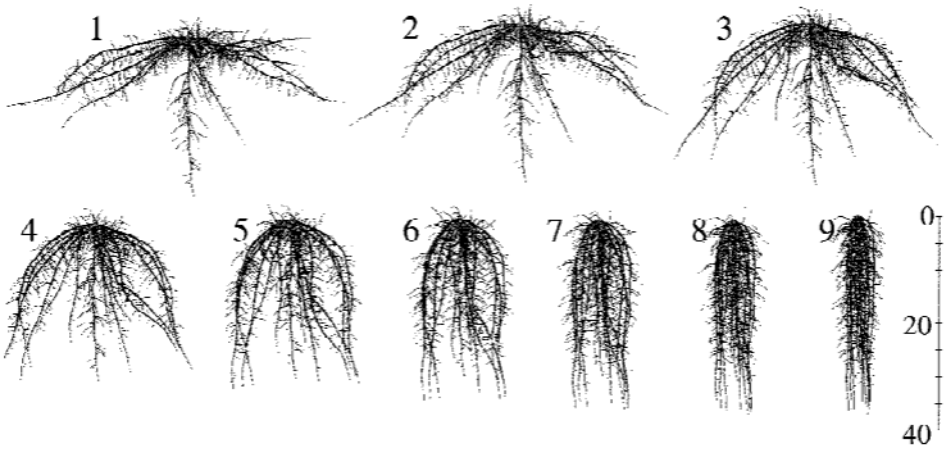

Figure III.6. Systèmes racinaires de *Phaseolus vulgaris L.* simulés avec Simroot en faisant varier le gravitropisme (Ge et al., 2000).

Au-delà de la disposition du système racinaire dans les différentes couches du sol, d'autres paramètres architecturaux et/ou morphologiques sont impliqués dans la capacité d'un génotype à prospecter le sol et à acquérir le Pi. L'acquisition du Pi est étroitement liée à la longueur des racines, leur diamètre, la densité de ramification ainsi qu'au volume des poils racinaires. Chez les plantes une bonne capacité à acquérir le Pi est généralement associée à un phénotype racinaire particulier. Il se définit par un système racinaire ramifié et composé de longs poils absorbants (Postma et al., 2014) (Figure III.7).

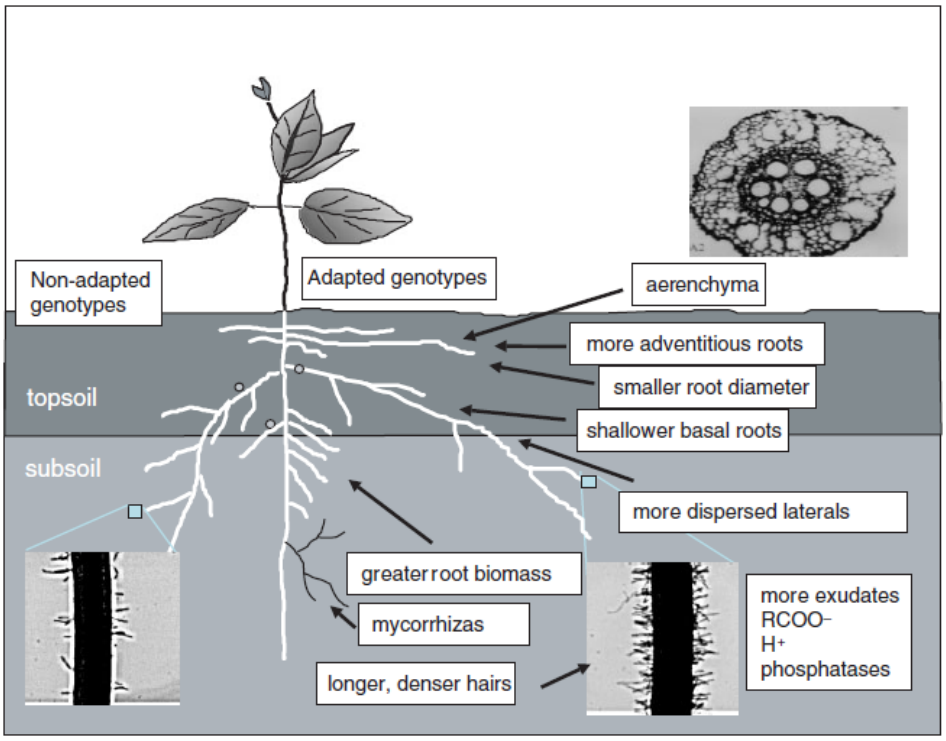

Figure III.7. Caractéristiques d'un système racinaire adapté à l'acquisition de Pi (Lynch, 2007).

Cependant, un système racinaire dont la densité de ramification est trop élevée, couplé à un fort gravitropisme, peut empêcher l'acquisition du P par un phénomène de concurrence inter-racinaire. En conséquence l'absorption racinaire provoque une zone de déplétion en P dans la rhizosphère, pouvant résulter par une forte diminution du Pi assimilable pour les racines aux alentours (Ge et al., 2000). Tout comme le gravitropisme, la densité de ramification et la morphologie racinaire sont très affectées par la disponibilité en eau et en nutriments du sol. Le rôle de ces facteurs environnementaux sur le développement racinaire a été mis en évidence, montrant une réponse de la plante propre au facteur limitant (Drew, 1975; López-Bucio et al., 2003; Shahzad and Amtmann, 2017). L'architecture racinaire

optimale pour l'acquisition des différents minéraux est étroitement corrélée avec leur capacité à diffuser dans le sol et à être assimilé par la plante. Par exemple, la densité de racines latérales est un paramètre essentiel dans l'acquisition des minéraux. Cependant chaque phénotype racinaire serait plus adapté à l'acquisition d'éléments nutritifs particulier. Les systèmes racinaires montrant une faible densité de ramification seraient plus efficients à acquérir l'eau, NO_3^- et SO_4^{2-} ; tandis que les systèmes racinaires ayant une forte densité de ramification seraient plus efficient dans l'acquisition du Pi, K^+, NH_4^+, Cu^{2+} ou Mn^{2+} (Postma et al., 2014). Une forte densité de ramification semblerait donc associée à une bonne capacité de la plante à acquérir le Pi. Ces résultats démontrent l'importance de l'architecture racinaire et sont en accord avec les modifications que cette dernière subit en réponse à la faible disponibilité en Pi. En effet, une réduction de la croissance des racines primaires et une augmentation de l'émergence des racines latérales sont généralement observées en réponse à la carence en P chez *A. thaliana* (Niu et al., 2013; Peret et al., 2011; Peret et al., 2014). Dans ce cas, la réduction de la croissance des racines primaires est la conséquence d'une diminution de l'élongation et de la division cellulaire des cellules du méristème ; alors que l'émergence des racines latérales est régulée par des signaux hormonaux reliés aux auxines (Peret et al., 2014). Cependant, la réponse à la faible disponibilité en Pi n'est pas aussi claire dans la littérature, due à la diversité des réponses observées. Suivant les espèces étudiées la réponse n'est pas la même, voire opposée. En effet, chez *Phaseolous vulgaris* la carence en P n'affecte pas la croissance des racines primaires et diminue l'initiation de racines latérales (Borch et al., 1999). Cette réponse serait même dépendante du génotype au sein d'une même espèce, comme démontré par exemple chez le maïs (Zhu and Lynch, 2004). Enfin, la morphologie intrinsèque des racines est elle-même affectée par la teneur en Pi. On observe une diminution du diamètre moyen des racines en réponse à la faible disponibilité en P (Xie and Yu, 2003).

L'acquisition des nutriments par le système racinaire, est assurée en partie par les poils absorbants, cellules différenciées spécialisées dans l'absorption des éléments minéraux. Ils permettent d'augmenter considérablement (près de 70%) la surface racinaire au contact du sol (Parker et al., 2000). La faible disponibilité en nutriments et particulièrement en Pi, induit une forte prolifération et une élongation des poils absorbants (Figure III.8) (Poirier and Bucher, 2002), augmentant ainsi la capacité d'acquisition et/ou d'interactions avec la rhizosphère permettant d'augmenter la concentration en Pi assimilable (Bates and Lynch, 1996; Ma et al., 2001; Vance et al., 2003).

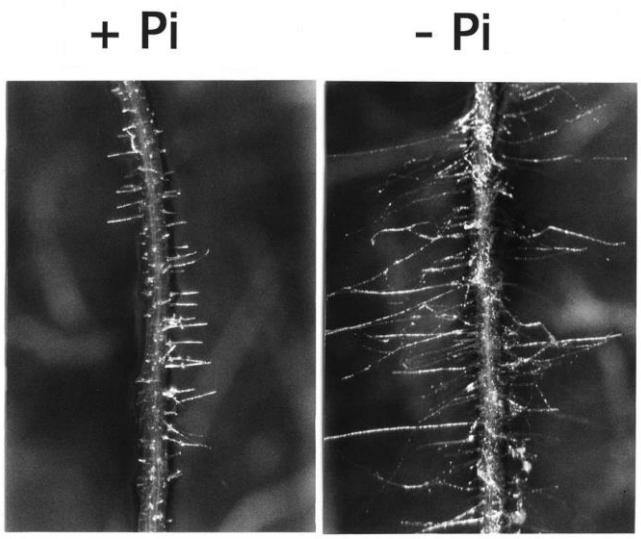

Figure III.8. Modification de la densité et de la longueur des poils absorbants chez *A. thaliana* après 10 jours de culture sur un milieu contenant 5 mM (+Pi) ou 5 µM (-Pi) de phosphate inorganique (Poirier and Bucher, 2002).

Interactions avec la rhizosphère

Le paragraphe précédent décrit la mise en place, la plasticité et le rôle du système racinaire dans l'acquisition du Pi. Les traits caractéristiques d'un système capable d'explorer une plus grande surface de sol et sélectivement des zones plus riches en P ont été mis en évidence, proposant des systèmes racinaires potentiellement plus efficients à acquérir le Pi. Cependant une large fraction du P du sol n'est pas directement assimilable par la plante. Etroitement lié à la capacité du système racinaire à se développer dans le sol, son fonctionnement et notamment sa capacité à modifier la rhizosphère est un facteur essentiel dans l'acquisition du Pi. Ces interactions se traduisent par l'exsudation d'un grand nombre de molécules dans la rhizosphère, modifiant les propriétés chimiques de cette dernière ou agissant directement sur la disponibilité en Pi.

Modification du pH

La disponibilité du Pi est étroitement liée au pH de la rhizosphère, lui-même soumis à des variations induites par l'activité racinaire via les processus suivants (Hinsinger et al., 2003) :

- Les échanges et la régulation entre anions et cations
- L'exsudation d'acides organiques
- La respiration cellulaire
- Les processus de réduction

L'impact de ces processus sur le pH de la rhizosphère peut être important et atteindre jusqu'à une variation de 2 à 3 unités de pH. Suite à l'acquisition d'éléments minéraux, les racines vont absorber ou exsuder des protons afin de réguler leur pH cytosolique. En effet, l'acquisition des nutriments nécessite des transporteurs actifs, due au gradient de concentration établi entre la rhizosphère et les cellules végétales, incluant des systèmes de transport fonctionnant grâce au couplage de pompes à protons et/ou des systèmes antiport ou symport entraînant une variation des concentrations de protons dans la rhizosphère (Marschner, 2011). L'équilibre des charges entre anions et cations n'est pas uniquement due à l'acquisition des minéraux. En effet, un grand nombre de molécules chargées positivement ou négativement sont exsudées par les racines, modifiant le pH intra-cellulaire. Les processus d'absorption et/ou d'exsudation de protons entrent en jeux enfin de réguler les différences de charges positives et négatives au sein de la cellule. C'est le cas pour les acides aminés ou les acides organiques qui sont exsudés de la cellule via deux processus distincts, une diffusion passive à travers la membrane plasmique ou à travers les canaux protéiques. La diffusion passive est régulée essentiellement par les pompes à protons modifiant le gradient électrochimique (Figure III.9) (Jones, 1998). Enfin l'influence de ces processus sur le pH de la rhizosphère est affectée par le pouvoir tampon du sol, dépendant en partie de la nature et de la composition du sol (Hinsinger et al., 2003; Schaller, 1987). Comme décrit précédemment, le pH de la rhizosphère est un paramètre crucial affectant la forme et la solubilité des Pi de la solution du sol et en définitive sur l'acquisition par la plante. La compréhension des mécanismes influençant le pH est donc indispensable à la caractérisation de la nutrition phosphatée chez la plante (Barrow, 2016).

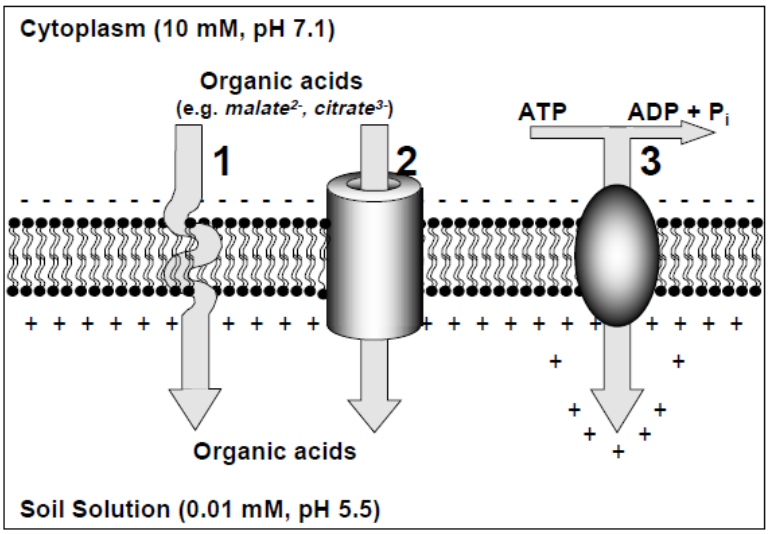

Figure III.9. Représentation de l'exsudation des acides organiques via (1) la diffusion passive à travers la membrane plasmique ou (2) les canaux protéiques. Ces deux processus sont contrôlés par le gradient électrochimique régulée par (3) les pompes à protons (Jones, 1998).

En réponse à la faible disponibilité en Pi et principalement dans des sols calcaires, il a été observé une acidification de la rhizosphère par de nombreuses espèces végétales (Gollany and Schumacher, 1993; Zhou et al., 2009). Cependant, une diversité de la réponse à P a été soulignée en termes d'intensité ou de la localisation, suivant l'espèce et/ou le génotype étudié et la forme de N dans le milieu (Figure III.10) (Liu et al., 2016a; Liu et al., 2016b). De plus, peu d'études ont pu mettre en avant l'origine de cette acidification et découpler l'effet de l'équilibre cation-anions, de l'exsudation de protons ou d'acides organiques.

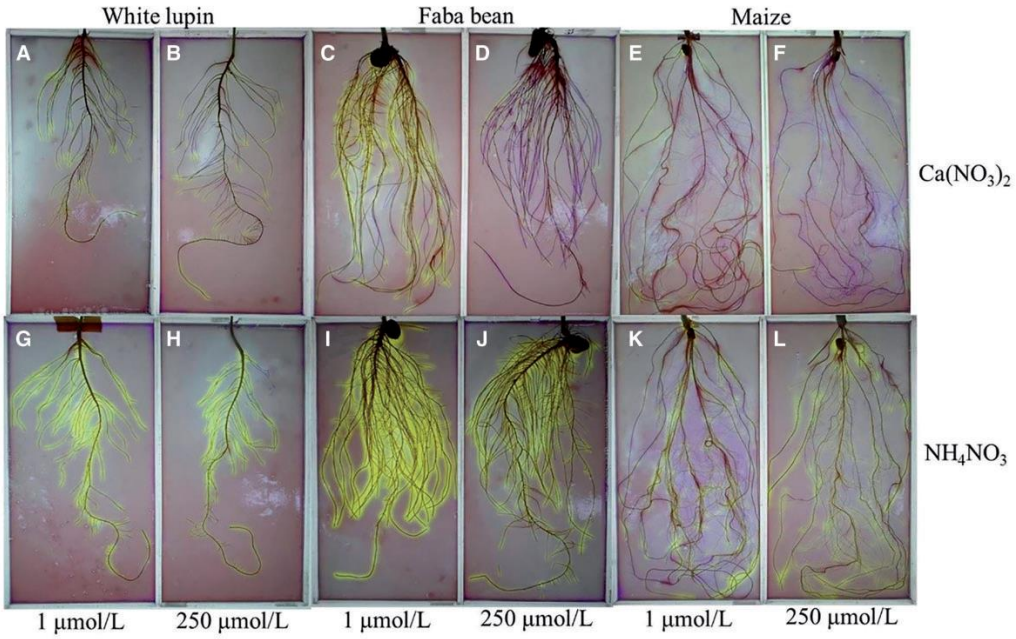

Figure III.10. Effet de la disponibilité en Pi et de la forme d'azote sur l'acidification de la rhizosphère chez *Lupinus albus* L., *Vicia faba* L. et *Zea mays* L., utilisation d'un indicateur de pH (pourpre de bromocresol). La couleur jaune indique un pH inférieur à 5.2, alors que le violet indique un pH supérieur à 6.8 (Liu et al., 2016a).

Exsudation d'acides organiques

L'acidification de la rhizosphère peut également être due à l'exsudation de composés carboxylés comme les acides organiques (Hinsinger et al., 2003; Hoffland et al., 1989). Ces composés sont présents en fortes concentrations dans les racines et jouent un rôle important dans la régulation du pH du cytosol (Marschner, 1995). En cas d'excès, les acides organiques sont envoyés vers les parties ariennes de la plantes ou sécrétés dans la rhizosphère (Neumann and Römheld, 1999). Cependant, l'exsudation de carboxylates dans la rhizosphère n'est pas sans conséquence sur la disponibilité en Pi. En effet, cela va affecter directement le pH de la rhizosphère et donc affecter la disponibilité en Pi comme décrit dans le paragraphe précédent. De plus certaines études ont mis en évidence un couplage entre l'exsudation d'acides organiques et de protons (Kania et al., 2003; Liu et al., 2016b; Neumann and Römheld, 1999; Tomasi et al., 2009). Au-delà de leur impact sur le pH de la rhizosphère, les acides organiques vont permettre la désorption des anions inorganiques tel que le Pi, initialement couplé à des cations, Ca sur sol calcaire, Al ou Fe sur sol acide (Figure III.11) (Bolan et al., 1994). L'exsudation d'acides organiques tels que le malate, le citrate, le tartrate ou encore le fumarate induit donc une

augmentation de la disponibilité en Pi. L'augmentation de la synthèse et de l'exsudation d'acides organiques est une réponse courante chez la plante durant un stress nutritionnel et en particulier en réponse à la faible disponibilité en Pi (Jones, 1998; Vance et al., 2003). Enfin, certaines espèces telles que le lupin blanc (*Lupinus albus* L.) ont la capacité de former des racines particulières appelées « cluster root », qui sont le condensé de petites racines latérales très rapprochées les unes des autres, induites en réponse à la faible disponibilité en Pi (Peret et al., 2014). Elles ont la capacité d'exsuder des quantités relativement importantes d'acides organiques comparées aux autres espèces végétales (Neumann and Römheld, 1999).

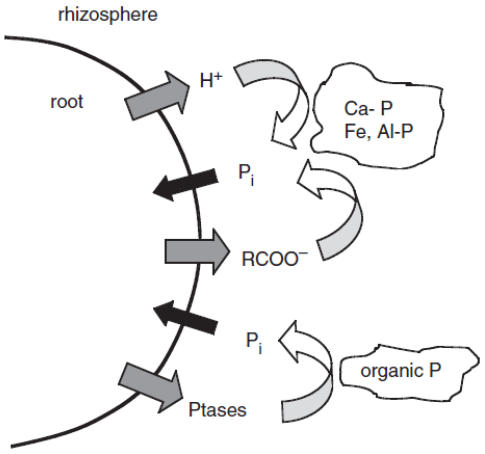

Figure III.11. Schéma simplifié du rôle des composés exsudés dans la rhizosphère dans la solubilisation du Pi (Lynch, 2007).

Exsudation d'acides phosphatases

L'augmentation de l'activité des APases dans la rhizosphère est une réponse courante à la faible disponibilité en Pi (Vance et al., 2003). Elles permettent l'hydrolyse des groupements phosphates contenus dans les molécules organiques présentes dans le sol (Figure III.11). Les APases sécrétées sont codées par les gènes de la famille des *PAP*. Les principales APases exsudées dans la rhizosphère ont été identifiées chez Arabidopsis comme étant *AtPAP26* et *AtPAP12* (Tran et al., 2010b). En réponse à la faible disponibilité en Pi, une surexpression de *AtPAP12* a été observée (Robinson et al., 2012a), contrairement à l'expression de *AtPAP26* qui semble peu régulée en cas de carence phosphatée. Cependant, l'étude d'un mutant *AtPAP26* a montré une très forte réduction de l'activité des APases exsudées, démontrant une possible une régulation protéique post-transcriptionnelle et/ou post-traductionnelle de ces enzymes (Tran et al., 2010b). D'autres gènes de la famille des *PAP*s semblent également coder des APases sécrétées en réponse à la faible disponibilité en Pi, tels que *AtPAP10*

(Wang et al., 2011b). Enfin, les espèces végétales montrent des réponses contrastées dans leur capacité à réguler l'activité des APases extra-cellulaires en réponse à la faible disponibilité en Pi, avec par exemple le maïs (*Zea mays* L.) dont l'efficacité des exsudats racinaires à modifier la teneur Pi de la rhizosphère est faible (Liu et al., 2016b).

Symbioses mycorhiziennes

La symbiose mycorhiziennes repose sur une relation avec des effets bénéfiques pour deux espèces impliquées, ici une plante et un champignon. La plante va apporter au champignon des assimilats carbonés issus de la photosynthèse, en échange de nutriments et en particulier Pi. Il est estimé que 70 à 80% des plantes terrestres sont capables de former des symbioses mycorhiziennes (Smith and Read, 2010). Ces symbioses apportent de nombreux avantages dans la régulation de la nutrition phosphatée. Due à la faible diffusion du P dans le sol, l'absorption racinaire induit rapidement une zone d'appauvrissement en Pi, obligeant le système racinaire à explorer le sol de façon constante (Bucking et al., 2012; Smith et al., 2011). Le développement de mycorhizes permet à la plante d'acquérir du Pi au-delà de sa zone d'appauvrissement (Figure III.12). De plus, le faible diamètre des hyphes permet l'acquisition de nutriments dans des zones non colonisables par les racines. L'effet bénéfique de ces associations n'est plus à démontrer (Plassard and Dell, 2010) et serait induit en réponse à la faible disponibilité en Pi via l'exsudation de phytohormones telles que les strigolactones (López-Ráez et al., 2008).

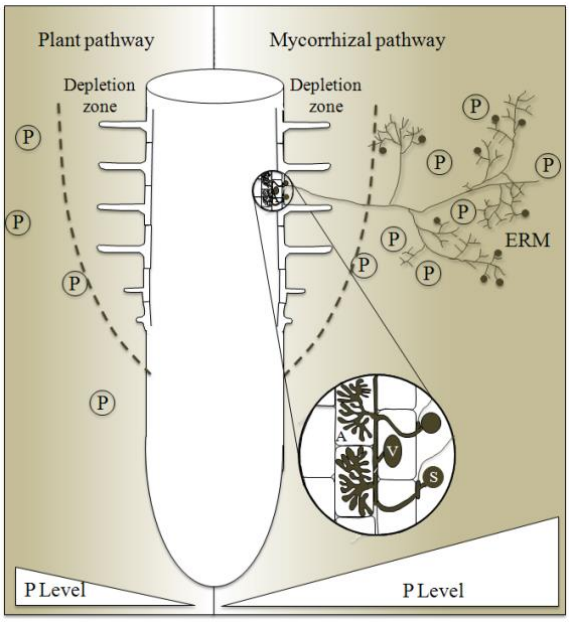

Figure III.12. Acquisition du Pi par la plante et par la plante mycorrhizée (Bucking et al., 2012).

Le phosphore chez la Vigne

Comme la majorité des espèces pérennes, les demandes en P de la Vigne sont relativement faibles pour être cultivée dans de bonnes conditions, de l'ordre de 3 à 10 kg/ha selon les objectifs de production (Delas, 2010; Reynier, 2011). De plus, la remobilisation en P depuis les parties pérennes participe fortement à la nutrition phosphatée de la plante durant les périodes de forte demande (Jackson, 2008). En conséquence, bien qu'observés en Australie (Tulloch and Harris, 1970), en Californie (Cook et al., 1983) ou en France (Champagnol, 1978), les symptômes de carences en P restent rares chez la Vigne (Delas, 2010). Ils se traduisent principalement par une réduction de croissance, un jaunissement du limbe de la feuille voire un rougissement dans le cas de symptômes sévères (Figure IV.1) (Ashley, 2011; Delas, 2010).

Figure IV.1. Symptômes foliaires de la carence en P observée chez la Vigne (Treeby et al., 2004)

Avant l'apparition de symptômes foliaires, un diagnostic pétiolaire permet de détecter la carence en P, basé sur la fait qu'une teneur pétiolaire en P est considérée comme faible lorsqu'elle est inférieure à 0,12 % de matière sèche (Cordeau, 1998), pouvant être facilement corrigée par une fumure de fond. Malgré le peu d'incidences détectées, les souches montrant de faibles teneurs en P sont généralement dotées d'une faible vigueur. Effectivement, les teneurs pétiolaires varient en fonction des porte-greffes utilisés. La vigueur conférée au greffon peut être positivement corrélées aux teneurs pétiolaires mesurées (Figure IV.2) (Cordeau, 1998).

Les faibles besoins en P chez la Vigne peuvent être expliqués par sa nature pérenne (Skinner et al., 1988). En effet, à l'échelle de la plante, le P est principalement contenu dans les parties boisées ou les racines ligneuses. Ces réserves peuvent contribuer de 10 à 50% aux besoins annuels pour le bon développement de la Vigne (Conradie, 2017; Schreiner and Scagel, 2006). Les réserves assurent principalement les premiers stades de croissance, jusqu'à l'optimum d'acquisition en P, compris entre la floraison et la véraison (Doolette and Smernik, 2016).

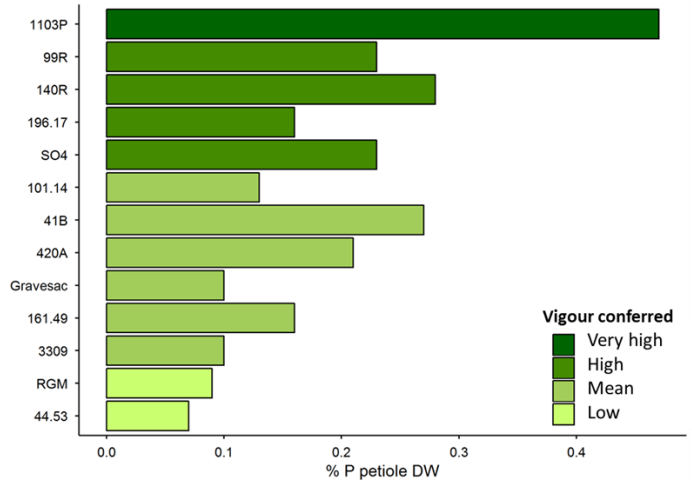

Figure IV.2. Teneurs pétiolaires en phosphore de *V. vinifera* cv. Merlot, au stade véraison en fonction du porte-greffe utilisé, sur le vignoble de Bordeaux (Adapté à partir de Cordeau, 1998)

L'impact de la faible disponibilité en P sur les cycles végétatif et reproductif de la Vigne a été peu étudié en raison des rares symptômes de carences observés. Cependant, de fortes réductions de croissances au niveau aérien ont été démontrées chez la Vigne cultivée sur de faibles teneurs en Pi (Grant and Matthews, 1996b; Skinner et al., 1987). De plus, un apport en P résulte d'une augmentation de la vigueur ainsi que du rendement (Skinner et al., 1988). Plus spécifiquement, la faible disponibilité en Pi induit une diminution de la longueur des tiges, de la surface foliaire et du nombre de feuilles, ainsi qu'une augmentation de la surface foliaire par rapport à la biomasse totale de la partie aérienne (Grant and Matthews, 1996b). Le système racinaire de la Vigne semble également impacté par la faible disponibilité en Pi. Les auteurs montrent une diminution de la longueur, de la superficie et du volume total du système racinaire. Ce dernier semble moins affecté, résultant d'une augmentation de la surface racinaire par rapport à la surface foliaire (Grant and Matthews, 1996a).

Une récente étude s'est intéressée à l'impact de la disponibilité en Pi sur le développement et le rendement de *V. vinifera* cv. Pinot noir greffé sur 101-14 MGt (Schreiner and Osborne, 2018). Elle montre une diminution du rendement, du nombre de baies par grappe et du poids moyen des baies dans le cas où les Vignes ne reçoivent pas de fertilisation phosphatée. De plus, cela semble impacter la qualité des moûts, avec une diminution de la concentration en acides totaux, d'azote assimilable et une augmentation du pH.

Enfin, l'ensemble de ces réponses sont dépendantes des génotypes du greffon et du porte-greffe étudiés, ce qui suggère des différences d'acquisition et d'utilisation en P au sein du genre *Vitis* (Grant and Matthews, 1996b; Skinner et al., 1988).

OBJECTIFS

Comme souligné précédemment, les porte-greffes de la Vigne sont à l'origine de fluctuations dans la composition minérale du greffon et plus particulièrement en P. Comprendre l'alimentation minérale des différents porte-greffes utilisés en viticulture est primordiale afin d'optimiser leur utilisation, de préserver la production de raisins de qualité et de permettre l'entière expression du terroir viticole.

Ce travail de thèse a pour objectifs de caractériser les processus impliqués dans l'alimentation phosphatée de la Vigne, et plus globalement de comprendre pourquoi les porte-greffes ayant pour origine *V. berlandieri* ou *V. rupestris*, confèrent des teneurs en P plus importantes à leur greffon que ceux issus de *V. riparia*. Pour cela, nous avons choisis de caractériser différents paramètres impliqués dans la nutrition phosphatée de la plante pérenne (Figure V.1) chez deux génotypes de porte-greffes cultivés en boutures simples, *V. riparia* cv. Riparia Gloire de Montpellier (RGM), connu pour apporter de faibles teneurs en P à son greffon, et *V. rupestris* x *V. berlandieri* hybride cv. 1103 Paulsen (1103P), connu pour apporter de fortes teneurs en P à son greffon. En parallèle, un troisième génotype est étudié en bouture simple, *V. vinifera* cv. Pinot noir (PN) habituellement cultivé en tant que greffon, afin de caractériser les paramètres de la nutrition phosphatée chez *V. vinifera*.

Le premier objectif est de comprendre l'origine de ces variations de teneurs aériennes en P, plus précisément si elles relèvent de l'acquisition, de l'utilisation, de la remobilisation et/ou de l'allocation du P entre les différents organes de la plante ; et si ces différences proviennent du génotype étudié ou bien de la disponibilité en Pi dans le milieu **(Chapitre 1)**. Nous nous sommes ensuite focalisés sur l'acquisition du P, et plus particulièrement sur le développement et le fonctionnement racinaire de nos différents génotypes **(Chapitre 2)**. A travers ce second chapitre, l'objectif est de caractériser des traits racinaires reliés à l'efficience d'acquisition du Pi, ainsi que l'impact des exsudats racinaires sur la rhizosphère et la disponibilité en Pi.

Après voir caractérisé l'ensemble de ces paramètres sur nos trois génotypes modèles, l'impact du greffage est étudié et plus particulièrement les influences réciproques du greffon et du porte-greffe sur les paramètres de l'acquisition en P et sur la réponse à la faible disponibilité en P **(Chapitre 3)**. Enfin une analyse du transcriptome racinaire est réalisée afin de comprendre plus précisément les rôles du porte-greffe et du greffon sur la réponse de la faible disponibilité en P dans le milieu **(Chapitre 4).**

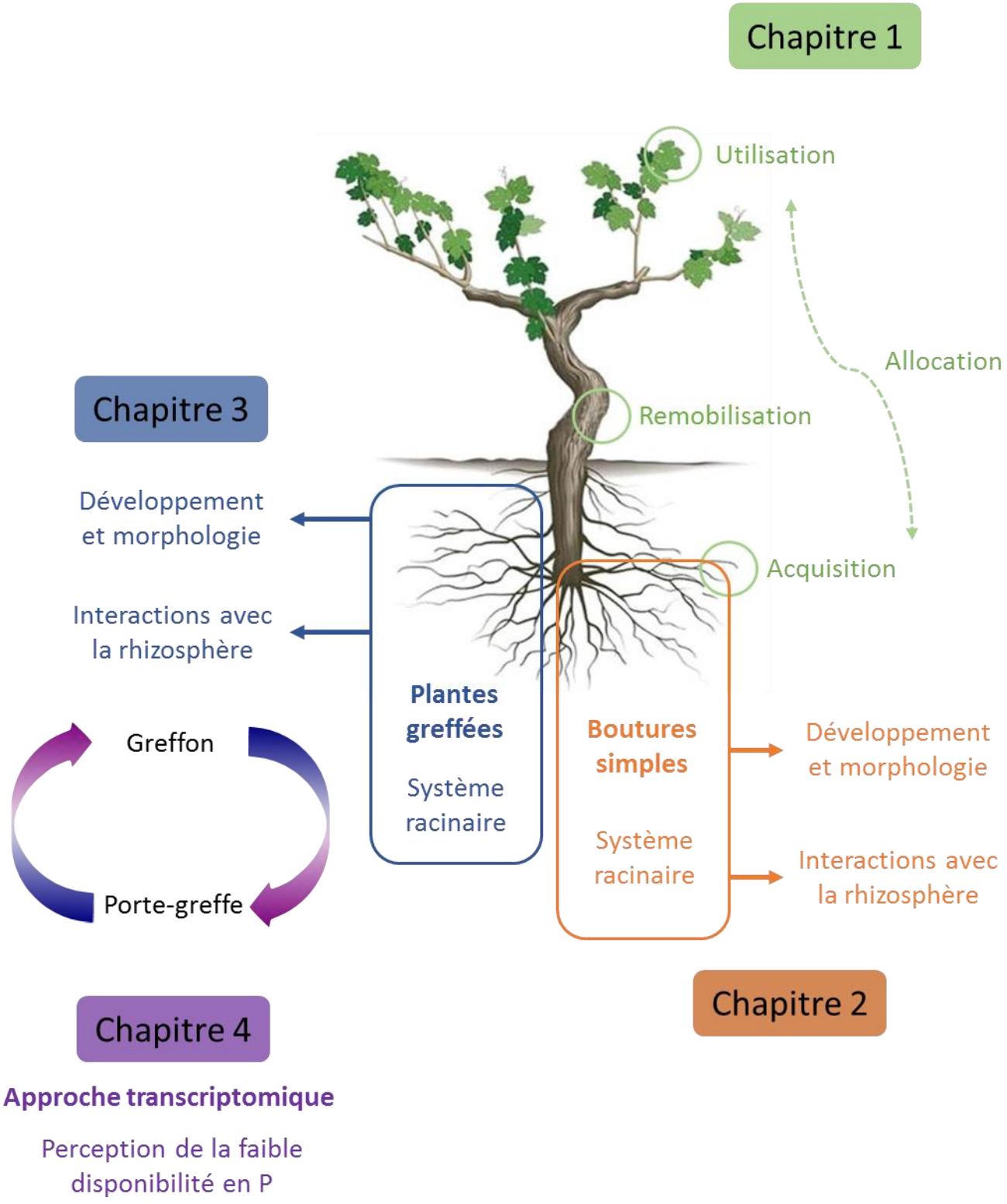

Figure V.1. Stratégie adoptée dans la caractérisation du rôle du greffon et du porte-greffe dans la réponse à la faible disponibilité en P

CHAPITRE 1

Influence du génotype et de la disponibilité en phosphore sur l'acquisition et la remobilisation en phosphore chez la Vigne

Avant-propos

Les porte-greffes issus de *V. riparia* confèrent des teneurs en P plus faibles à leur greffon que ceux issues de *V. rupestris* ou *V. berlandieri*. Ce phénomène peut être relié aux différents paramètres impliqués dans la nutrition phosphatée et plus globalement à des différences entre génotypes en termes de PAE (Phosphate Acquisition Efficiency), c'est-à-dire la capacité de la plante à absorber le Pi disponible dans le sol, ou encore en terme de PUE (Phosphorus Use Efficiency), c'est-à-dire la capacité de la plante à utiliser le P acquis pour sa croissance via l'allocation du P entre les différents organes ou via la remobilisation depuis les parties pérennes.

Dans ce premier chapitre, nous cherchons à caractériser les propriétés des génotypes étudiés, en termes d'acquisition du Pi disponible, de remobilisation du P depuis les organes de réserves, ainsi que leur capacité à assurer leur développement aérien et racinaire au cours des premiers stades de développement de boutures simples.

Pour cela, un apport régulier en ^{32}P a été effectué sur les 21 premiers jours de développement racinaires, permettant de distinguer le P provenant de l'acquisition et le P remobilisé depuis la bouture. L'absence de P externe a également été étudié, permettant de mettre en avant l'adaptation des différents génotypes de Vignes, sur l'utilisation de leur réserve interne.

Cette expérimentation a permis de mettre en évidence des différences génotypiques dans l'acquisition et l'utilisation du P en présence de Pi externe, ainsi que la plasticité des génotypes à réguler leur utilisation en P en absence de ce nutriment dans le sol. L'ensemble de ces résultats a conduit à la rédaction d'un article scientifique publié dans le journal *Tree Physiology* en juillet 2018 (https://doi.org/10.1093/treephys/tpy074).

Article 2: Phosphorus acquisition efficiency and phosphorus remobilisation mediate genotype-specific differences in shoot P content in grapevine

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Running head (50 characters and spaces) Grapevine shoot P related to PAE & remobilization

Abstract

Crop productivity is limited by phosphorus (P) and this will probably increase in the future. Rootstocks offer a means to increase the sustainability and nutrient efficiency of agriculture. It is known that rootstocks alter petiole P concentrations in grapevine. The objective of this work was to determine which functional processes are involved in genotype-specific differences in scion P content by quantifying P uptake, P remobilisation from the reserves in the cutting and P allocation within the plant in three grapevine genotypes.

Cuttings of two American rootstocks and one European scion variety were grown in sand and irrigated with a nutrient solution containing either high P (0.6 mM) or low P (0 mM). The high P solution was labelled with ^{32}P throughout the experiment.

The grapevine genotypes studied show variation in the inhibition of shoot and root biomass in response to low P supply, and P supply also affected shoot, but not root, P concentrations. Genotype-specific differences in total P content were related to differences in P acquisition and utilisation efficiencies (PAE and PUE respectively). P allocation within the plant was not affected by genotype or P supply.

The rootstock genotype known to confer high petiole P content in the vineyard was associated with a high PAE under high P, and a high PUE under low P. This suggests that the petiole P concentrations in the vineyard are related to genotype-specific differences in PAE and PUE that these traits could be used for rootstock selection programs in the future.

Keywords: *Vitis spp.*, *V. vinifera, ^{32}P labelling*

Introduction

Phosphorus (P) is considered as a major limiting factor of crop production. Phosphorus deficiency is currently mitigated by application of P fertilizers. However, P fertilizers are mainly produced from non-renewable phosphate rock, and concerns have been expressed that this natural resource will be exhausted in near future (Cordell and White, 2015; Ulrich and Frossard, 2014). Another concern is that the application of P fertilizer has caused severe environmental problems such as eutrophication (Bennett et al., 2001). Thus, strong economic and environmental reasons exist to improve efficiency of P use in agriculture (Lu and Tian, 2017; Wissuwa et al., 2016).

Alternative plant-based strategies to mitigate P deficiency require the identification of plant traits that enhance the uptake and utilization efficiency of P. Plant species and genotypes differ in the efficiency in which they acquire and utilize P (Narang et al., 2000). Breeding cultivars that efficiently acquire P from soil with low available P has been the focus research efforts to reduce P fertilizer use (Richardson et al., 2009). A number of quantitative trait loci (QTL) for enhanced P acquisition by roots have been identified across a range of crops (Gong et al., 2016; Su et al., 2009; Yuan et al., 2017), but their application in breeding programs has been limited (Wissuwa et al., 2008).

Many crops are cultivated grafted on to rootstocks, which are chosen on the basis their ability to provide biotic and/or abiotic stress tolerance or conferring dwarfing or reduced vigour to the scion (Warschefsky et al., 2016). Selection of scion genotypes is generally restricted as a certain fruit quality or typicity is required by the consumer, however, rootstocks can be selected from a more diverse genetic background. As a consequence, rootstock selection offers opportunities to increase the sustainability of agriculture and the nutrient efficiency of crops (Gregory et al., 2013). It is well known that in many grafted cultivated species, the vegetative growth of the scion and the mineral nutrition are altered by the rootstock genotype (Nawaz et al., 2016), yet we know little of the mechanisms underlying these differences. Rootstock-specific differences in scion P concentrations have been observed in apples (Amiri et al., 2014; Zarrouk et al., 2005), grapevine (Bavaresco et al., 2003; Grant and Matthews, 1996a; Ibacache G. and Sierra B., 2009) and *Prunus spp*. (Hrotkó et al., 2014). In general, these studies have been field trials or experiments using soil-filled pots and not conducted under very limited P conditions, it is possible that under low P soils differences between rootstocks will be reduced.

Rootstock-specific differences in tissue P concentrations could be explained by a number of processes (as reviewed by (Wang et al., 2010)), such as, their capacity to provide P to the shoot, defined by P acquisition efficiency (PAE). Furthermore, in perennial woody plants, new growth can also be

supported by the remobilisation of P reserves from trunk and roots (Schreiner and Scagel, 2006), so the ability to store and remobilise P also has the potential to alter P contents. In addition to differences in the amount of P present in a plant, plants can also differ how P resources are allocated within the plant and in their PUE, the ratio of the biomass relative to the P absorbed by plants; *i.e.* more P investment in growth can lead to a higher PUE, while greater P storage in plant cells can lead to a lower PUE (for example in poplar (Gan et al., 2015)).

In this study we hypothesised that rootstock-specific differences in scion P concentrations are due to differences in P uptake, P remobilisation from the cutting and/or P allocation within the plant. The objective of this work was to determine which processes are involved by supplying labelled exogenous P (^{32}P) and quantifying the uptake and allocation of the newly acquired ^{32}P along with the remobilisation and allocation of P from reserves in three grapevine genotypes. The two grapevine rootstock genotypes were chosen according to their ability to confer differences in petiole P concentration to the scion when grown in the calcareous soil of South West France (Cordeau, 1998). When grafted with *Vitis vinifera* cv. Merlot, the *V. rupestris* x *V. berlandieri* hybrid cv. 1103 Paulsen (1103P) confers high petiole P content (0.47 % P) and *V. riparia* cv. Riparia Gloire de Montpellier (RGM) confers low petiole P content (0.09 % P) (Cordeau, 1998).

Materials and methods

Plant material

Overwintering canes of two American rootstock genotypes, 1103P and RGM, and one typical European scion genotype, *V. vinifera* cv. Pinot noir (PN), were collected from Bordeaux, France and stored at 4°C.

Growing conditions

The experiment was carried out in greenhouse in Bordeaux, France from April to June 2017. Cuttings of the three genotypes of grapevine were rooted in vigorously aerated water at 25 °C for 21 to 28 days (any inflorescences that developed were removed). When the first root was approximately 1 cm in length, five plants per genotype were harvested and 15 plants per genotype were transferred to sand-filled pots and were irrigated daily with 25 mL of nutrient solution with either 0.6 mmol of P (HP) or without P (LP). 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

46.25 µmol H_3BO_3, 9.1 µmol $MnCl_2$, 2.4 µmol $ZnSO_4$, 0.5 µmol $CuSO_4$, 14 nmol $(NH_4)_6Mo_7O_{24}$ and iron was supplied as 8.5 mg/L Sequestrene 138 (Syngenta Agro S.A.S., Guyancourt, France) [i.e. 31.3 µmol ethylenediamine-N,N'-bis (2-hydroxyphenylacetic acid) NaFe].

A known quantity of ^{32}P (Radioactivity at t0 (*R t0)* = 84 MBq) was added to 20 L of HP solution for labeling. The initial specific activity at time 0 (SA t0) was 7 kBq (µmol P)$^{-1}$.

Plants were grown under natural light with the addition of four sodium lamps (400 W each) functioning for 16 h per day (daily maximum photosynthetically active radiation typically between 450 and 610 µmoles cm^{-2} s^{-1}). The temperature was maintained by controlling the opening the roof of the greenhouse, the average maximum daily temperature during the experiment was 34 °C. Given that the differences in temperature during the experiment were minimal, time is expressed in days rather than degree days.

Plant measurements and chemical analysis

After 0, 7, 14 and 21 days of treatment (DAT), shoots (leaves and stems), trunks and roots of five plants per genotype per P treatment were harvested, rinsed, weighed (fresh weight, FW), dried (in an oven at 60 °C until they reached a constant mass) and weighed again (dry weight, DW). To reduce the time spent handling ^{32}P labelled plant material and to have sufficient material for analysis, the leaf and stems were not separated at harvest.

Dried samples were reduced to ashes at 550 °C for 5 h. The resulting ashes were dissolved in 3 mL nitric acid and placed on a hotplate to evaporate, then washed with 5 mL of distilled water until only a few drops were left. Mineralized solutions were filtered and diluted to a final volume of 50 mL with distilled water. P content was measured colorimetrically using malachite green method (Van Verdhoven and Mannaerts, 1987). Exogenous P uptake (P_{exo}) was quantified by labelling the P in the nutrient solution with ^{32}P radioisotope. Assuming that no $^{32}P/^{31}P$ fractionation occurred during exogenous P uptake by roots and P transport within plant, the amount of P in each plant compartment taken from external nutrient solution was calculated as follows:

$$P_{exo} = \frac{r_t}{\left(\frac{R_t}{P_{ns}}\right)}$$

Where Rt/Pns is the specific radioactivity SA t measured at harvest time t in nutrient solution, rt is ^{32}P radioactivity measured in the mineralized plant compartment at harvest time t. Rt and Pns are the amount of carrier-free ^{32}P and ^{31}P measured in nutrient solution. The Rt and rt values were counted

using a scintillation cocktail (Insta-gel Plus Packard, Perkin-Elmer) by a Packard TR 2100 (Canberra Industries, Meriden, CT). The maximum counting time was 60 min.

The total exogenous P uptake is the sum of exogenous P measured in shoot, root and trunk. The initial P stock in cuttings ($P_{stock,0}$) was calculated as the difference between the measured whole plant P content at time t and accumulated exogenous P uptake measured by ^{32}P labelling.

The amount of P from internal remobilization (P_{remob}, µg P) in each organ was calculated as the difference between the measured total P and P_{exo} in shoot, root and trunk respectively at time t:

$$P_{remob} = P_{total} - P_{exo}$$

The rate of P remobilization from cutting (trunk) to the shoot and root was calculated as the sum of amount of P remobilized to the shoot and root divided by the initial P stock in cuttings ($P_{stock,0}$).

Definitions and calculations

Relative Growth Rate (RGR) was estimated from the slope of linear regression between log-transformed biomass of new organs (DW_{shoot} + DW_{root}) and the duration of growth in days (n = 20).

PUE (mg DW (µg P)$^{-1}$) was obtained from the slope of linear regression between P total (µg) in new organs and biomass of new organs (DW_{shoot} + DW_{root}) (Rose et al., 2011; Rose and Wissuwa, 2012).

PAE (µg P in the shoot g root (FW)$^{-1}$) was obtained from the slope of linear regression between P uptake (µg) of the shoot and biomass FW (g) of root (as calculated by (Zambrosi et al., 2012))

Statistical analysis

Genotype and P supply effects on biomass, P concentration, P content, P uptake, rate of P remobilized and P allocation were determined using a two-way analysis of variance (ANOVA $p < 0.05$, with Holm-Sidak test), using Sigma Plot (Version 10, Systat Software). Slope and standard error of linear regression of RGR, PAE and PUE were obtained using Sigma Plot (Version 10, Systat Software). T-tests on these parameters were performed to determine significant effect of genotype or P supply using Sigma Plot (Version 10, Systat Software).

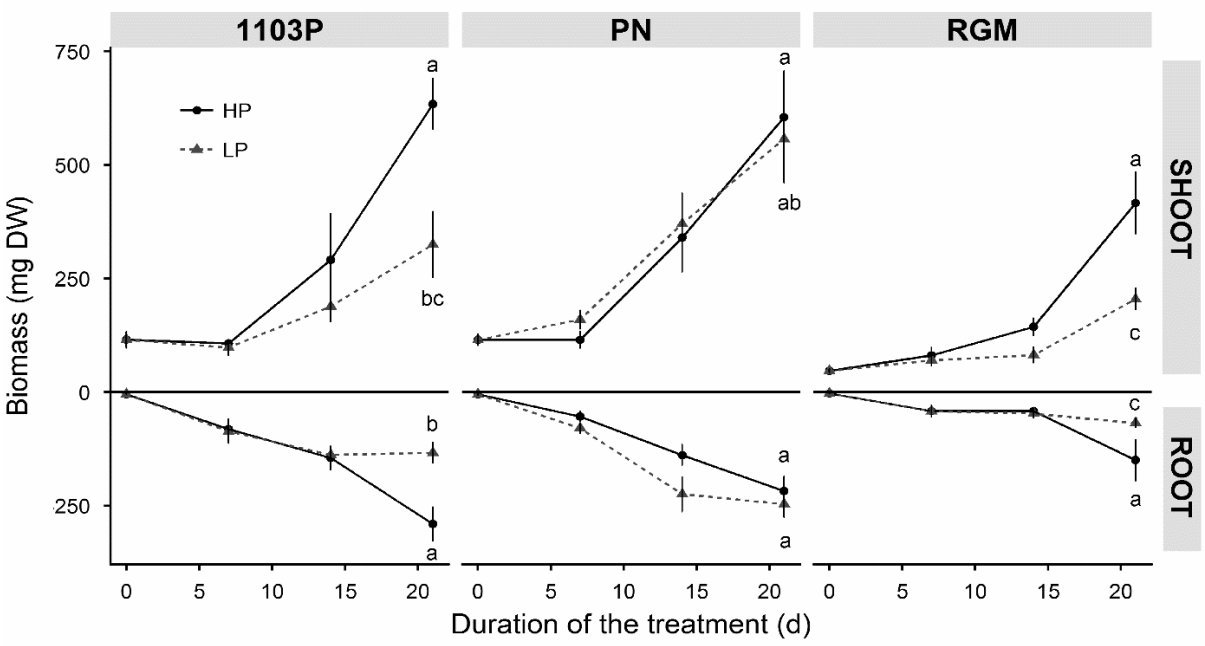

Figure VI.1. Changes over time in shoot and root biomass of three grapevine genotypes (1103P, PN and RGM) under different P supplies, high P (HP, black circles connected by solid lines) or low P (LP, grey triangles connected by dashed lines). Means and standard errors shown (n = 5). Data were log-transformed for two-way ANOVA analyses (with genotype and P supply as factors). For the root data which had a significant interaction, different letters indicate significant differences at $P < 0.05$ for a given time point. For the shoot data which had no significant interaction, letters indicate genotype effects and stars significant treatment effects at $P < 0.05$ for a given time point.

Results

Grapevine genotypes display different degrees of growth inhibition in response to low P supply

Phosphorus supply did not significantly affect shoot or root growth for the first 14 d after treatments began for all three genotypes studied (Figure VI.1). However, a decrease of biomass of new organs (shoot and root) in response to LP was observed after 21 d of treatment, biomass was approximately 50 % reduced for the shoot and root of RGM and 1103P, whereas the growth of PN was slightly less affected (however the interaction of the ANOVA was not significant) (Figure VI.1). Accordingly, the relative growth rate (RGR, Figure VI.2) was significantly lower in LP treatment for RGM and 1103P. Interestingly, under HP, the RGR of the three genotypes was similar despite the fact that the shoot biomass of RGM was generally lower than the other two genotypes; this is because the shoot biomass of RGM was lower at the beginning of the experiment. The genotype-specific differences between the

shoot biomass at the beginning of the experiment were not due to the size of the cutting as no significant differences in trunk biomass were observed (Table VI.1). Shoot/Root ratio was not affected by P treatment (data not shown).

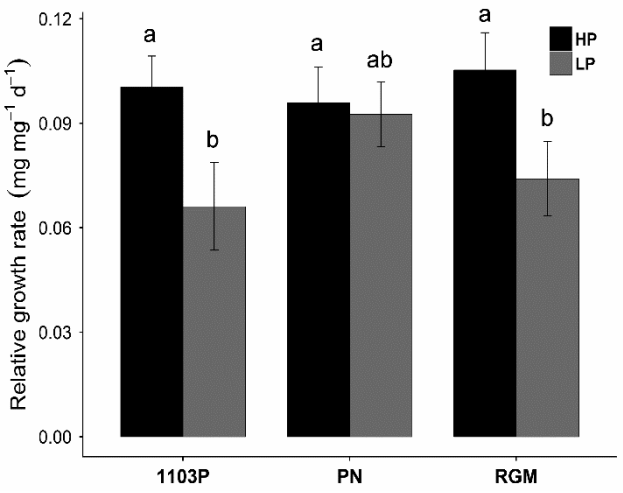

Figure VI.2. Relative growth rate (RGR) of three grapevine genotypes (1103P, PN and RGM) grown under different P supplies, high P (HP, black bars) or low P (LP, grey bars). RGR was calculated as the slope of linear regression between ln(shoot + root dry biomass) and the duration of treatment. Different letters indicate significant differences at $P < 0.05$ (t-test).

P supply and grapevine genotype affected shoot, but not root P concentrations

Root P concentration was not affected by either P supply or grapevine genotype after 21 d of treatment (Figure VI.3). However, shoot P concentration after 21 d of treatment was different between the genotypes and was affected by P supply; in addition there was a significant genotype x treatment interaction (Table VI.1). Under HP, P concentration in the shoot is two times higher in 1103P and PN than RGM; this concentration was decreased significantly under P starvation for 1103P and PN, but not for RGM (Table VI.1). In addition, total P content in shoot of RGM was approximately 3.6 and 2.3 less than 1103P and PN respectively (Table VI.1); while shoot DW was not significant different between the genotypes. These results suggest a difference in PUE between genotypes. In HP, PUE of RGM was higher than 1103P and PN (Figure VI.4), i.e. RGM produced more biomass for the same quantity of P in the new tissues. However in response to LP, the PUE of 1103P and PN increased, whereas it remained unchanged for RGM.

	1103P				PN				RGM				ANOVA		
	HP		LP		HP		LP		HP		LP		Genotype	Condition	GxC
Dry Weight (mg)															
Shoot	634.3 ± 109.7	ab	325.2 ± 143.2	ab*	605.5 ± 227.0	a	557.4 ± 217.3	a	416.4 ± 95.0	b	204.9 ± 51.8	b*	**0.010**	**0.003**	0.135
Trunk	1500.8 ± 249.5	a	1079.8 ± 385.7	a	1262.6 ± 439.6	a	1772.8 ± 628.0	a	1777.0 ± 543.1	a	1189.0 ± 213.9	a	0.577	0.273	**0.041**
Root	290.3 ± 74.1	a	133.4 ± 43.7	b	217.9 ± 73.7	a	246.3 ± 62.9	a	149.7 ± 63.0	a	67.9 ± 21.1	c	**< 0.001**	**0.002**	**0.008**
Whole plant	2425.3 ± 395.7	a	1538.3 ± 567.5	b	2086.0 ± 734.3	a	2576.6 ± 895.6	ab	3243.1 ± 571.7	a	1461.8 ± 262.7	bc	0.512	**0.010**	**0.011**
Total P (µg)															
Shoot	2113.3 ± 244.5	a	315.0 ± 235.3	d	1328.3 ± 587.9	b	350.1 ± 194.0	d	574.2 ± 290.0	c	277.7 ± 141.9	cd	**0.001**	**< 0.001**	**0.011**
Trunk	381.9 ± 268.8	a	231.8 ± 208.7	a	301.5 ± 282.6	a	315.9 ± 253.6	a	567.4 ± 94.5	a	156.3 ± 59.7	a	0.817	0.302	0.300
Root	357.0 ± 284.4	a	18.4 ± 5.3	a	161.4 ± 154.3	a	63.0 ± 54.1	a	73.9 ± 41.6	a	37.3 ± 27.8	a	0.239	0.385	0.918
Whole plant	2852.2 ± 151.7	a	565.2 ± 433.6	c	1791.2 ± 652.9	b	729.0 ± 311.8	c	1215.5 ± 342.9	a	471.2 ± 211.6	cd	**0.003**	**< 0.001**	**0.009**
Initial P stock															
µg	682.6 ± 89.6	a	736.6 ± 107.3	a	659.8 ± 101.5	a	728.9 ± 62.3	a	513.2 ± 46.3	a	508.8 ± 42.3	a	0.471	0.787	0.976
µg/mg DW	0.475 ± 0.04	a	0.486 ± 0.03	a	0.474 ± 0.04	a	0.434 ± 0.02	a	0.315 ± 0.02	a	0.413 ± 0.02	a	0.294	0.716	0.661

Table VI.1. Biomass (DW) and total P content (µg) of different plant parts (shoot, trunk, root and whole plant) of three grapevine genotypes (1103P, PN and RGM) after 21 d of growth under high P (HP) or low P supply (LP). Means and standard errors shown (n = 5). Different letters indicate significant differences at $P < 0.05$, tested using a two-way ANOVA with genotype and P supply as factors. For the shoot DW data (which had no significant interaction) letters indicate genotype effects and stars significant treatment effects at $P < 0.05$.

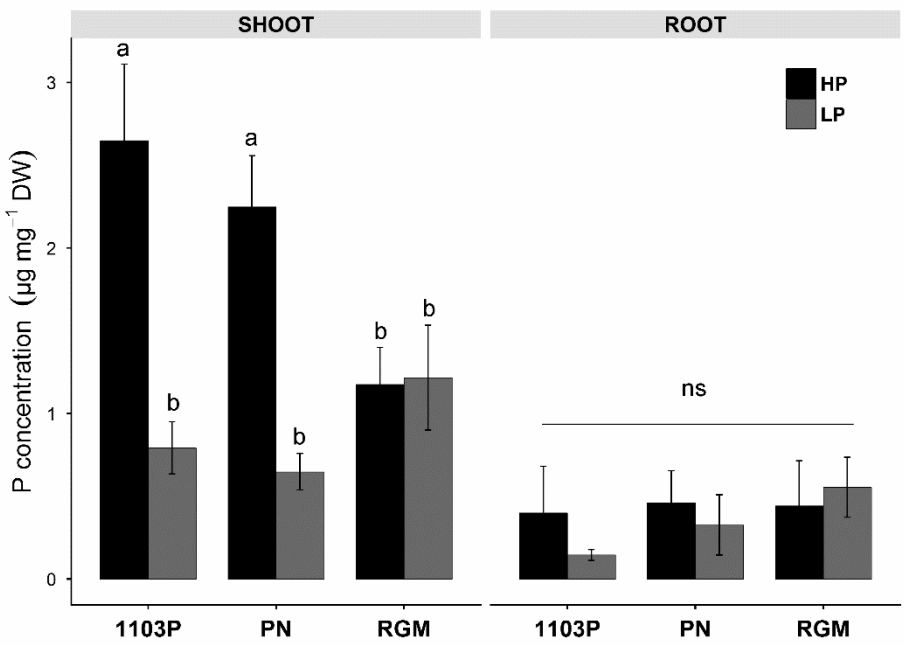

Figure VI.3. Total P concentration in shoot and root of three grapevine genotypes (1103P, PN and RGM) after 21 d of growth under high P (black) or low P supply (grey). Means and standard errors shown (n = 5). Different letters indicate significant differences at $P < 0.05$, ns = not significant, tested using a two-way ANOVA with genotype and P supply as factors.

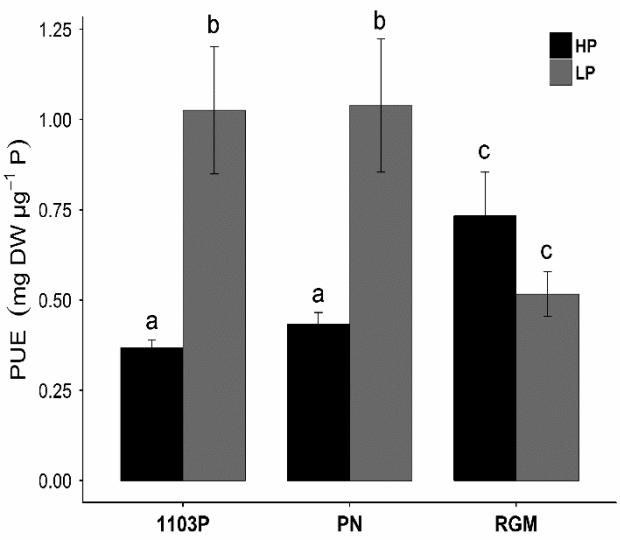

Figure VI.4. P utilization efficiency (PUE) of three grapevine genotypes (1103P, PN and RGM) during the first 21 d of growth under high P (HP, black bars) or low P supply (LP, grey bars). PUE is the slope of linear regression between shoot + root biomass and the P content. Different letters indicate significant differences at $P < 0.05$ (t-test).

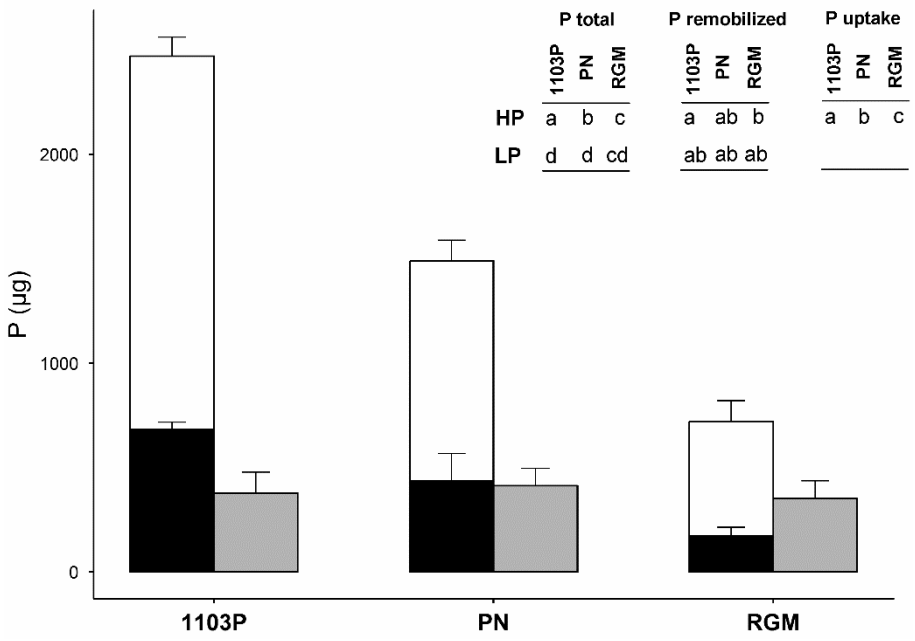

Figure VI.5. Total P content and its origins in the shoot and root of three grapevine genotypes (1103P, PN and RGM) after 21 d of growth under high P (black bars, P remobilized from the cutting, white bars, P from exogenous uptake) and low P supply (grey bars, only P remobilized from the cutting). Means and standard errors shown (n = 5). Insert, results from one- or two-way ANOVA analysis (with either genotype or P supply as factors), different letters indicate significant differences at $P < 0.05$.

Genotype-specific differences in total P contents were related to differences in P uptake, but the proportion of P derived from uptake and remobilisation remained constant

Use of ^{32}P labelling permits the identification and the quantification of the origin of P within a tissue, i.e. from exogenous ^{32}P uptake or from the remobilization of P reserves from the cutting. After 21 d of treatment, effects of genotype and P supply were observed on the total P content in shoots and roots (Figure VI.5). As expected the total P content was drastically lower in LP treatment compare to HP treatment. Under LP, no significant difference was observed for total shoot and root P between genotypes, this P was entirely derived from remobilisation of P from the cutting. Whereas under HP, total plant P was highest for 1103P, intermediate for PN and lowest for RGM, and these differences were mainly explained by the differences in exogenous P uptake. Interestingly, under HP, proportion of P from uptake and remobilization remained constant (3:1) across all genotypes.

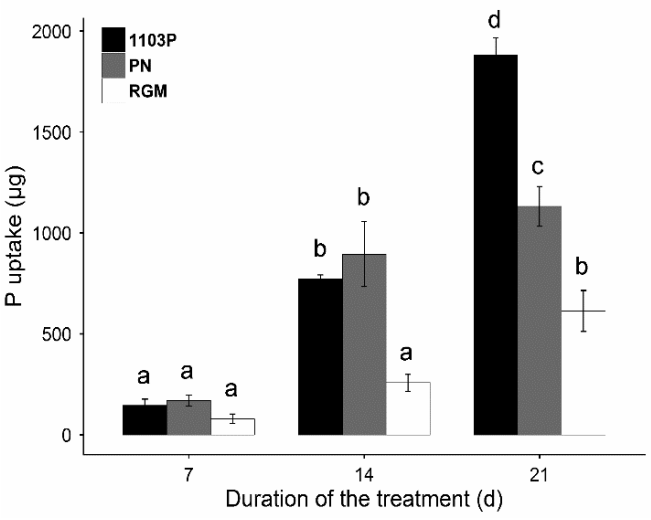

Figure VI.6. Total P uptake over time of three grapevine genotypes grown under high P supply, the genotypes are 1103P (black bars), PN (grey bars) and RGM (white bars). Means and standard errors shown (n = 5). Different letters indicate significant differences at $P < 0.05$ (two-way ANOVA with genotype and time as factors).

Grapevine genotypes differed in their P acquisition efficiency

After 7 d of treatment, P uptake had begun for all genotypes (no significant differences, Figure 6). Phosphorus uptake increased over time, especially for 1103P and PN. After 14 d of treatment, differences between genotypes were observed, with 1103P and PN showing more P uptake than RGM and these differences were increased at 21 d of treatment. Phosphorus acquisition efficiency was highest for 1103P intermediate for PN and lowest for RGM (Figure VI.7).

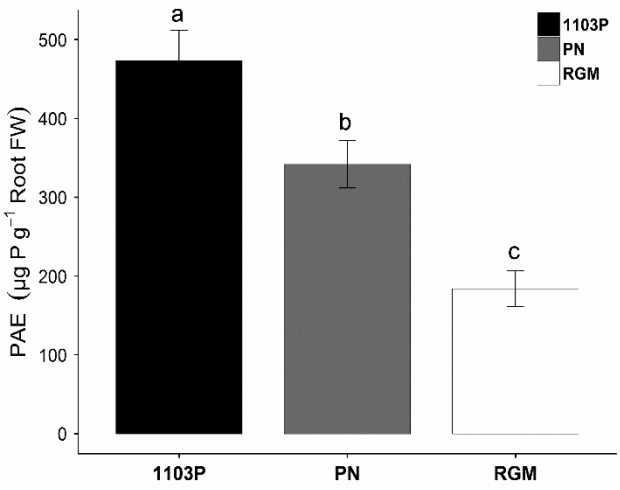

Figure VI.7. P acquisition efficiency (PAE) during the first 21 d of growth under high P supply of three grapevine genotypes: 1103P (black bars), PN (grey bars) and RGM (white bars). PAE is the slope of linear regression between exogenous P in the shoot and root fresh biomass. Different letters indicate significant differences at $P < 0.05$ (t-test).

Only one genotype differed in the rate of P remobilised from the cutting in response to P supply

Rate of remobilization was calculated as the proportion of loss of P from the cutting during the experiment for each plant. After 21 d of treatment, approximately 60 % of P reserves of the cutting were remobilized to new organs (Figure VI.8). No significant effect of P treatment was observed for 1103P and PN, whereas the rate of remobilisation of P was lower in HP than LP for RGM.

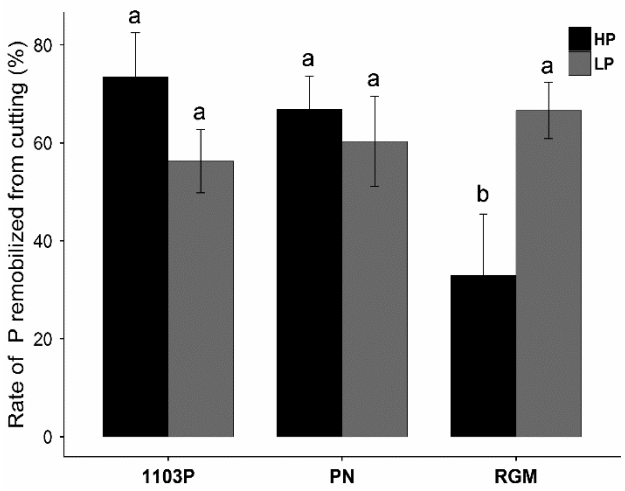

Figure VI.8. Rate of P remobilized from cutting of three grapevine genotypes (1103P, PN and RGM) after 21 d of growth under high (HP, black bars) and low P supply (LP, grey bars). Means and standard errors shown (n = 5). Different letters indicate significant differences at $P < 0.05$ (two-way ANOVA with genotype and P supply as factors).

P allocation within the plant was not affected by genotype or P supply

After 21 d of treatment, allocation of newly acquired exogenous P was studied under HP; no difference between genotypes was observed, with approximately 75 % of exogenous P allocated to the shoot and 12 % to the roots (Figure VI.9A). Allocation of P from cutting remobilization was also studied, P remobilized was preferentially allocated to shoot (80-90 %), without significant effect of P supply or genotype (Figure VI.9B). However, an increase of P allocation to the root was observed in LP for RGM.

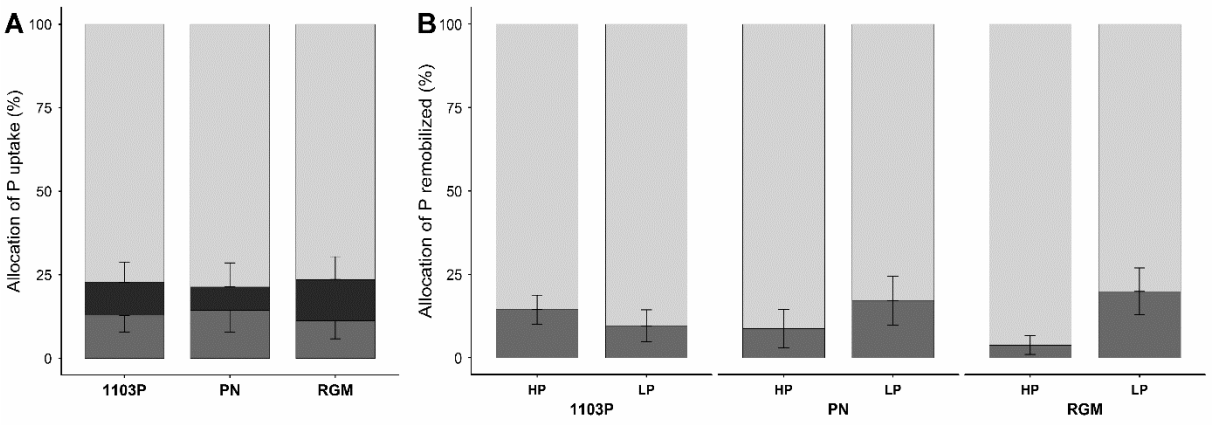

Figure VI.9. Allocation of exogenous P from uptake (A) and P from cutting remobilization (B) of three grapevine genotypes (1103P, PN and RGM) after 21 d of growth under high (HP) or low P (LP) supply; in shoot (light grey), trunk (black) and root (dark grey). No significant differences observed.

Discussion

Genotype-specific reduction of growth and P content in response to LP supply

The growth reduction reported in this work is a typical response to nutrient starvation in plants (Hermans et al., 2006; Lambers et al., 2006; Vance et al., 2003). The difference in shoot P concentration between 1103P and RGM in agreement with what is known about how these genotypes alter scion petiole P contents in the vineyard, with 1103P, and other *V. berlandieri* hybrids, increasing petiole P concentrations (Cordeau, 1998). However, root P contents were low and not significantly modified by P supply, which is unusual as for many species root and shoot P concentrations change concomitantly in response to P supply (e.g. *Lupinus spp.*, (Abdolzadeh et al., 2010); *Populus spp.*, (Gan et al., 2015)). In this study, the root systems were very young with the oldest root being 3 weeks old, so could explain low root P concentrations.

Shoot P concentrations are related to differences in PAE

As described in the introduction, it is well known that rootstocks can alter petiole or leaf P contents, but little is known about how rootstocks alter tissue P concentrations. A number of mutants have been identified in model annual species that show altered shoot P concentrations suggesting that many processes are involved including Pi uptake, translocation and remobilization as well as potential regulators like transcriptional factors or signaling molecules (Lin et al., 2009). Here we show that the differences in the concentration of P in the shoot of the three grapevine genotypes are positively

related to their PAE. In this study, PAE was assessed in conditions of high and homogeneous orthophosphate concentration with a restricted root volume, i.e. in conditions largely independent of rhizosphere interactions and root architecture. This suggests that grapevine genotypes have different Pi transport uptake efficiencies. This has been shown for both the high and low affinity nitrate uptake systems of excised roots of different grapevines genotypes (in which both the scion and rootstock genotype can alter nitrate uptake (Tomasi et al., 2011)). However, genotype-specific differences in uptake efficiencies are not necessarily independent of root system architecture because it has been shown that nitrate uptake rapidly declines in roots of over 7 days old (Volder et al., 2005). If this is also the case of Pi transport, the proportion of young versus old roots could also impact whole plant P uptake efficiency. In addition, as the development of root hairs is important to PAE (Bates and Lynch, 2000; Gahoonia and Nielsen, 2003), it is possible that the genotype-specific differences are due to differences in root hair development and differences in root surface area per unit of root biomass.

Differences in PAE, and hence shoot P content, may also be due to a shoot derived signals, particularly in grafted plants, to date the only identified shoot derived signal of P homeostasis is microRNA 399, which moves from the shoot to the root under low P conditions to increase P uptake and translocation to the shoot (as reviewed by (Ko and Helariutta, 2017)). MicroRNA 399 has been identified in grapevine, but its predicted target gene is different from that of Arabidopsis (Wang et al., 2011a) and its function is unknown.

Phosphorus utilization efficiency

Numerous mechanisms have been implicated in control of PUE in plants (Veneklaas et al., 2012) and many studies have also investigated genotype-specific variation for PUE in crop species (Rose and Wissuwa, 2012). In grapevine, the PUE of the rootstocks Freedom (a complex hybrid) and St George (*V. rupestris*) was quantified under two nutrient regimes; PUE was increased in response to LP, but not affected by the rootstock genotype (Grant and Matthews, 1996a). In the current work, PUE increased in response to LP in two genotypes (1103P and PN), but remained constant for RGM, being high under HP and low under LP in comparison to the other two genotypes studied. This suggests that RGM lacks the capacity to increase PUE in response to low P supply.

Under HP, RGM is inefficient at acquiring P from the growing medium (low PAE) so may suffer a greater degree of P deficiency stress and subsequently produce more biomass per unit of P acquired. As such, it is difficult to conclude that differences in PUE under HP in this work are real. However, under LP when all shoot and root P originates only from internal remobilization and is independent of PAE, PUE

was higher in 1103P and PN than RGM. This demonstrates that 1103P and PN can more efficiently use P to accumulate biomass under P starvation conditions than RGM. Plants have evolved numerous strategies to cope with nutritional stresses such as P deficiency, as can be observed in the PUE data presented here, differences under HP supply do not necessarily occur under LP supply.

Only one genotype differed in the rate of P remobilized from the cutting in response to P supply

Seasonal nutrient cycling is a requisite component of the perennial lifestyle, stored nutrient reserves are mobilized in the trunk and roots of support bud break and plant growth during the spring. In grapevine, the degree of P remobilization of reserves may vary considerably from year to year depending on the climatic conditions and the availability of P in the soil. In two successive years of growth of PN in a vineyard, remobilized P can account for 20 % of the P present in the shoot one year and 50 % the following year when the climate was warmer and drier and P was less available for growth (Schreiner and Scagel, 2006). In this study on the growth of cuttings of grapevine, approximately 60 % of the stored P was remobilized to support new growth in the LP conditions after 21 days. However, under HP conditions, RGM remobilized a smaller proportion of the reserves available in the cutting than the two other genotypes and less than under LP conditions, whereas the P remobilization rate did not change for PN or 1103P. Similarly, in grafted trees of *Citrus spp.* generally the fraction of P remobilized was higher under P sufficient conditions than under P deficiency, but it was genotype dependent (Zambrosi et al., 2012).

The proportion of P in new tissues originating from uptake and remobilization is the same across the three genotypes

Under HP conditions, the proportion of P from uptake and remobilization of reserves remains constant across the three genotypes studied despite the fact that the amount of P taken up varied over 3-fold and the quantity of P in the cuttings was not different between the three genotypes. This could suggest that there is a demand signal for remobilization of P reserves in the simplified system of studying three weeks of growth from cuttings (i.e. without root P reserves).

P allocation within the plant is not affected by genotype or P supply

There were no differences among the three genotypes in the allocation of exogenous P and P remobilized from reserves within the plant during the experiment with approximately 80 % of the P in the new organs allocated to the shoots. In trees, young leaves represent a strong sink for P in the spring suggesting that this is a typical allocation pattern in woody perennials (Zambrosi et al., 2012; Zavisic and Polle, 2018).

Conclusion

Grapevine rootstocks like RGM and 1103P are known to alter scion mineral P concentrations, but the mechanisms underlying these differences were unknown. For the first time we show that a genotype known to confer high petiole P content in the vineyard, 1103P, when grown as un-grafted cuttings had a higher PAE and rate of P remobilization under HP than RGM, a genotype known to confer low petiole P content. This suggests that when P is available, 1103P will take up as much P as possible and that this uptake is not subject to the same degree of feedback control as RGM. Furthermore, under LP, 1103P also has a higher PUE than RGM so it has a greater potential to accumulate biomass under limited P supply. The molecular mechanisms explaining genotype-specific differences in PAE, P remobilization and PUE in woody perennial plants are currently unknown. In model annual species such as Arabidopsis and rice, numerous mutants have been characterized and QTLs detected that control PAE and PUE (Gong et al., 2016; Yuan et al., 2017), it is probable that similar molecular players are also involved in grapevine. However, we have no knowledge of the regulation of P remobilization from woody tissues in perennials although it merits further attention.

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Perspectives

A travers cet article, nous mettons en évidence des différences génotypiques en termes d'acquisition, d'utilisation et de remobilisation du P. Tout d'abord, cultivé en boutures simples et avec un apport régulier en Pi, 1103P (*V. rupestris* x *V. berlandieri*) montre des concentrations en P plus fortes dans ses parties aériennes que RGM (*V. riparia*), comme observé au vignoble. Ces différences de concentration sont associées à un taux de remobilisation depuis la partie pérenne plus importante, ainsi qu'une efficience d'acquisition plus élevée. Il est intéressant d'observer qu'un rapport constant entre le P provenant de la remobilisation interne et le P venant de l'acquisition dans les tissus en croissance est maintenu chez les trois génotypes. Des différences importantes d'acquisition en P externe ont été mis en évidence, où 1103P montre au cours de cette expérimentation une efficience d'acquisition en P quasiment 3 fois supérieure à celle de RGM.

En réponse à l'absence en P dans le sol, les deux porte-greffes étudiés montrent des stratégies d'adaptations différentes. Dans le premier cas, 1103P montre une augmentation de sa capacité d'utilisation du P, c'est-à-dire de sa capacité de maintenir une croissance avec des teneurs en P très fortement réduites ; alors que RGM montre une augmentation du taux de remobilisation du P disponible dans la partie boisée. Dans les deux cas de Figures, ces adaptations ne sont pas suffisantes pour maintenir une croissance optimale, conduisant toutes deux à une réduction de croissance des parties aérienne et racinaire. Une variété de *V. vinifera* (PN) habituellement cultivée en tant que greffon a également étudié sur ces paramètres, montrant un comportement intermédiaire aux deux porte-greffes contrastés en termes d'acquisition, mais un comportement similaire à 1103P en termes d'utilisation et de remobilisation. Finalement, aucune différence d'allocation du P entre les racines et les feuilles n'a été mis en évidence chez les trois génotypes.

Ces résultats révèlent de réelles différences d'acquisition impliquant certainement l'activité des transporteurs PHT1. En effet, dans nos conditions expérimentales, les génotypes étudiés ont été cultivés sur un sol neutre (sable) et irriguées avec une solution nutritive n'apportant que du Pi immédiatement disponible pour l'absorption racinaire. Ces différences ne reflètent donc peu ou pas l'implication du développement racinaire et de son fonctionnement via l'exsudation de molécules induisant une augmentation la disponibilité en Pi dans le milieu.

CHAPITRE 2

Développement racinaire et modification de la rhizosphère en réponse à la disponibilité en phosphore chez différents génotypes de Vigne non greffés

Avant-propos

Les différences d'acquisition en Pi observées entre les génotypes de Vigne étudiés peuvent provenir de différents facteurs tels que l'efficacité des transporteurs de Pi de la famille des PHT1, une architecture et une morphologie racinaire favorable à l'absorption du Pi ou des modifications de la rhizosphère par le système racinaire induisant une augmentation de la teneur en Pi assimilable.

L'objectif de ce chapitre est de caractériser ces différents paramètres chez 1103P, RGM et PN cultivés en boutures simples, ainsi que l'influence de la disponibilité en Pi dans le milieu. Un système de culture en hydroponie a été mis au point, permettant de suivre l'évolution du pH des solutions nutritives au contact du système racinaire tout au long de l'expérimentation, ainsi que de faciliter l'étude du développement racinaire et l'échantillonnage des exsudats tels que les acides phosphatases et les acides organiques.

De plus, une analyse de la composition des différents tissus de la plante a été effectuée par chromatographie ionique en collaboration avec Patrick Doumas du laboratoire BPMP (Biochimie et Physiologie Moléculaire des Plantes – Montpellier), afin de déterminer les concentrations en phosphate, nitrate et acides organiques de la plante et de comprendre les caractéristiques phénotypiques observées. Enfin, une approche de biologie moléculaire a été conduite, ciblant des gènes connus pour leur implication dans la réponse à la faible disponibilité en Pi chez la plante.

Les résultats obtenus sont présentés sous forme d'un article scientifique et certains d'entre eux devront être confirmées avant soumission de l'article pour publication.

Article 3: Root system development and rhizosphere modifications are related to differences in phosphorus acquisition efficiency in grapevine

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Running head: Root development and functioning of ungrafted *Vitis spp.*

Abstract

Grapevine rootstocks are known to alter petiole phosphorus (P) concentration in the scion and this is related to differences in P acquisition efficiency (PAE). Phosphorus acquisition efficiency could be dependent on parameters such as the efficiency of phosphate transporters, the extent of soil exploration by roots and/or the modification of rhizosphere to increase Pi availability. The objective of this work was to determine which functional processes are involved in genotype-specific differences in PAE by quantifying root system development and root functioning in different grapevine genotypes. Cuttings of two American rootstocks and one European scion variety were grown in hydroponic culture and subjected to two contrasted P supply, high P (600 µM) or low P (1 µM). All genotypes studied modified root morphology and root functioning (acidification of the media and exudation of acid phosphatases) under low P supply. Furthermore, transcriptomic and metabolic responses were analyzed in response to low P supply. The rootstock genotype *Vitis berlandieri* x *V. rupestris* hybrid cv. 1103 Paulsen, known to confer high P concentration to the scion, produced a larger root system, so potentially had an advantage in soil exploration. This suggests that P concentration in the grapevine scion is related to PAE and foraging efficiency of rootstocks, and that this trait may be important for rootstock selection in the future.

Keywords: Root system architecture, Biomass partitioning, pH, Acid phosphatases, Organic acids

Introduction

Phosphorus (P) is considered as one of the most limiting macronutrients for crop production and it is involved in numerous metabolic pathways due to its role in energy transfer, including photosynthesis (Marschner, 2011). However, a large fraction of P is not directly available for plant acquisition in most soil conditions, and P is heterogeneously distributed and is the least mobile nutrient in the soil (Hinsinger, 2001; Marschner, 2011; Vance et al., 2003). In the soil, P is only taken up by root under this inorganic form (orthophosphate, Pi), but a large fraction Pi in the soil is unavailable, because it can be strongly sorbed into oxides and hydroxides of iron (Fe) and aluminum (Al) in acid soils; or the strong interaction of Pi with calcium (Ca) results in the formation of insoluble complexes in alkaline soils (Barrow, 1999; Borie and Rubio, 2003). In addition, the predominant form of P in soil solution is in an organic form, up to 80%, which comprises a mixture of chemical compounds such as sugar phosphates, mononucleotides, phospholipids and phytates (Richardson et al., 2009; Tran et al., 2010a; Turner et al., 2005; Turner and Engelbrecht, 2010). Unfortunately, organic-P is not directly available for root uptake and needs to be hydrolyzed before assimilation by plants.

Plant species and genotypes differ in their P acquisition efficiency (PAE), defined as the ability of crops to take up P from soils. Two types of strategy are described to enhance acquisition of this highly immobile nutrient: (1) increased root system development and consequently the soil exploration; and (2) mobilization of Pi in the rhizosphere (Lynch, 2011). These strategies are closely linked, because the first strategy determines the placement of root exudates and thereby their functional benefit.

Pi is taken up from the soil by phosphate transporters of PHT1 multigene family. Pi import relies on H^+-Pi cotransport (Sakano, 1990), therefore Pi import is an energy-demanding process that is tightly associated with the activity of proton ATPase pumps (Gaxiola et al., 2007). Several orthologues have been identified in different species (Nussaume et al., 2011), for example 9 genes have been identified in *A. thaliana* (*PHT1;1* to *PHT1;9*) and 12 in poplar (*Populus trichocarpa*) (Loth-Pereda et al., 2011; Poirier and Bucher, 2002). In addition, PAE is also related to the number and the activity of PHT1 transporters. In fact, some *PHT1* genes were induced in plants grown under Pi deficient conditions, to increase the P acquisition by root system, such as *PHT1;1* and *PHT1;4* or *PHT1;8* and *PHT1;9* in *A. thaliana* (Misson et al., 2004; Remy et al., 2012), resulting in an increase of potential Pi acquisition.

Root system development and architecture are important factors for soil exploration and consequently for P uptake. Morphological plasticity of the root system is a characteristic response to nutrient starvation. Commonly under low P (LP) supply, plants allocate more carbon to root growth compared to the shoot, and consequently altering shoot:root ratio, in the order to increase relative soil

exploration capacity (Hermans et al., 2006; Mollier and Pellerin, 1999). For the same purpose, higher root branching and increased production of fine roots are typical responses to LP supply (Brewster et al., 1976; Peret et al., 2011; Peret et al., 2014). Usually under LP supply, the root system is affected by reducing shoot:root ratio, increasing the production of fine roots and the emergence of lateral roots, this results in a highly branched root system with an increase of specific root length due to a decrease of root diameter (Fernandez and Rubio, 2015; Peret et al., 2011; Peret et al., 2014; Wen et al., 2017; Zhang et al., 2012; Zhu and Lynch, 2004).

The pH of the rhizosphere influences the nutrient availability and particularly P. Roots can modify pH within the rhizosphere via proton efflux or influx (Hinsinger et al., 2003), which influences the surface charges on metal oxides, ultimately leading to the release of Pi available for the plant (Geelhoed et al., 1999). Acidification could be also related to release of carboxylates, such as citric, malic or tartaric acid. They are involved in the Pi release by ligand exchange on P-sorption sites; and complexation of metal ions such as Ca, Al or Fe involved in P sorption (Bolan et al., 1994; Jones, 1998). Carboxylate release is known to be a common plant response to LP supply (Vance et al., 2003). In fact, an increase of carboxylate concentration in the rhizosphere of plants submitted to LP supply was observed for many species. However, the quantity and the proportion of different carboxylates released can be genotype dependent (Pearse et al., 2006). Enzymes such as acid phosphates (APases; EC 3.1.3.2) are able to catalyse the hydrolysis of Pi groups attached from organic P (Duff and Sarath, 1994). These enzymes are encoded by genes of the purple acid phosphatase family (*PAP*), and diverse subcellular localisations were known. In addition, some APases were secreted from root to the rhizosphere to hydrolyse organic-P present in the soil. Predominant PAP enzymes secreted were identified in *A. thaliana* as AtPAP12 and AtPAP26 (Tran et al., 2010b). An increase of transcript abundance of *PAPs* is a common plant response to low Pi conditions to increase the Pi availability for root acquisition (Del Pozo et al., 1999; Robinson et al., 2012a; Wang et al., 2011b).

In addition to maximise external Pi acquisition, plants reprioritise internal Pi use in response to LP supply (Morcuende et al., 2007; Vance et al., 2003). In fact, pools of Pi of the cell are recycled to maintain growth in P-deficient plants by different mechanisms. Phospholipids are the principal compounds of cellular membranes and constitute a rich pool of P for remobilization. Under P deficiency, phospholipids of cellular membranes are replaced by sulfolipides, and conversely, under S deficiency sulfolipides are replaced by phospholipids (Essigmann et al., 1998; Härtel et al., 1998; Sugimoto et al., 2007; Yu et al., 2002).

Grapevine is cultivated grafted, using American grapevine species as rootstocks historically introduced for their tolerance to the American aphid pest, Phylloxera (Ollat et al., 2016). The use of rootstocks in

crops, including viticulture, affects scion vigour, yield, fruit quality and tolerance to biotic or abiotic environmental conditions such as water and nutrient limitation (Nawaz et al., 2016; Warschefsky et al., 2016). As a consequence, rootstock selection offers opportunities to increase the sustainability of agriculture and the nutrient efficiency of crops (Gregory et al., 2013). Grapevine rootstocks have long been known to modify the mineral element profile of the scion, particularly in P concentration (Bavaresco et al., 2003; Cordeau, 1998; Ibacache G. and Sierra B., 2009). More specifically, differences in responses of grapevine rootstocks to P supply were observed (Grant and Matthews, 1996a; Grant and Matthews, 1996b). Grant and Matthews (1996a) have shown that rootstocks differ in their plasticity to LP supply by differences in P uptake, root system characteristics and shoot growth efficiency. In our previously study, we have shown that grapevine rootstocks have different capacities to take up Pi from the soil, to remobilize P from perennials parts and to use P for growth (Gautier et al., 2018). However, we have no knowledge of the capacity of grapevine rootstocks to alter rhizosphere to potentially increase Pi availability or modify its root system architecture or metabolism in response to LP supply. The objective of this work is to determine whether different grapevine genotypes differ in their responses to LP supply and whether these differences could explain the characteristics of two rootstock genotypes known to confer differences in P content to the scion in the vineyard.

Materials and methods

Plant material and growing conditions

Three genotypes of grapevines (*Vitis spp.*) were studied: *V. berlandieri* x *V. rupestris* cv. 1103 Paulsen (1103P), *V. riparia* cv. Riparia Gloire de Montpellier (RGM) and *V. vinifera* cv. Pinot noir (PN). Plants were propagated *in vitro* on McCown Woody Plant Medium (Duchefa) supplemented with 30 g L^{-1} sucrose and 0.27 µM 1-naphthalene acetic acid, and with 0.4 % agar, in a growth chamber at 22 °C and subjected to a photoperiod of 16 h light/8 h dark with a light intensity of 55 µmol m^{-2} s^{-1}. Six-week-old plantlets were then acclimated to perlite-filled pots, irrigated with water for four weeks, in a growth chamber at 26 °C and subjected to a photoperiod of 16 h light/8 h dark with a light intensity of 145 µmol m^{-2} s^{-1}. Plants were then transferred into hydroponic culture; each pot contained 2 plants of the same genotype with 700 mL of HP nutrient solution (described below) for 4 d. Nutrient solutions were oxygenated to 6.5 mg of O_2 dissolved per liter. Finally, plants were subjected to two P treatments: 0.6 mM of P (HP) or 0.001 mM of P (LP). 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 46.25 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 (Syngenta Agro S.A.S., Guyancourt, France) (i.e., 31.3 µmol ethylenediamine-N,N'-bis (2-hydroxyphenylacetic acid) NaFe). Nutrient solutions were refreshed each week for the 28 d of the experiment.

Plant growth measurements

After 1, 7, 14 and 28 d of treatment (DOT), leaves, stems and roots of 15 plants per genotype per P treatment were harvested, rinsed and weighed (fresh weight, FW), frozen in liquid nitrogen and kept at -80 °C for metabolic and transcript profiling. Shoot FW was calculated as leaf plus stem FW; and Plant FW was calculated as shoot plus root FW. Relative growth rate (RGR), expressed in $g.g^{-1}.d^{-1}$, was calculated as follows (Hunt, 1982):

$$RGR = \frac{Ln(Plant\ FWd) - Ln(Plant\ FWd1)}{d - d1}$$

Where *d* was the number of DOT, *Plant FWd1* was the mean plant FW after 1 DOT.

Gas exchange measurements

After 14 and 28 DOT, CO_2 assimilation rate, transpiration rate, water vapour conductance and intracellular CO_2 concentration were measured on mature leaves of 3 plants per genotype per condition, using a portable open-system infrared gas analyser (GFS 3000, WALZ). Three measurements were performed for each leaf. For each plant, the same mature leaf was used for measurements at 14 and 28 DOT.

Root system morphology analysis

After 28 DOT, five plants per genotype per P treatment were used for root system morphology analysis. Images were captured using an Epson 1640XL scanner and Epson scan software. Images were analysed using *WinRHIZO* software (Regent Instruments Inc., 1996). Number of tips, root average diameter, length, surface and volume of total root system were measured. Roots were indexed by diameter into different size classes. The percentage of each root class was calculated based on total root length. Correlations between root surface and root FW were calculated for each genotype.

Variation of the concentration of protons in nutrient solution

Three times per week, the volume and pH of nutrient solution in each pot was measured for five pots per genotype per condition and in pots without plants. pH values were transformed into proton concentrations using buffering capacity of the nutrient solutions at the beginning of week. Buffer capacities of the two nutrient solutions were calculated by the progressive addition of HCl and measurement of pH change. Variation of protons in the solution was calculated by taking into account the change in pH of the solutions without plants and volume of the solution. The surface of root system was estimated from linear regression between root FW and root surface obtained with root system morphological analysis for each genotype grown in the two P treatments.

Visualisation of the spatial distribution of root acidification was performed on roots excised after 28 DOT and placed on 0.4 % agar plates with 0.1 % (w/v) of the pH indicator bromocresol purple for 1 h. Images were captured using scanner Epson 1640XL and Epson scan software.

Activity of acid phosphatases released from the roots

After 7, 14 and 28 DOT, five plants per genotype per P treatment 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 and root surface area was calculated using from linear regression between root FW and root surface area described above. p-nitrophenyl (pNP) concentration was measured using a spectrophotometer at 405 nm.

Metabolic profile in plant tissues

The root, stem and leaf samples from 28 DOT were ground using a *Retsch^{TM} 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, tartrate and fumarate) were identified and quantified by ion exchange chromatography ICS-5000 HPIC system (Thermo Scientific Dionex), using an isocratic gradient of KOH from 4 to 28 mM for 16 min, with a volume of sample injected of 20 µL.

Transcript level quantification

After 1, 7 and 28 DOT, three pools of three root tips per plant (~15 mm in length) 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 (q) PCRs 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. Gene expression was calculated as normalized relative quantities (NRQs) as defined by Hellemans et al. (2007), with the reference genes ACTIN, GAPDH and SAND3' for normalization. Primer sequences are listed in Supplementary Table VII.1.

Statistical analysis

All statistical analyses were performed using the software R (R Core Team, 2016). When assumptions for parametric tests were not respected, genotypic differences for a given P supply were analysed by a multiple comparison test after a Kruskal- Wallis test at $P < 0.05$, 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. P-treatment effects for each genotype were analysed by a Wilcoxon non-parametric test at $P < 0.05$, with the Bonferroni correction. When assumptions for parametric test were respected, data were analysed using a two or three-way analysis of variance (ANOVA $P < 0.05$, with Tukey's Honest Significant Difference test), with genotype, P supply and days of treatment as factors).

Results

Grapevine genotypes differed in their growth and photosynthetic activity under non-limiting HP condition

There were intrinsic differences in plant growth under non-limiting conditions (HP) between the genotypes studied. 1103P had a higher biomass (Figure VII.1A, D, E, F, G), more leaves (Figure VII.1I) and, correspondingly, higher RGR (Figure VII.1B) than the two other genotypes at 28 DOT, although many differences appeared earlier (Supplementary Table VII.2). The shoot:root biomass ratio was higher for RGM compared to the other two genotypes (Figure VII.1C) and these differences were observed from the 1 DOT (Supplementary Table VII.2). The genotypes also differed in the allocation of biomass between the leaves and stems, with 1103P allocating more biomass to leaves than RGM (Figure VII.1H).

After 14 and 28 DOT under non-limiting conditions, CO_2 assimilation rate, transpiration rate, water vapour conductance and intracellular CO_2 concentration were measured on mature leaves (Table VII.1). Significant differences between genotypes growing under HP supply were observed; after 14 DOT CO_2 assimilation rate, transpiration rate, and water vapour conductance were two-fold higher in 1103P and PN compared to RGM. After 28 DOT, measurements done on the same leaves showed an increase in CO_2 assimilation rate, transpiration rate and water vapour conductance values for 1103P grown under HP supply compared to RGM and PN.

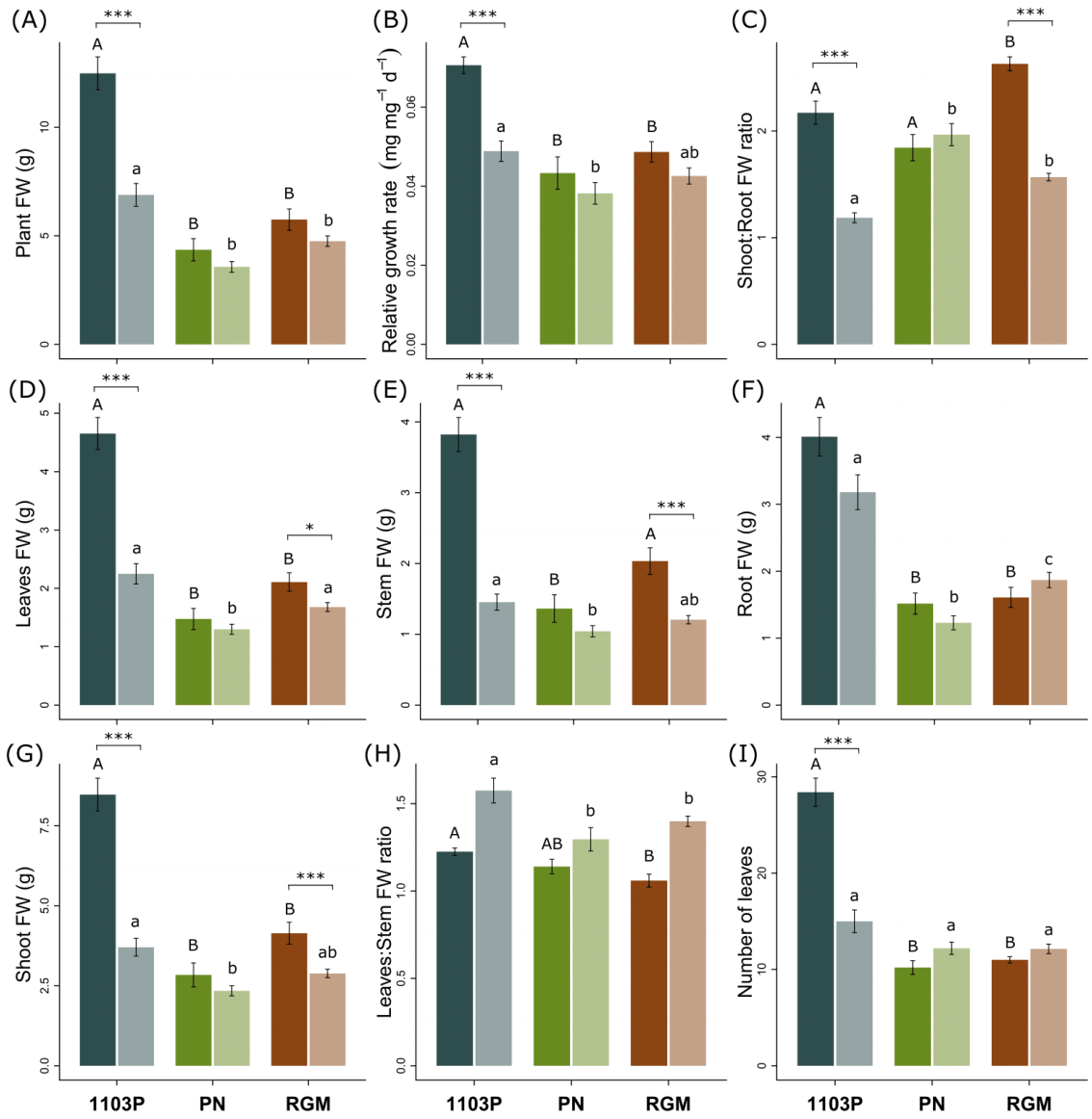

Figure VII.1. (A) Whole plant fresh weight (FW); (B) relative growth rate; (C) shoot:root FW ratio; (D-G) leaf, stem, root and shoot FW; (H) leaves:stem FW ratio and (I) number of leaves of 1103P, PN and RGM cultivated in hydroponic culture under different P supplies, high P (dark colours) or low P (light colours), after 28 days of treatment. Means and standard errors shown (n=10). For each P-treatment, genotypic differences were analysed using a multiple comparison after Kruskal-Wallis tests at $P < 0.05$, uppercase letters indicate differences under HP supply while lowercase letters indicate differences under LP supply. For each genotype, stars indicate an effect of P supply using Wilcoxon test with the Bonferroni correction (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

	1103P				PN				RGM			
	HP		LP		HP		LP		HP		LP	
14 DOT												
A (μmol m^{-2} s^{-1})	3.77 ± 0.71 ^{A}		4.80 ± 0.32 ^{a}		4.28 ± 0.55 ^{A}		3.56 ± 0.41 ^{ab}		1.57 ± 0.13 ^{B}		2.74 ± 0.23 ^{b}	
E (mmol m^{-2} s^{-1})	1.13 ± 0.11 ^{A}		1.39 ± 0.04 ^{a}		0.97 ± 0.03 ^{A}		0.89 ± 0.09 ^{b}		0.55 ± 0.06 ^{B}		0.78 ± 0.09 ^{b}	
GH_2O (mmol m^{-2} s^{-1})	77.99 ± 8.36 ^{A}		95.27 ± 4.44 ^{a}		68.90 ± 2.41 ^{A}		63.83 ± 6.83 ^{b}		38.07 ± 3.84 ^{B}		55.41 ± 7.03 ^{b}	
C_i (ppm)	317.54 ± 7.95 ^{A}		312.01 ± 4.27 ^{a}		297.54 ± 11.98 ^{A}		307.97 ± 4.68 ^{a}		322.24 ± 9.37 ^{A}		298.97 ± 17.61 ^{a}	
28 DOT												
A (μmol m^{-2} s^{-1})	5.45 ± 0.28 ^{A}		2.71 ± 0.20 ^{a}	***	3.55 ± 0.12 ^{B}		1.42 ± 0.04 ^{b}	***	2.00 ± 0.08 ^{B}		1.11 ± 0.06 ^{b}	***
E (mmol m^{-2} s^{-1})	1.32 ± 0.08 ^{A}		0.84 ± 0.05 ^{a}	***	0.73 ± 0.03 ^{B}		0.44 ± 0.01 ^{b}	***	0.85 ± 0.02 ^{B}		0.42 ± 0.01 ^{b}	***
GH_2O (mmol m^{-2} s^{-1})	100.37 ± 5.93 ^{A}		62.82 ± 4.32 ^{a}	***	53.51 ± 1.86 ^{B}		31.74 ± 0.89 ^{b}	***	67.14 ± 4.76 ^{B}		30.30 ± 0.69 ^{b}	***
C_i (ppm)	304.62 ± 0.93 ^{A}		322.11 ± 3.75 ^{ab}		284.49 ± 9.08 ^{B}		317.72 ± 3.02 ^{b}		338.05 ± 1.99 ^{A}		334.60 ± 4.81 ^{a}	

Table VII.1. CO_2 assimilation rate (A), transpiration rate (E), water vapour conductance (GH_2O) and intracellular CO_2 concentration (C_i) of 1103P, PN and RGM cultivated in hydroponic culture under different P supplies, high P (HP) or low P (LP); after 14 and 28 days of treatment. Means and standard errors shown (n=9). For each P-treatment, genotypic differences were analysed using a multiple comparison after Kruskal-Wallis tests at $P < 0.05$, uppercase letters indicate differences under HP supply while lowercase letters indicate differences under LP supply. For each genotype, stars indicate an effect of P supply using Wilcoxon test with the Bonferroni correction (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Low P supply affected some growth and gas exchange parameters in 1103P and RGM, but not PN

Low P supply reduced the biomass of the whole plant (Figure VII.1A), shoot (Figure VII.1C), stem (Figure VII.1E) and leaves (Figure VII.1D) of 1103P but did not alter root biomass (Figure VII.1F); this was associated with a corresponding decrease in leaf number (Figure VII.1I) and RGR (Figure VII.1B). Low P supply also reduced shoot biomass of RGM (both the leaf and stem FW, Figure VII.1D and VII.1D respectively), but it did not reduce whole plant biomass (Figure VII.1A), RGR (Figure VII.1B) or the number of leaves (Figure VII.1B). The reduction in shoot FW of both 1103P and RGM from 14 DOT resulted in a significant decrease of shoot:root biomass ratio (Figure VII.1C). Conversely, growth and biomass allocation of PN were not affected by LP supply (Supplementary Table VII.2 and Figure VII.1). At 28 DOT, CO_2 assimilation rate, transpiration rate and water vapour conductance were decreased between 40 and 60 % under L P supply for the three genotypes (Table VII.1).

Root system morphology is genotype dependent and affected by low P supply

After 28 DOT, the root system morphology was different between the genotypes. Length, surface area and volume of root system were calculated (Supplementary Figure VII.1). The length, surface area and volume of the root system of 1103P were higher than RGM and PN, in agreement with the root FW data (Figure VII.1F). Total root system surface area was strongly correlated with root FW and not affected by P treatment. Consequently, the correlation between root FW and total root system surface for each genotype was calculated (Supplementary Figure VII.2). The total number of tips on the root system was significantly different between genotypes, where 1103P had two times more root tips than RGM and PN, but LP supply did not affect the number of tips for any of the genotypes (Figure VII.2A). However, the tip density (number of tips per cm of root) and the root average diameter were not different between the three genotypes but were decreased under LP supply (Figure VII.2B-C). During the analysis, roots were categorized into nine classes depending on their diameter. The distribution of these different diameter classes in the root system was calculated using the cumulated root length of each of the classes. Under HP supply, 1103P and RGM had different root class distributions, a significantly higher proportion of roots with a diameter ranging between 0.4 and 0.6 mm was observed for RGM compared to 1103P (Figure VII.3). Conversely, 1103P had a higher proportion of roots with a diameter ranging between 0.2 and 0.4 mm than RGM (Figure VII.3). In response to LP supply, only 1103P modified significantly its root diameter distribution with an increase of roots with a diameter ranging between 0.4 and 0.6 mm.

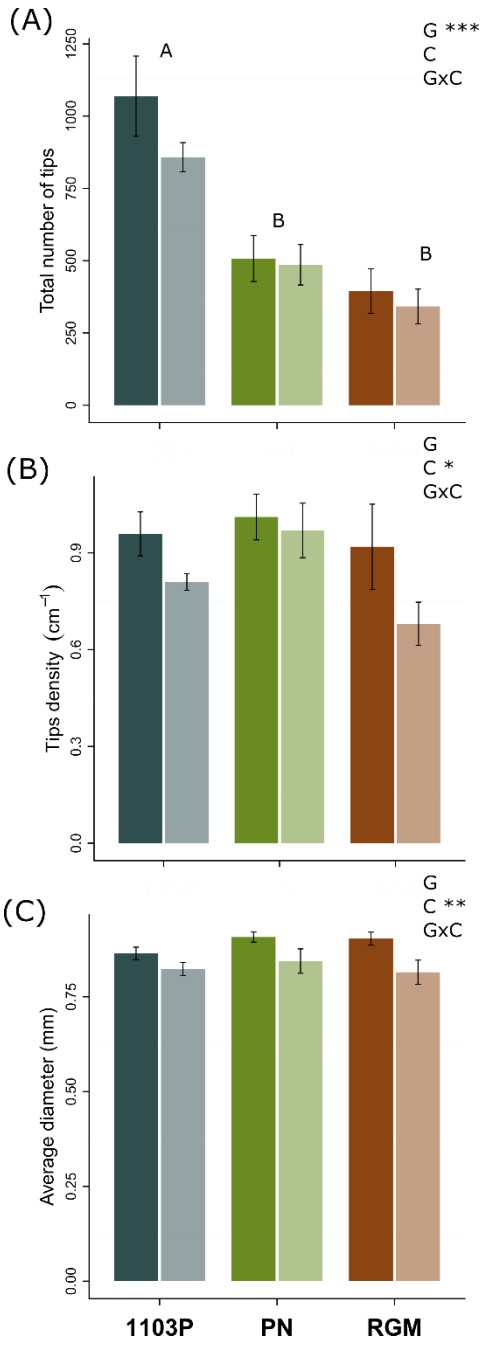

Figure VII.2. (A) Number of tips, (B) tip density and (C) average diameter of total root system of 1103P, PN and RGM cultivated in hydroponic culture under different P supplies, high P (dark colours) or low P (light colours); after 28 days of treatment. Means and standard errors shown (n=5). Data were analysed using a two-way ANOVA, with genotype (G) and P supply (C) as factors, stars indicate significant differences (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Uppercase letters indicate differences between genotypes analysed using a Tukey test.

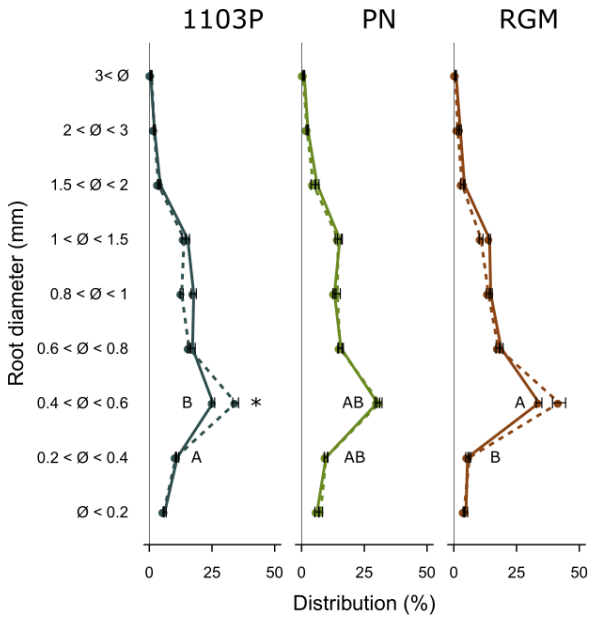

Figure VII.3. Percentage of each root diameter class of the total length of root system of 1103P, PN and RGM cultivated in hydroponic culture under different P supplies, high P (dark colours connected by solid lines) or low P (light colours connected by dashed lines); after 28 days of treatment. Means and standard errors shown (n=5). For each P-treatment, genotypic differences were analysed using a multiple comparison after Kruskal-Wallis tests at $P < 0.05$, uppercase letters indicate differences under HP supply. For each genotype, stars indicate an effect of P supply using Wilcoxon test with the Bonferroni correction (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

pH of the nutrient solutions was affected by root functioning and depends of P supply

The buffering capacity of each nutrient solution was calculated using HC1 1 M within the pH range of 8 to 3 (Supplementary Figure VII.3). HP solution had a higher buffering capacity than LP solution. Each week, nutrient solutions were refreshed, and the pH was adjusted to 5.8. The changes of pH of the two nutrient solutions without plants were measured and an increase of pH was observed for both solutions (Supplementary Figure VII.4). pH measurements were done 2, 4 and 7 d after changing the nutrient solution for 4 weeks. Taking account of the buffer capacity of the solution, the variation of pH without plants and the volume of solution in each pot, the flux of protons in the solution relative to the fresh solution was calculated and normalized by the estimated root surface area per pot (Figure VII.4). Under HP, the three genotypes alkalinised the nutrient solution. Conversely, under LP supply the content of protons in the nutrient solution did not change for the first 18 DOT, from 18 DOT there

was an increase in the flux of protons to the nutrient solution for RGM and 1103P. Significant differences between the two P treatments for each genotype were observed for all measurement points. Phosphate supply affects the localization of acidification by roots (Supplementary Figure VII.5). Under HP supply acidification of rhizosphere was localized around tips, while under LP supply acidification was localized all along the root.

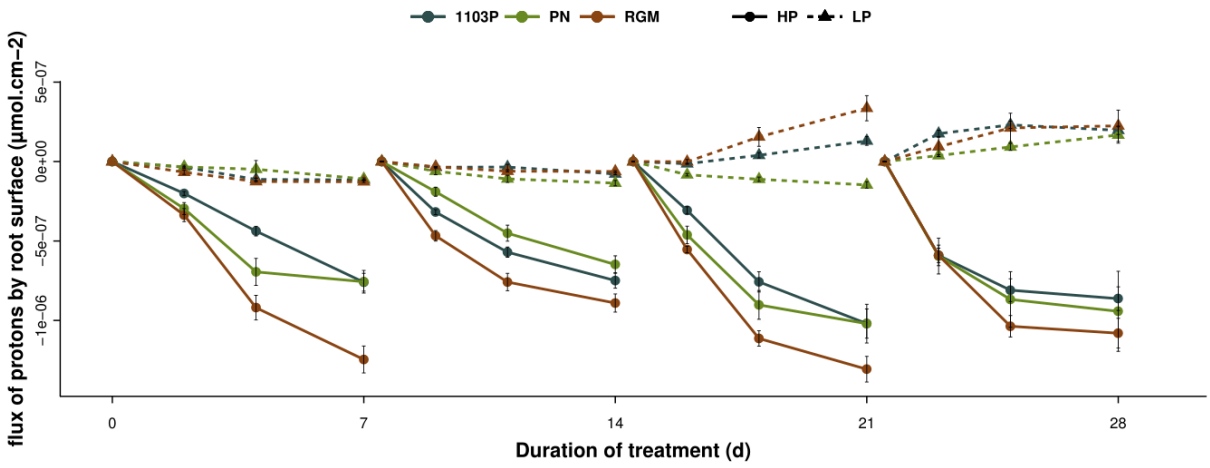

Figure VII.4. Variation of proton content in the nutrient solution surrounding roots of 1103P, PN and RGM cultivated in hydroponic culture under different P supplies, high P (dark colours connected by solid lines) or low P (light colours connected by dashed lines); during 28 days of treatment. Means and standard errors shown (n=5). For a given time point (except at the replacement of nutrient solution), significant differences were observed between P supply using Wilcoxon test at $P < 0.05$ with the Bonferroni correction.

Activity of APases released by the roots increased under low P supply

The activity of APases released into the media was calculated after 7, 14 and 28 DOT (Figure VII.5). After 14 DOT, the activity of APases released into the media by the roots was increased two-fold for each genotype under LP compared to HP supply; there was no significant difference between genotypes. However, after 28 DOT, the activity of APases released into the media by the roots of PN was significantly greater than RGM and 1103P, but the level of increase under LP supply was the same for each genotype.

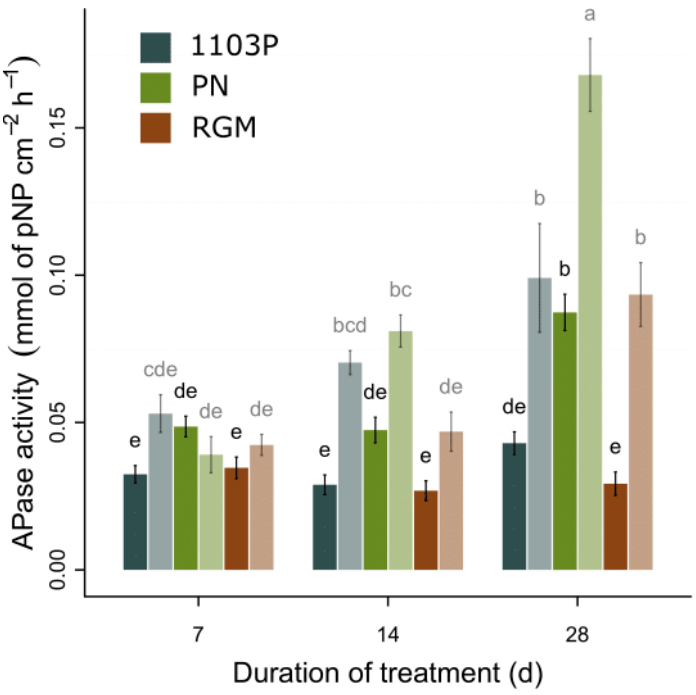

Figure VII.5. Activity of acid phosphatases released by roots of 1103P (blue), PN (green) and RGM (brown) cultivated in hydroponic culture under different P supplies, high P (dark colours) or low P (light colours); after 7, 14 and 28 days of treatment. Means and standard errors shown (n=5). Letters indicate significant differences ($P < 0.05$) using a Tukey test after a three-way ANOVA, with genotype, P supply and duration of treatment as factors.

Metabolite profile of the plants was affected by P supply

After 28 DOT, ethanolic extracts of leaves, stems and roots were analysed by ion exchange chromatography to determine the concentration of Pi, nitrate, sulphate and organic acids in plants tissues. Under HP, differences in Pi content in whole plant were observed between genotypes, 1103P had a higher Pi content than RGM, and PN was intermediate, between the two American grapevine genotypes (Figure VII.6). Pi content was higher under HP compared to LP supply. Under HP, the concentration of nitrate was not different between the three grapevine genotypes in all parts of plants. However, LP supply increased the nitrate concentration of stem of PN and RGM two-fold (Figure VII.7A). The concentration of sulphate was generally not different under HP supply between the genotypes, but a significant increase (approximately 1.5 to 2-fold) of sulphate concentration was observed in stem and root tissues under LP supply for all genotypes (Figure VII.7B). Concentration of total organic acids (the sum of malate, tartrate, citrate and fumarate concentrations) was not different

in the different organs of plant between the different genotypes but was increased in the leaves of 1103P and RGM under LP (Figure VII.8). However, differences in the proportions of the different organic acids between genotypes were observed. 1103P had lower malate concentrations in leaves compared to PN and RGM (Figure VII.9). Furthermore, concentration of citrate in all organs was lower for 1103P compared to PN and RGM (Figure VII.9). Finally, tartrate concentration was higher in the leaves of 1103P than PN and RGM (Figure VII.9).

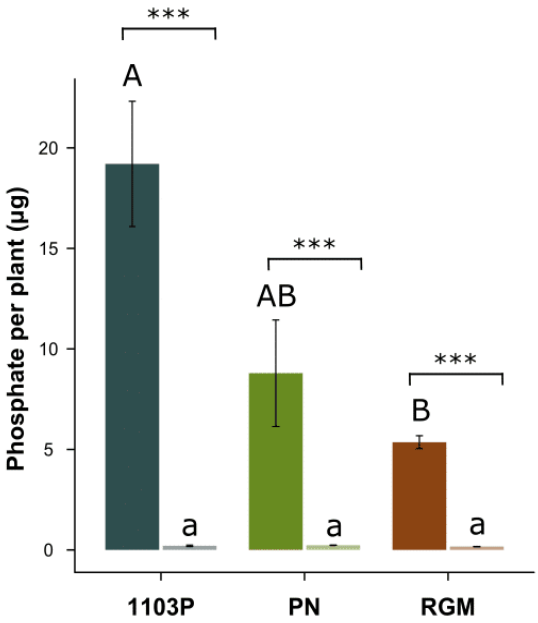

Figure VII.6. Total phosphate content per plant of 1103P, PN and RGM cultivated in hydroponic culture under different P supplies, high P (dark colours) or low P (light colours); after 28 days of treatment. Means and standard errors shown (n=5). For each P-treatment, genotypic differences were analysed using a multiple comparison after Kruskal-Wallis tests at $P < 0.05$, uppercase letters indicate differences under HP supply while lowercase letters indicate differences under LP supply. For each genotype, stars indicate an effect of P supply using Wilcoxon test with the Bonferroni correction (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

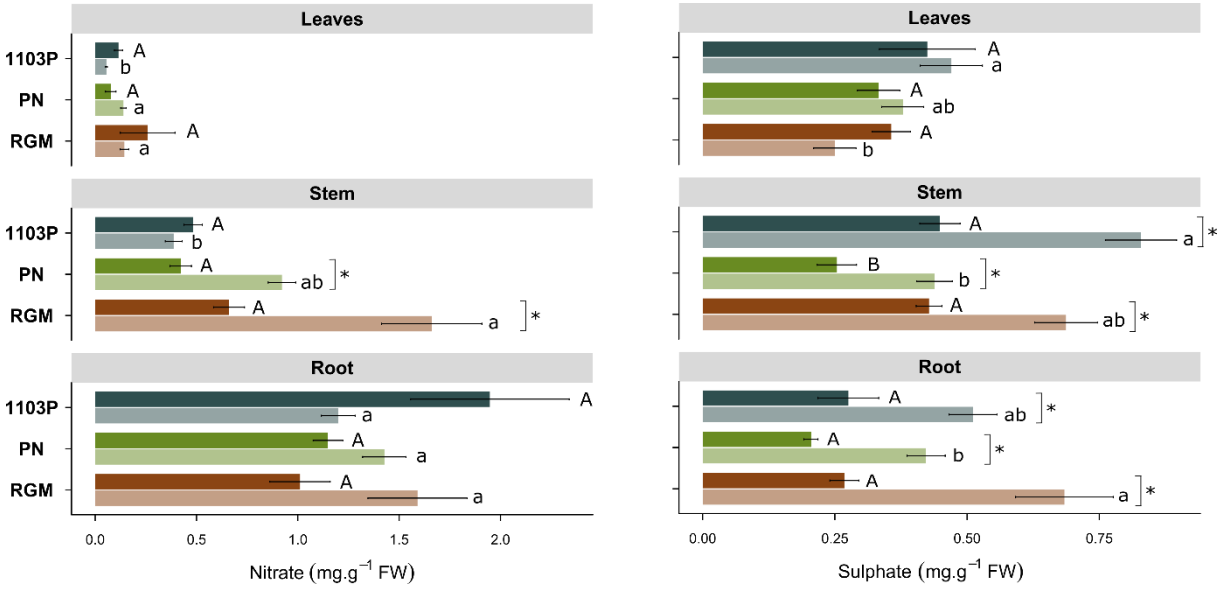

Figure VII.7. Nitrate and sulphate concentration in leaves, stems and roots of 1103P, PN and RGM cultivated in hydroponic culture under different P supplies, high P (dark colours) or low P (light colours); after 28 days of treatment. Means and standard errors shown (n=5). For each P-treatment, genotypic differences were analysed using a multiple comparison after Kruskal-Wallis tests at $P < 0.05$, uppercase letters indicate differences under HP supply while lowercase letters indicate differences under LP supply. For each genotype, stars indicate an effect of P supply using Wilcoxon test with the Bonferroni correction (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

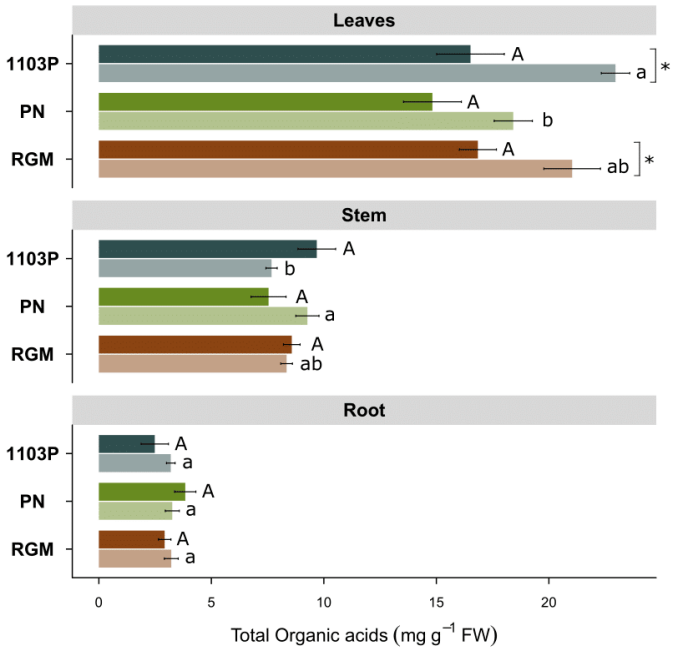

Figure VII.8. Total organic acid (malate, tartrate, citrate and fumarate) concentration in leaves, stems and roots of 1103P, PN and RGM cultivated in hydroponic culture under different P supplies, high P (dark colours) or low P (light colours); after 28 days of treatment. Means and standard errors shown (n=5). For each P-treatment, genotypic differences were analysed using a multiple comparison after Kruskal-Wallis tests at $P < 0.05$, uppercase letters indicate differences under HP supply while lowercase letters indicate differences under LP supply. For each genotype, stars indicate an effect of P supply using Wilcoxon test with the Bonferroni correction (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

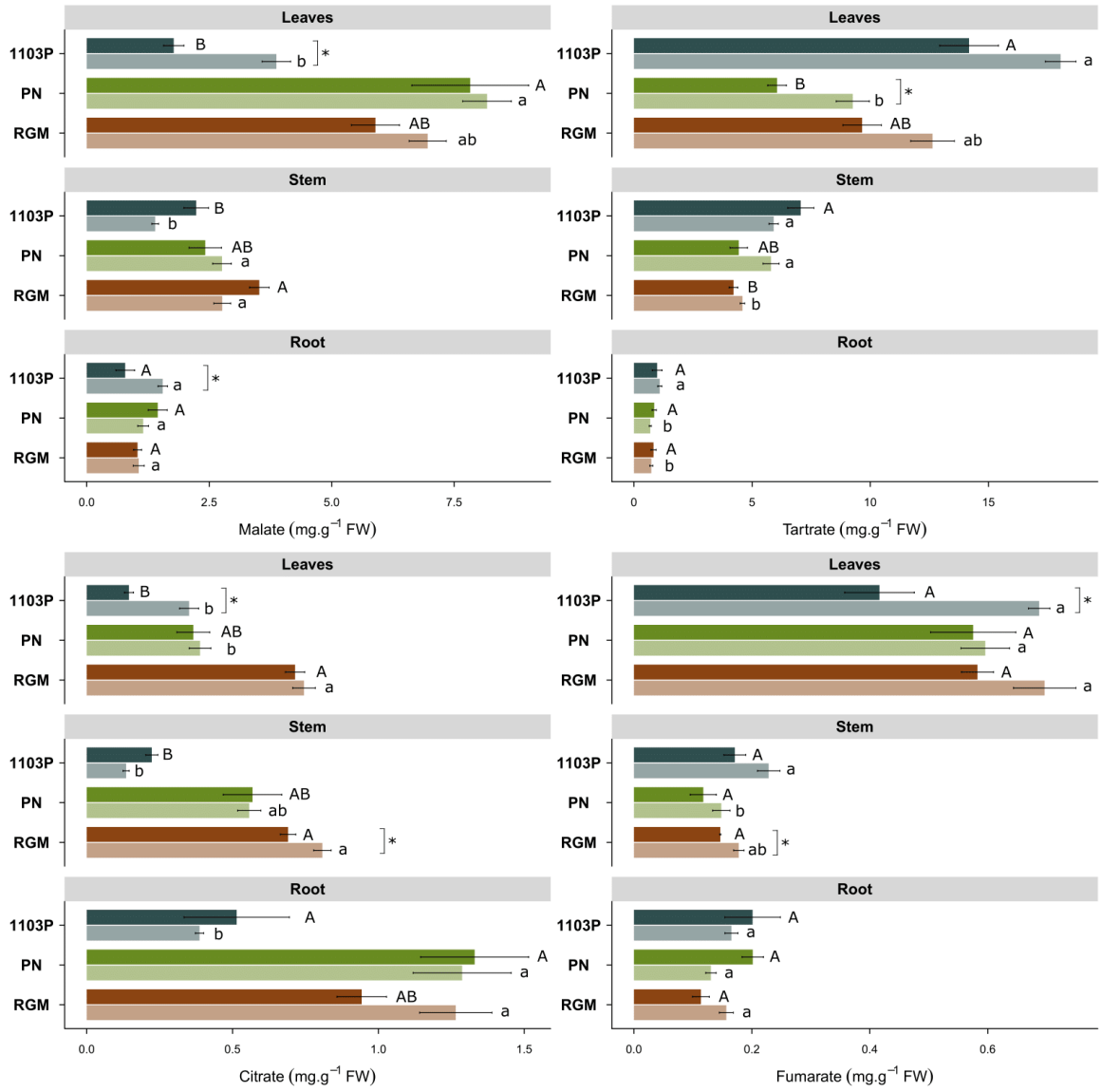

Figure VII.9. Malate, tartrate, citrate and fumarate concentrations in leaves, stems and roots of 1103P, PN and RGM cultivated in hydroponic culture under different P supplies, high P (dark colours) or low P (light colours); after 28 days of treatment. Means and standard errors shown (n=5). For each P-treatment, genotypic differences were analysed using a multiple comparison after Kruskal-Wallis tests at $P < 0.05$, uppercase letters indicate differences under HP supply while lowercase letters indicate differences under LP supply. For each genotype, stars indicate an effect of P supply using Wilcoxon test with the Bonferroni correction (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Gene expression

The transcript abundance of different genes involved in phosphorus nutrition were analysed in the roots after 1, 7 and 28 DOT (Figure VII.10). The expression of transcripts of seven *PHT1* genes was quantified, under HP supply there were genotypic differences in the level of expression. Furthermore, the transcript abundance of some of the *PHT1* family increased under LP supply; *PHT1;4a* and *PHT1;4b* were up-regulated in all genotypes, whereas *PHT1;3b* and *PHT1;4e* were up-regulated by only 1103P and RGM. The expression of four members of the *PHO1* gene family (involved in Pi loading to xylem for transport to shoot) did not differ between the genotypes or P treatments. Proteins containing SPX domains are known to play a crucial role in the P signalling, under LP supply, SPX transcripts abundance was increased more than 32-fold compared to HP supply. Phospholipase D (PLD) transcript abundance did not differ between the genotypes and did not change under LP supply. The abundance of *PAP* transcripts differed between genotypes and P-treatments. In fact, *PAP10bc* and *PAP12c* were induced in response to LP supply for all genotypes, while *PAP10a*, *PAP26a* and *PAP26b* did not show a change of transcript level under LP supply. The expression of *HA* genes, encoding H^+-ATPase, mainly showed difference between the genotypes under HP supply, with PN showing a higher-level expression than RGM and 1103P.

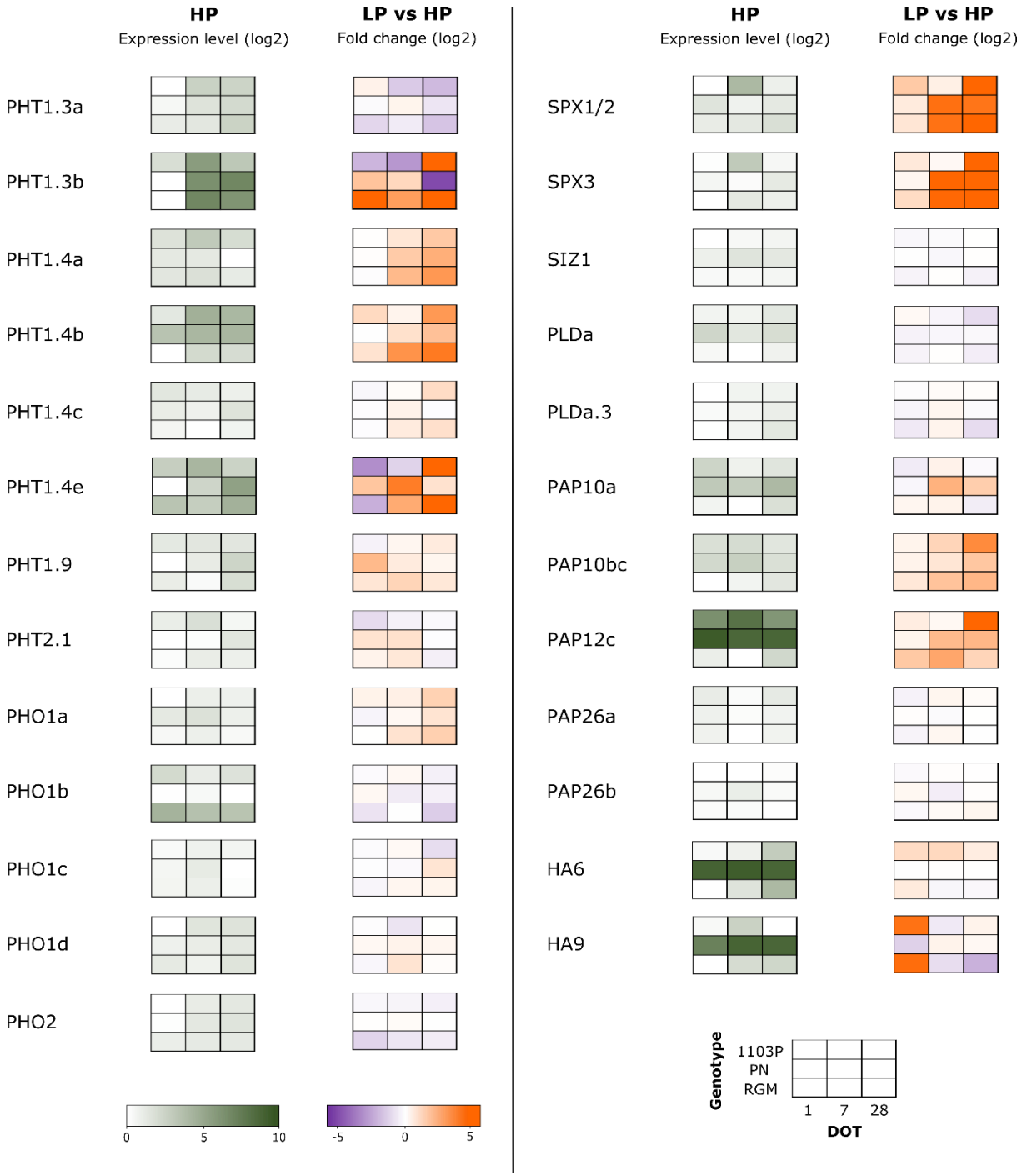

Figure VII.10. Heatmaps of the abundance of transcripts for some genes involved in phosphorus nutrition in roots of 1103P, PN and RGM cultivated in hydroponic culture and their response to low P (LP) supply after 1, 7 and 28 days of treatment. For transcript abundance under high P (HP) supply, green shade indicates the level of expression relative to the lowest value (n = 3). For transcript abundance changes in response to LP supply, the purple and orange shades indicate the extent of down- and up-regulation respectively (n = 3).

Discussion

The genotypes differ in growth and photosynthesis under non-limiting conditions

There were differences in plant biomass between the different genotypes studied under the HP treatment; 1103P had the highest biomass and RGR. The high biomass of 1103P was correlated with an increase in photosynthetic activity which could underlie the high RGR of this genotype compared to PN and RGM. 1103P is known to have a greater biomass than RGM when grown as un-grafted cuttings (Gautier et al., 2018) and to confer higher shoot biomass to the scion (Cochetel et al., 2017; Lecourt et al., 2015). These differences in development could be related to nutrient use and/or acquisition efficiency under sufficient supply, as already demonstrated for these genotypes for nitrogen (Lecourt et al., 2015) or for P (Gautier et al., 2018). In addition, when grown grafted with the scion *V. vinifera* cv. Cabernet Sauvignon, 1103P is known to increase the assimilation of CO_2 compared to RGM (Shtein et al., 2017), in agreement with our results.

Genotype-specific reduction of growth in response to low P supply, but photosynthesis is inhibited similarly in the three genotypes studied

In our study, the responses of plants to P supply were assessed in homogenous, hydroponic system in which all P was present as Pi and directly available for plant uptake. The LP treatment reduced total plant biomass for 1103P, but not for the other genotypes, this reduction in biomass was primarily due to reductions in shoot growth. Shoot growth was also reduced in RGM, but this did not reduce overall plant biomass as there was a slight increase in root biomass. The decrease of shoot:root FW ratio observed for the two American grapevine genotypes, 1103P and RGM, is a common response to LP supply. In fact, under low P availability, plants can decrease their shoot:root ratio to explore a greater surface of soil to improve Pi acquisition related to shoot growth requirement (Hermans et al., 2006; Liu et al., 2016b; Mollier and Pellerin, 1999). This modification in biomass partitioning suggests that P-deficient plants invested more carbon to form new roots and increase the P foraging capacity (Fernandez and Rubio, 2015). These results are in agreement with the growth reduction during the first step of development of grapevine woody cuttings, where PN was not affected by LP supply concerning growth and biomass partitioning (Gautier et al., 2018). Such genotype-specific morphological traits and the plasticity of root system can underlie differences in PAE.

After 28 DOT, CO_2 assimilation rate, transpiration rate and water vapour conductance was decreased in response to LP supply for all genotypes, a reduction in photosynthetic activity in response to LP is well known (Foyer and Spencer, 1986; Wissuwa et al., 2005). Reduction of photosynthetic activity could explain reduction in shoot growth of grapevine.

There are genotypic differences in P uptake, but not P allocation within the plant

Differences in total P content in the plants under HP supply reveal genotype-specific differences in Pi uptake independently of the capacity of the genotypes to explore the soil and/or increase the Pi availability with rhizosphere modifications (i.e. releases of APases and/or organic acids). This suggests that differences in P content were related to difference in capacity to take up P. These results are in agreement with our previous study which used ^{32}P to quantify PAE in the same grapevine genotypes (Gautier et al., 2018). In fact, a higher PAE was observed for 1103P compared to PN, itself higher than RGM, therefore explaining the differences in P content observed in HP supply between genotypes. Under HP supply, 28 DOT, the relative expression of genes from *PHT1* family that we identified in the grapevine genome showed differences between the three genotypes studied. *PHT1.4a* and *PHT1.4b* were more expressed in 1103P than RGM and could be potentially be involved in the higher PAE of 1103P. Other *PHT1* transporters such as *PHT1.3a*, *PHT1.4c*, *PHT1.4e* or *PHT1.9* did not show clear differences in expression between the different genotypes. All genes of *PHT1* family are known to show differences in response to LP supply (Teng et al., 2013) Furthermore, these results concern only the relative expression of *PHT1* genes, but do not consider the quantity of proteins synthesised, their trafficking to the plasma membrane or their activity. In our case, all genes with a homology to *PHT1.4* of *A. thaliana* were strongly up-regulated under LP supply, but the level of induction was genotype dependent. Furthermore, enhancement of *PHT1;4* expression level is a known response to LP supply to increase P uptake (Misson et al., 2004).

In addition to the PHT1 family, PHO1 is also important as it is involved in loading Pi from root cells to the xylem. In fact, *A. thaliana pho1* mutant shows several Pi deficiencies in shoot tissues with a normal P concentration in roots (Hamburger, 2002). In our case, the relative expression of four *PHO1* homologues was not different between genotypes under HP condition. These results agree with the Pi distribution in the different parts of plants, without difference between genotypes. Furthermore, in our previous study on ^{32}P uptake and allocation, we found no different in P allocation between the genotypes (Gautier et al., 2018).

Low P supply does not alter root biomass, but modifies root morphology

Root system development and architecture are important factors for soil exploration and consequently for P uptake. Morphological plasticity of the root system is a characteristic response to nutrient starvation. Our results shown that grapevine genotypes maintain their total root system length but reduced the root average diameter under LP supply. These responses are characteristics of P deficiency responses and already observed on different species (Cao et al., 2016). The decrease of root diameter, usually associated with a higher specific root length, indicates the development of a root system with a decreased metabolic demand per unit of root length (Pang et al., 2010; Zobel et al., 2007). The proportion of fine roots (from 0.4 to 0.6 mm in diameter) was significantly increased by LP supply for 1103P. For mature grapevines in the field, fine roots (less of 1mm in diameter), are critical for uptake of water and nutrients (Keller, 2015; Richards, 1983). A highly branched root system is a common response to LP supply; however, in this study the tip density (number of tips per cm of root) was decreased by LP supply.

All genotypes increase the concentration of sulphate and nitrate in response to LP

In our study, under low P supply the concentration of sulphate in stem and root of all genotypes was increased. We can suppose that under LP supply, phospholipids of cellular membranes in stem and root are replaced by sulpholipids. Proteins from the phospholipase D family are known to catalyse the degradation of phospholipids of cellular membranes to remobilize P under LP supply. However, the abundance of *PLDa* and *PLDa.3* were not increased under LP supply in our study. LP supply increased also the nitrate concentration of stem of PN and RGM, this may be due to the considerable cross-talk between nitrate and phosphate signalling in plants (Jeschke et al., 1997; Rufty et al., 1990).

The exudation of APases was increased in response to LP

In this study, APase activity in root exudates was increased approximatively two-fold under LP supply from 14 DOT compared to non-limiting P supply. Differences between the genotypes were observed; PN released more APases than the two American grapevine genotypes, independently of P supply. This result was related to the expression of *PAP10a*, which was more highly expressed in PN than 1103P and RGM. PAP10 is a secreted PAP, known to be induced by Pi starvation in different species such as *A. thaliana*, tomato or rice (Tian et al., 2012). In our case, the expression of *PAP10bc* and *PAP12c* increased under LP supply for all genotypes. The expression of *PAP10a* increased only for PN under LP

supply. This suggests that PN has an intrinsically higher capacity to mineralise Po from the soil. The transcript abundance of *PAP26a* and *PAP26b* was not increased by LP supply, this is in agreement with the literature as the expression of *PAP26* does not increase under LP supply, but the activity of the enzyme does increase (Tran et al., 2010a; Tran et al., 2010b; Veljanovski et al., 2006).

LP supply induces rhizosphere modification by roots

The uptake of nutrients by plants and release of macro-molecules from roots to the rhizosphere are often coupled with the efflux or influx of protons. Under HP supply, the pH of nutrient solutions was increased, potentially due to nutrient uptake. For example, Pi and nitrate are taken up from the soil by transporters, functioning with the symport of protons. In our case, all the genotypes affect the pH of rhizosphere differently in LP compared to HP. While the content of protons in the solution decreased under HP supply, its stayed constant under LP condition during the first 14 DOT and increased from 19 DOT, revealing an acidification of rhizosphere. Release of protons is a common response of plants to LP supply. Low P supply, and consequently low Pi uptake, results in excess uptake of cations over anions, and a release of protons to maintain charge balance in the cytosol (Heuwinkel et al., 1992). This adaptive trait of plants under LP supply has been connected with the activity of the plasma membrane H^+-ATPase (Santi and Schmidt, 2009). However, the expression of *HA6* and *HA9*, encoding H^+-ATPase (Pii et al., 2014), were not correlated with the increase of proton content in response to LP supply. In grapevine, Fe starvation responses have been well studied and several authors have shown that grapevine species have different strategies of adaptation, such as modifying pH of the rhizosphere, or increase carboxylate concentration in the root (Covarrubias et al., 2016; Covarrubias and Rombolà, 2015; Jiménez et al., 2007); this indicates that grapevine is able to modify the rhizosphere in response to low nutrient availability

In grapevine, differences in organic acid concentration in root tips were previously observed under Fe deficiency and in response to different N sources (Covarrubias and Rombolà, 2015; Jiménez et al., 2007). In our case, there were no significant differences in organic acid concentration in the root between genotypes or P treatments. To maintain intracellular pH stability, plants show an accumulation in carboxylate anions in the root tissue (Marschner, 1995). Excess of carboxylate anions could be translocated to the shoot, to maintain the charge balance in root tissues (Jeschke et al., 1997); or released into the rhizosphere (Neumann and Römheld, 1999). Under LP supply an increase of organic acid concentration in shoot was observed, supporting the hypothesis that excessive carboxylate concentration was compensated for by shoot translocation. In several species, the major organic acid in root tissues is citric acid and malic acid (Neumann and Römheld, 1999) whereas tartaric

acid is a characteristic organic acid restricted to a small number of species including the *Vitaceae* (Bennet-Clark, 1933).

Conclusion

Grapevine rootstocks are known to confer various P content to the scion because they differ by their PAE, but the mechanisms underlying these differences were unknown (Gautier et al., 2018). We show that two *PHT1* are more highly expressed in the genotype with a higher PAE, 1103P, when grown as un-grafted cuttings. Furthermore, 1103P had a lower shoot:root ratio so it has a greater potential to confer high P concentration to the shoot. However, grapevine genotypes studied in this study did not show differences in their efficiency to modify the rhizosphere to enhance P availability in a homogeneous growth medium. However, we have no knowledge of real adaptative capacity of these genotypes in responses to a heterogeneous distribution of P in the soil, although it merits further attention.

Funding

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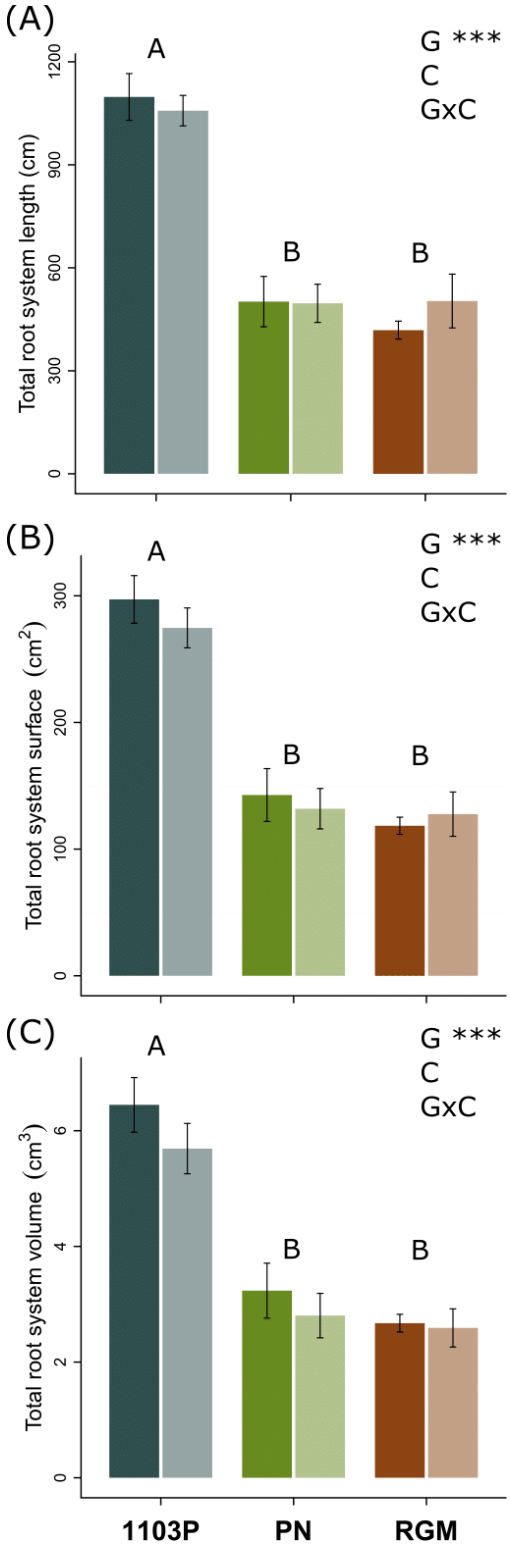
Gene name	Accession number	Forward primer	Reverse primer	Efficiency
SPX 1 / SPX 2	VIT_11s0016g05330	GATGGACAGGAAGGGTGTGG	TTTCCTTCAGAGCCCGCAAT	91
SPX 3	VIT_15s0048g00190	CAGGAACACAGTTGCAGCAC	TGGGGATGGGAGAGTGGAAT	91.5
PHT 1;3a	VIT_13s0067g03280	GGGCAATTGTGGCTTTGTCT	TGGTCCTCCCCAGAGAGTTG	96.5
PHT 1;3b	VIT_16s0050g02380	CCCCACTAAAGATAAGCCTGGG	TCCTACTAGGGCAACCCCAA	103.2
PHT 1;4a	VIT_05s0049g00920	TTGTGCTCGGGGTAGTCAAC	CCAGTCCTGGTAGAAGGGGA	90
PHT 1;4b	VIT_05s0049g00930	CCCACCTGGTATTGGAATGAGA	TAGTAGGAGGCTGCATGTCCA	85
PHT 1;4c	VIT_05s0049g00940	CCCGAGTCCAAGGGAAAGTC	GGAACAGTCCTAGCCTGCTG	99.1
PHT 1;4e	VIT_16s0050g02370	TTGGTGACGGAGACCAAAGG	GTGTGCTAGGCATCTGGGTT	94.5
PHT 1;9	VIT_18s0122g00780	CCCGTGAAACCAAGGGAAGA	GAATCCGCGATGAGTTTCGC	95.8
PHT 2;1	VIT_00s0291g00060	TGCGGCTTCCGTTGTTCTAT	TAAGTCCCCTTGCAAACCCC	100
PHO1a	VIT_01s0011g02520	CGGGCTATTTTTAGCTGCCC	ACAGCTCTGAACTTGCCTGC	90.9
PHO1b	VIT_01s0182g00150	GCTTGGAGATCATCCGTCGT	GACTTGAATGCGCGGTATGC	96.8
PHO1c	VIT_05s0049g01410	AGTTGCTTGGGTGGAGACAG	CCAGTGCCCACGTCTGATAA	91.9
PHO1d	VIT_14s0108g01130	CAGCATTGGTTGCAGTGGTT	AATGCCCTGTACTTCCCCAC	96.3
PHO2a	VIT_00s0265g00070	TTGTGCTGTGGAAGCAGGAT	GTGCAGGCAAACTCAAACCA	80.2
SIZ1	VIT_06s0004g03180	CAGTGGGGGTCATGCTGAAT	AGCAAAGAAGCAGTGTCAGC	91
PAP10a	VIT_03s0038g00220	AGCCAGTTTTGGACACGCTA	GTGGGTCCACAAACCTTCCA	96.9
PAP 10bc	VIT_03s0038g00230	TGGTTTTTCAACAGGTTCTGGC	AAGGTTGATAACATCATGACTGGG	96.3
PAP12c	VIT_18s0001g13340	GGACCTCTTCAGAAAAATCCTCT	ACCAAATGGGACAGTGAACAT	100.1
PAP26a	VIT_04s0008g06520	ACAAATGGCGATCGGTATCCT	AGACCTTCCTGATTCCCTCCA	107.2
PAP26b	VIT_11s0118g00240	TGGGCAAGTAAGCTGGGAAG	AAGGAGCAGTCCAGCCAATC	92.4
HA6	VIT_01s0011g01030	GGTTCAGCCTCTGTCACCG	TCCAAACCTGAAGGACATCAGTC	94.6
HA9	VIT_02s0012g00950	TAGGGCTTCCTGGGTTGAGA	GCGTCCTCCAACTGTCTCTG	86
PLDα-3	VIT_04s0008g05450	TCCAAGTCAACCAGAATGGCA	ACTCAGATACGGTGCCAAGG	90.8
PLDα	VIT_09s0002g06760	TCGCCGATGGCAAGTACTATG	GCCTTCTTCTTAAGCAACTCACC	96.7
ACTIN	VIT_04s0044g00580	CTTGCATCCCTCAGCACCTT	TCCTGTGGACAATGGATGGA	93.6
GAPDH	VIT_17s0000g10430	CCACAGACTTCATCGGTGACA	TTCTCGTTGAGGGCTATTCCA	91
SAND3'	VIT_06s0004g02820	TGCTGGGTTACCCCGGAGTTTGA	CAGACCCGGTTGCACGTCCG	89.9

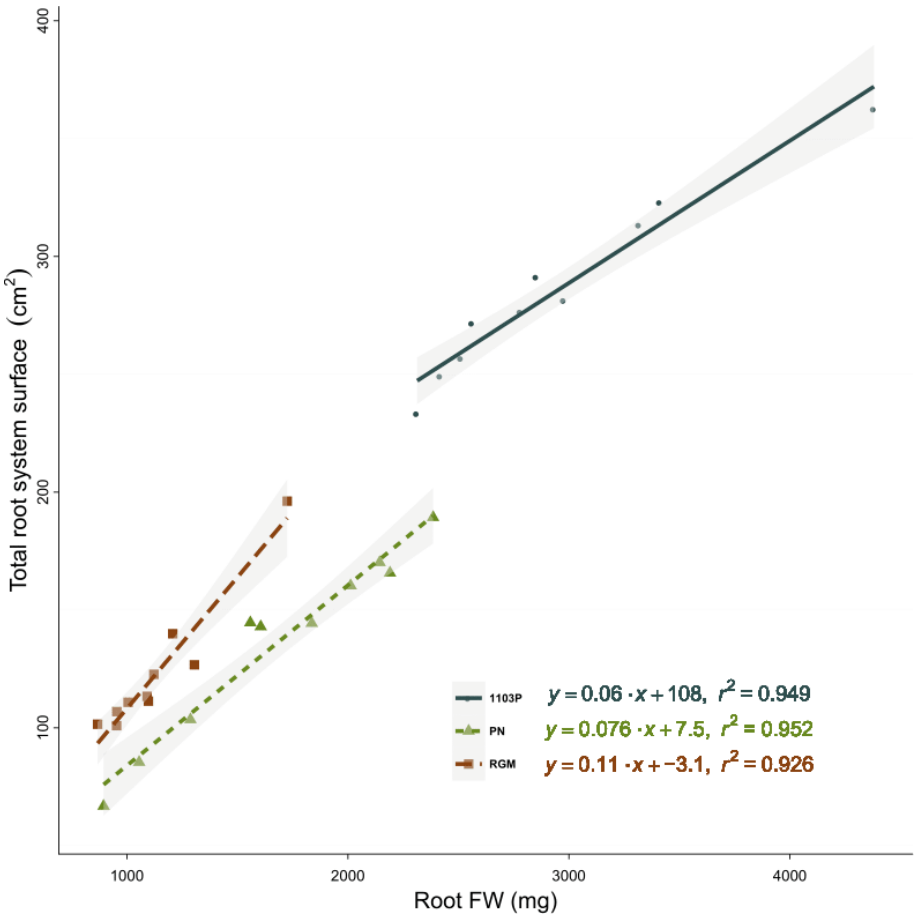
Supplementary Table VII.1. List of primers used for RT-qPCR experiments.

	1103P				PN				RGM				
	HP		LP		HP		LP		HP		LP		
1 DOT													
Plant FW (g)	1.83 ± 0.14	A	1.65 ± 0.13	a	1.26 ± 0.07	B	1.13 ± 0.04	b	1.54 ± 0.05	AB	1.32 ± 0.07	ab	
Shoot FW (g)	0.91 ± 0.06	A	0.82 ± 0.06	a	0.55 ± 0.05	B	0.53 ± 0.02	b	0.92 ± 0.04	A	0.78 ± 0.04	a	
Leaves FW (g)	0.54 ± 0.04	A	0.50 ± 0.03	a	0.27 ± 0.02	B	0.27 ± 0.01	b	0.50 ± 0.02	A	0.44 ± 0.02	a	
Stem FW (g)	0.37 ± 0.03	AB	0.33 ± 0.02	a	0.28 ± 0.02	A	0.27 ± 0.01	a	0.41 ± 0.02	B	0.34 ± 0.02	a	*
Root FW (g)	0.93 ± 0.09	A	0.83 ± 0.08	a	0.71 ± 0.04	AB	0.60 ± 0.04	ab	0.62 ± 0.03	B	0.54 ± 0.03	b	
Shoot:Root FW ratio	1.02 ± 0.07	A	1.02 ± 0.04	a	0.77 ± 0.06	A	0.92 ± 0.06	a	1.49 ± 0.07	B	1.45 ± 0.05	b	
Leaves:Stem FW ratio	1.45 ± 0.06	A	1.53 ± 0.05	a	0.96 ± 0.04	B	1.00 ± 0.04	b	1.23 ± 0.03	A	1.33 ± 0.07	a	
Number of leaves	7.40 ± 0.34	A	7.20 ± 0.29	a	6.50 ± 0.31	A	7.00 ± 0.26	a	6.90 ± 0.18	A	6.90 ± 0.23	a	
7 DOT													
Plant FW (g)	2.75 ± 0.18	A	2.73 ± 0.16	a	1.48 ± 0.08	B	1.54 ± 0.09	b	2.14 ± 0.12	A	2.18 ± 0.07	a	
Shoot FW (g)	1.46 ± 0.08	A	1.37 ± 0.06	a	0.72 ± 0.06	B	0.83 ± 0.07	b	1.38 ± 0.08	A	1.32 ± 0.05	a	
Leaves FW (g)	0.92 ± 0.05	A	0.85 ± 0.04	a	0.40 ± 0.04	B	0.47 ± 0.04	b	0.81 ± 0.04	A	0.77 ± 0.03	a	
Stem FW (g)	0.55 ± 0.04	A	0.52 ± 0.03	a	0.32 ± 0.02	B	0.36 ± 0.03	b	0.57 ± 0.04	A	0.54 ± 0.02	a	
Root FW (g)	1.28 ± 0.10	A	1.36 ± 0.10	a	0.76 ± 0.03	B	0.71 ± 0.04	b	0.76 ± 0.04	B	0.87 ± 0.03	b	
Shoot:Root FW ratio	1.17 ± 0.03	A	1.05 ± 0.05	a	* 0.95 ± 0.07	A	1.17 ± 0.10	a	1.81 ± 0.04	B	1.52 ± 0.03	b	***
Leaves:Stem FW ratio	1.71 ± 0.05	A	1.64 ± 0.04	a	1.23 ± 0.06	B	1.29 ± 0.04	b	1.45 ± 0.04	B	1.43 ± 0.04	b	
Number of leaves	9.13 ± 0.55	A	8.67 ± 0.47	a	6.27 ± 0.36	B	7.60 ± 0.21	b	8.13 ± 0.26	A	7.73 ± 0.18	a	
RGR ($mg.mg^{-1}.d^{-1}$)	0.065 ± 0.010	A	0.064 ± 0.010	a	0.030 ± 0.008	B	0.034 ± 0.009	b	0.056 ± 0.009	AB	0.061 ± 0.005	ab	
14 DOT													
Plant FW (g)	5.31 ± 0.29	A	4.40 ± 0.31	a	1.93 ± 0.12	B	2.26 ± 0.17	b	3.23 ± 0.17	C	3.01 ± 0.15	b	
Shoot FW (g)	3.34 ± 0.17	A	2.30 ± 0.16	a	*** 1.11 ± 0.09	B	1.38 ± 0.12	b	2.21 ± 0.11	C	1.83 ± 0.08	ab	*
Leaves FW (g)	1.93 ± 0.09	A	1.40 ± 0.09	a	*** 0.62 ± 0.05	B	0.79 ± 0.06	b	1.23 ± 0.06	C	1.08 ± 0.04	a	
Stem FW (g)	1.40 ± 0.09	A	0.90 ± 0.07	a	*** 0.49 ± 0.04	B	0.59 ± 0.06	b	0.97 ± 0.05	A	0.75 ± 0.04	ab	**
Root FW (g)	1.98 ± 0.13	A	2.10 ± 0.15	a	0.82 ± 0.04	B	0.88 ± 0.05	b	1.02 ± 0.06	B	1.19 ± 0.07	b	
Shoot:Root FW ratio	1.72 ± 0.05	A	1.11 ± 0.03	a	*** 1.35 ± 0.09	A	1.54 ± 0.07	b	2.19 ± 0.05	B	1.57 ± 0.04	b	***
Leaves:Stem FW ratio	1.40 ± 0.04	A	1.61 ± 0.04	a	1.28 ± 0.03	B	1.40 ± 0.04	b	1.28 ± 0.02	B	1.46 ± 0.04	ab	
Number of leaves	14.80 ± 0.85	A	11.07 ± 0.85	a	* 7.07 ± 0.34	B	9.00 ± 0.67	a	9.33 ± 0.36	C	8.60 ± 0.24	a	
RGR ($mg.mg^{-1}.d^{-1}$)	0.080 ± 0.004	A	0.066 ± 0.006	a	0.033 ± 0.004	B	0.044 ± 0.006	b	0.058 ± 0.004	C	0.053 ± 0.004	ab	

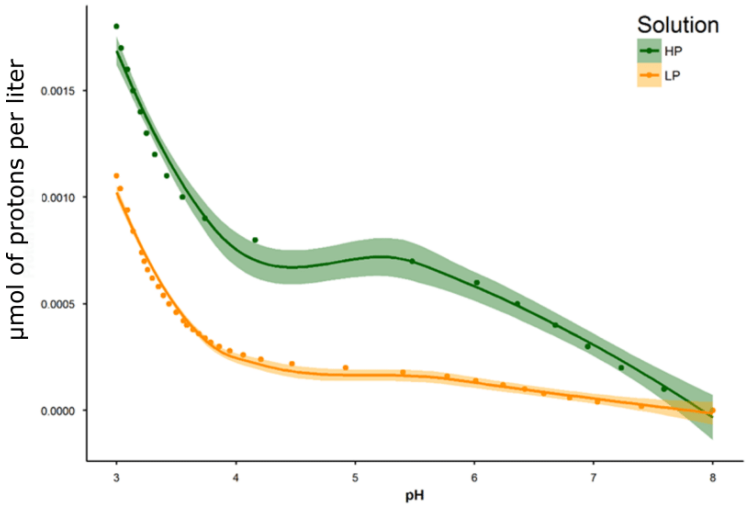
Supplementary Table VII.2. Whole plant, shoot, leaf, stem and root fresh weight (FW); shoot:root and leaves:stem FW ratio, number of leaves and relative growth rate of 1103P, PN and RGM cultivated in hydroponic culture under different P supplies, high P (HP) or low P (LP); after 1, 7 and 14 days of treatment. Means and standard errors shown (n=10). For each P-treatment, genotypic differences were analysed using a multiple comparison after Kruskal-Wallis tests at $P < 0.05$, uppercase letters indicate differences under HP supply while lowercase letters indicate differences under LP supply. For each genotype, stars indicate an effect of P supply using Wilcoxon test with the Bonferroni correction (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).



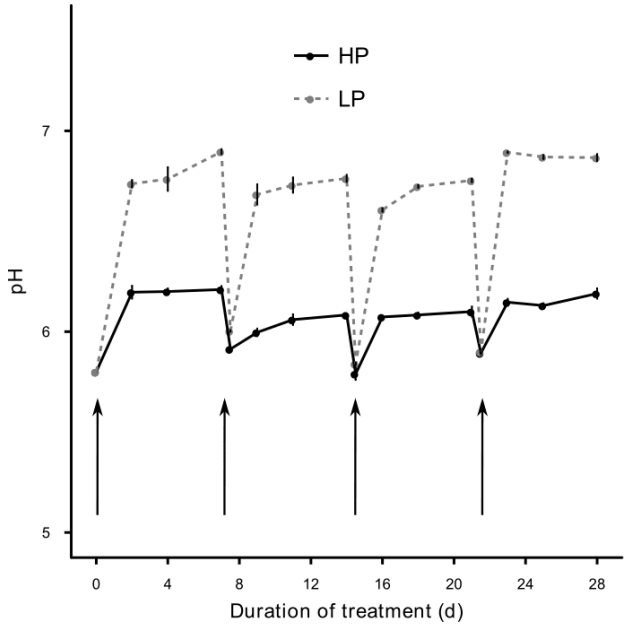
Supplementary Figure VII.1. (A) Length, (B) surface and (C) volume of total root system of 1103P, PN and RGM cultivated in hydroponic culture under different P supplies, high P (dark colours) or low P (light colours); after 28 days of treatment. Means and standard errors shown (n=5). Data were analysed using a two-way ANOVA, with genotype (G) and P supply (C) as factors (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Uppercase letters indicate differences between genotypes analysed using a Tukey test.



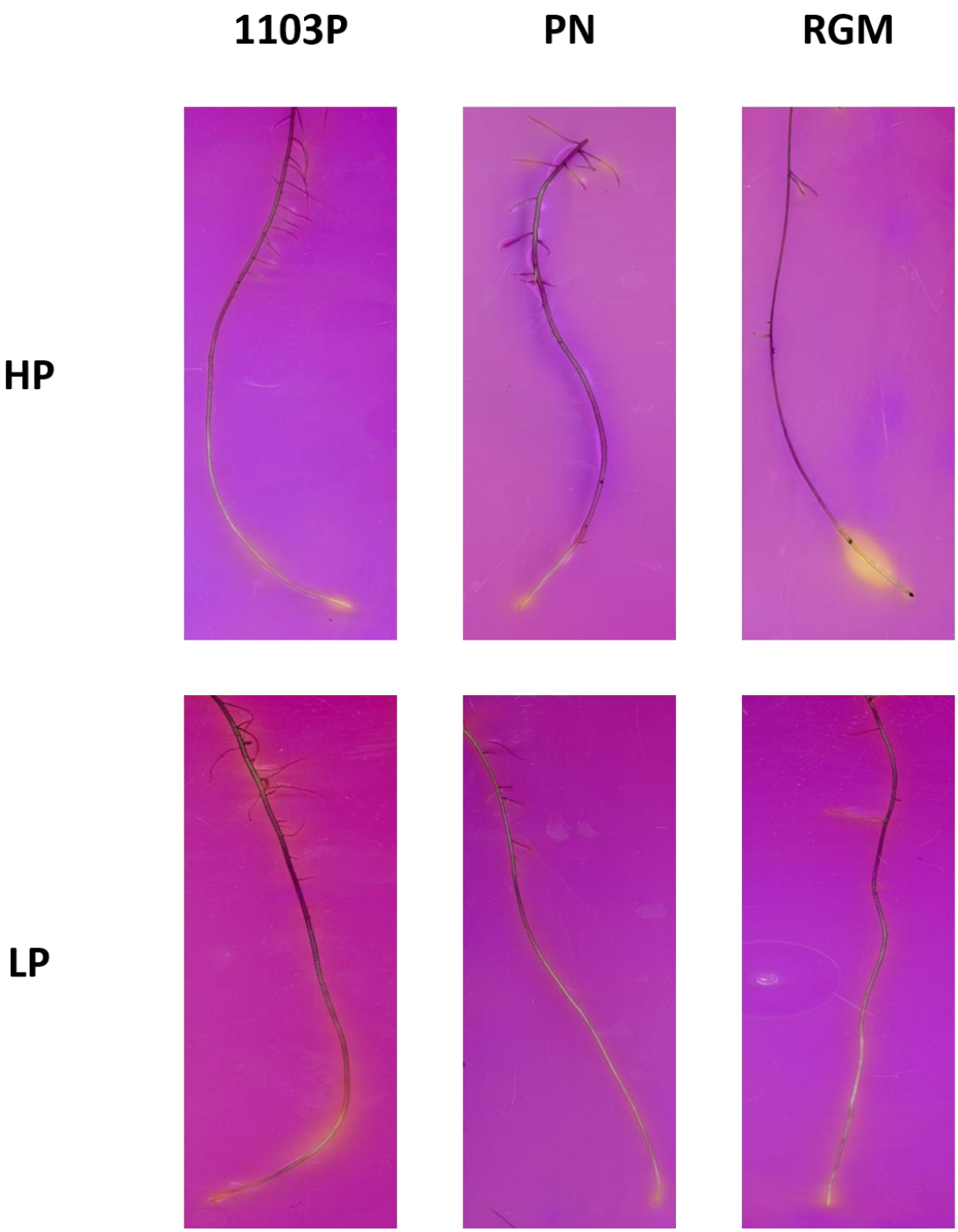
Supplementary Figure VII.2. Linear regression between fresh weight and surface of total root system of 1103P, PN and RGM cultivated in hydroponic culture under different P supplies, high P or low P; after 28 days of treatment.



Supplementary Figure VII.3. Buffering capacities of the two nutrient solutions containing two different P concentrations, 600 µM (HP, green) or 1 µM (LP, orange), calculated by the progressive addition of HCL and measurement of pH change.



Supplementary Figure VII.4. pH change of nutrient solutions containing two different P concentrations, high P (HP, black circles connected by solid lines) or low P (LP, grey circles connected by dashed lines), without plants. Arrows represent removing and adjusting pH of nutrient solutions.



Supplementary Figure VII.5. The rhizosphere acidification by roots of 1103P, PN and RGM cultivated in hydroponic culture under different P supplies, high P (HP) or low P (LP); after 28 days of treatment, using bromocresol purple pH indicator. Yellow indicate a decrease of pH.

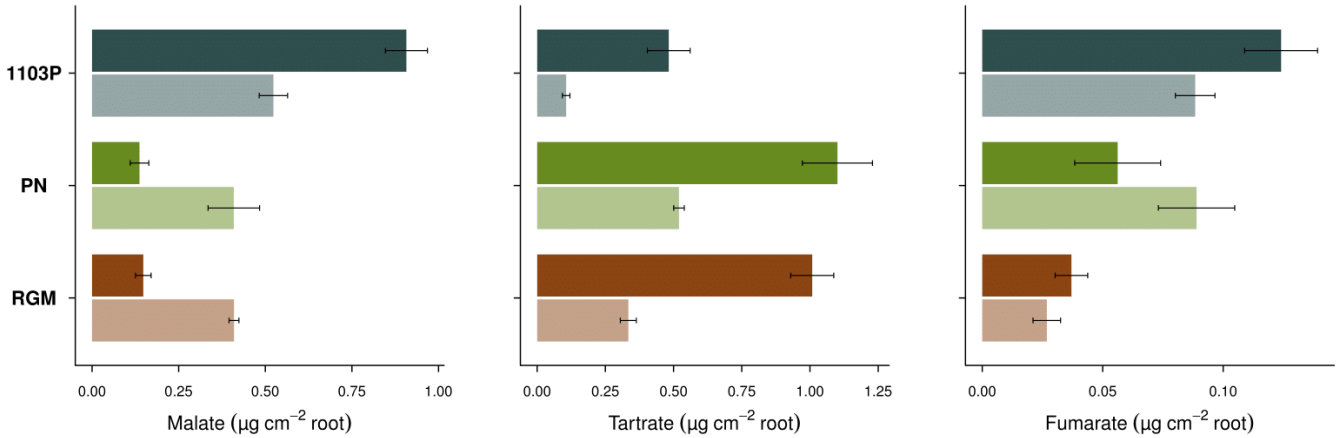
Perspectives

Les résultats de ce chapitre mettent en évidence des différences génotypiques marquées en termes de développement et de fonctionnement racinaire. Plus spécifiquement, la capacité de 1103P à conférer des teneurs en P plus forte à son greffon comparé à RGM, peut être reliée à une plus forte expression de certains gènes de la famille *PHT1* et donc potentiellement un plus grand nombre de transporteurs à Pi. De plus, 1103P montre un ratio biomasse aérienne sur biomasse racinaire inférieure, reflétant une partie « source » en nutriments supérieure à la partie « puits », avantageux pour l'acquisition des nutriments.

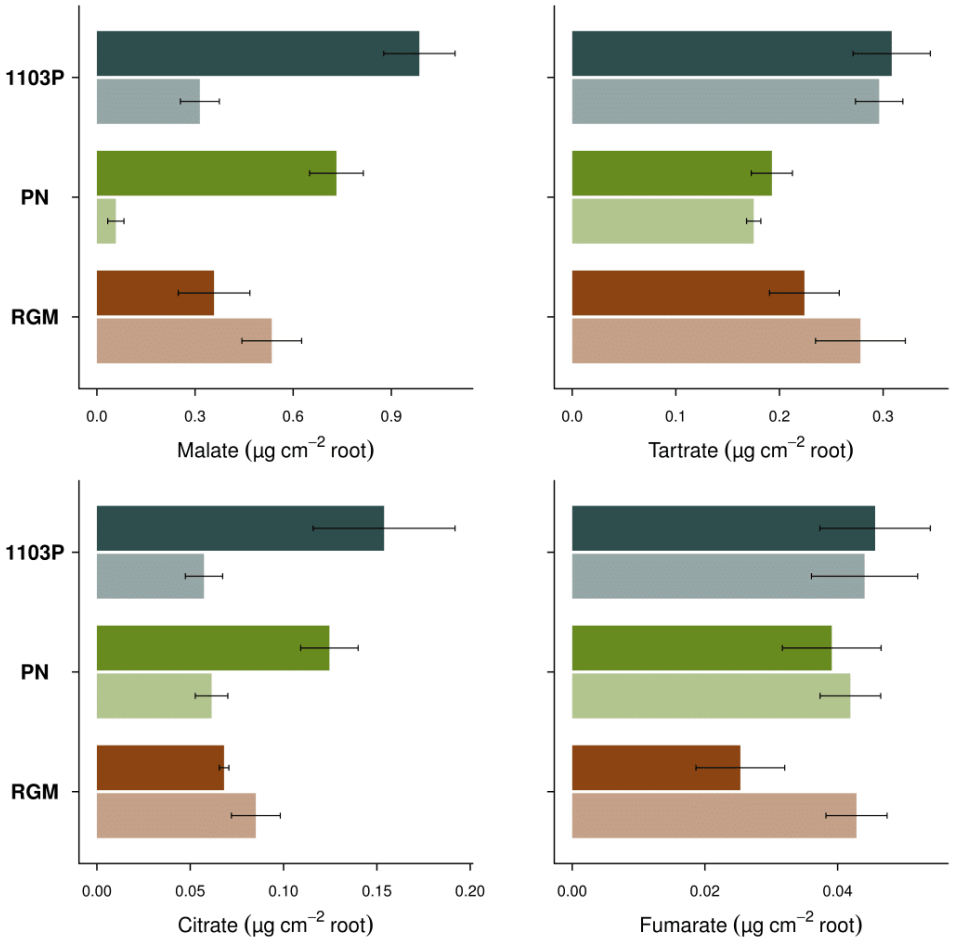
En termes d'interactions avec la rhizosphère, peu de différences génotypiques sont constatées. Cependant, l'adaptation de génotypes étudiés en réponse à la faible disponibilité en Pi est soulignée, montrant une acidification de la rhizosphère, ainsi qu'une augmentation de l'activité des APases exsudées.

Malheureusement, l'identification et la quantification des acides organiques exsudés n'ont pas pu être mené à termes. Deux méthodes de prélèvement ont été testées après 28 jours de traitement. Pour la première méthode, le système racinaire a été transféré dans 40 ml d'eau ultrapure pendant 6 heures. Pour la seconde, des pointes racinaires excisées (environ 1 cm de longueur) ont été transférées dans 1 ml d'eau ultrapure pendant 6 heures. Dans les deux cas, la biomasse et la surface racinaire ont été mesurées. Les exsudats récoltés ont été analysés par chromatographie, utilisant la méthode décrite pour l'analyse métabolique des tissus (cf. « Metabolic profile in plant tissues », Page 92).

Les acides malique, tartrique et fumarique ont été identifiés dans les deux cas, alors que l'identification de l'acide citrique n'a été possible qu'en utilisant les pointes racinaires (Supplementary data VII.6 et VII.7). La comparaison des deux méthodes de prélèvement montre des variations du double au triple des concentrations en acides tartrique et de fumarique respectivement. Des variations en quantités d'acides organiques exsudés entre génotypes et disponibilités en P sont observées. Cependant, les méthodes de prélèvement des exsudats racinaires semblent influer fortement ces résultats. Une amélioration du protocole et l'identification des zones d'exsudation semblent donc indispensable afin de pouvoir déterminer les quantités d'acides organiques exsudées dans les différentes conditions de l'étude.



Supplementary Figure VII.6. Acides malique, tartrique et fumarique exsudés par les racines de 1103P, PN and RGM cultivés en hydroponie avec différentes disponibilités en P, 600 µM (couleurs foncées) ou 1 µM (couleurs claires); après 28 jours de traitement. Les moyennes et erreurs standards sont calculées sur n=5. Les acides organiques ont été prélevés à partir de tout le système racinaire transféré dans 40 ml d'eau ultrapure pendant 6h.



Supplementary Figure VII.7. Acides malique, tartrique, citrique et fumarique exsudés par les racines de 1103P, PN and RGM cultivés en hydroponie avec différentes disponibilités en P, 600 µM (couleurs foncées) ou 1 µM (couleurs claires); après 28 jours de traitement. Les moyennes et erreurs standards sont calculées sur n=5. Les acides organiques ont été prélevés à partir de pointes racinaires transférées dans 1 ml d'eau ultrapure pendant 6h.

CHAPITRE 3

Influence du greffon et du porte greffe sur le développement racinaire et la modification de la rhizosphère en réponse à la disponibilité en phosphore chez la Vigne greffée

Avant-propos

A travers les chapitres précédents, des différences entre les porte-greffes étudiés ont été observés permettant d'apporter des éléments de réponse sur la capacité des hybrides issus de *V. berlandieri* ou *V. rupestris* à apporter de plus fortes concentrations en P à leur greffon, comparés aux porte-greffes issus de *V. riparia*. 1103P montre de plus fortes capacités à remobiliser ses réserves en P depuis les parties pérennes et à acquérir le Pi dans le sol via un système racinaire plus développé comparé à sa surface aérienne, adapté à l'acquisition des minéraux.

Cependant les observations sur la variation des teneurs en P en fonctions des porte-greffes proviennent de plantes greffées (cf. Article 1 : Petiole phosphorus concentration is controlled by the rootstock genetic background in grapevine). Le greffage inclut la connexion de deux génotypes ayant chacun un rôle précis sur le second. En effet, le porte-greffe permet l'alimentation hydrique et minérale du greffon, qui lui est responsable de l'apport carboné assurant la croissance et le fonctionnement racinaire. Le greffage et le greffon utilisé peuvent donc modifier les caractéristiques racinaires des porte-greffes observées précédemment.

Le dispositif expérimental utilisé dans le chapitre 2 a été réemployé afin de comprendre l'effet du greffage et des rôles des deux partenaires impliqués. Pour cela, des micro-greffes ont été réalisées en utilisant 1103P (*V. berlandieri* x *V. rupestris*) et *V. vinifera* cv. Pinot noir. Les quatre combinaisons de greffage possibles avec ces deux génotypes ont été effectuées en homogreffe et hétérogreffe afin de caractériser les effets du génotype de greffon sur le porte-greffe et inversement.

Les résultats obtenus sont présentés sous forme d'un article scientifique présentant les mêmes limites expérimentales que le précédent.

Article 4: Roles of the scion and the rootstock in root development and functioning under low phosphorus supply in grapevine

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Running head : Root development and functioning of grafted *Vitis spp.*

Abstract

Grapevine rootstocks are known to modify phosphorus (P) composition of the scion and have been shown to differ in their P use/acquisition efficiency, ability to explore the soil and root morphology. However, the influence of the scion on rootstock development is poorly understood. The objective of this work was to investigate roles of both rootstock and scion on parameters involved in P nutrition in grafted grapevines. Using two grapevine genotypes, homo-grafts and hetero-grafts were propagated resulting in 4 scion/rootstock combinations. Plants were grown on hydroponic culture and subjected to two contrasted Pi supplies, high P (600 µM) or low P (1 µM). Under non-limited P supply, scion genotype has a major influence on regulating plant growth and metabolite profile and this is correlated with differences in photosynthesis. The scion regulates root biomass accumulation, while the morphology of roots (such as tip density or the proportion of fine roots) is not affected by the scion genotype. Low P supply affected growth, metabolite profiling, enhanced the activity of acid phosphatases released. Rootstock genotypes showed differences in responses to low P supply, but scion play also a role in rootstock adaptations.

Keywords: *Vitis spp*., Grafting, Organic acid, Root morphology, Photosynthesis

Introduction

Grafting is an old horticultural technique used for the cultivation of perennial fruit crops (*e.g.* grape, apple, citrus, etc.) and more recently of annual vegetables and fruits (*e.g.* tomatoes, cucumber, pepper, etc.) (Bie et al., 2017; Mudge et al., 2009). Grafting allows us to combine desirable traits of both scion and rootstock in a single plant. However, grafting is a complex biological process including molecular exchange and signalling between two different genotypes. Rootstock affects several agronomical traits of the scion such as growth, architecture, water use efficiency, drought tolerance, mineral element composition, yield and fruit quality; and provides biotic stress tolerance (Nawaz et al., 2016; Warschefsky et al., 2016).

Since the 19th century, the Eurasian grapevine species (*Vitis vinifera*) is cultivated in a grafted system using North American *Vitis spp.* as rootstocks, historically selected for their tolerance to the American aphid pest Phylloxera. Today grapevine rootstocks are selected to provide abiotic and/or biotic stress tolerance, to control fruit production and scion growth (Ollat et al., 2016). Rootstocks are responsible of water and nutrient acquisition, and the translocation to the scion. Grapevine rootstocks have long been known to modify mineral element composition in the scion (Bavaresco et al., 2003; Cordeau, 1998). Nitrogen (N), phosphorus (P) and potassium (K) concentrations in petioles of four cultivars grafted onto ten rootstocks shown variations of 2 or 3-fold (Ibacache G. and Sierra B., 2009). However, the rootstocks did not have the same influence on N, P and K concentrations for the different scion cultivars, suggesting scion or scion/rootstock combination effects on whole plant mineral nutrition. Furthermore, some studies have shown differences in rootstocks responses to nutrient availability, such as N (Cochetel et al., 2017; Lecourt et al., 2015), K (Ruhl, 1989; Ruhl, 1991), P (Gautier et al., 2018; Grant and Matthews, 1996a; Grant and Matthews, 1996b) or iron (Covarrubias et al., 2016; Covarrubias and Rombolà, 2015; Jiménez et al., 2007). Different grapevine genotypes show different strategies in responses to low nutrient supply, such as a modification in the shoot:root ratio (Covarrubias et al., 2016; Lecourt et al., 2015), an increase of remobilization rate from perennial organs (Gautier et al., 2018); acidification of the rhizosphere (Jiménez et al., 2007); or an enhancement of organic acids concentration in roots (Covarrubias and Rombolà, 2015; Jiménez et al., 2007).

Phosphorus is an essential macronutrient for plant growth, involved in the constitution of macromolecules (*e.g.* nucleic acids or phospholipids) and in the transfer of energy required for metabolic pathways (Marschner, 2011). Phosphorus is taken up from the soil by root under this inorganic form (Pi, orthophosphate) by the phosphate transporters of the PHT1 family (Nussaume et al., 2011). However, available Pi accounts for a small fraction of total P present in the soil. In fact, a large fraction of Pi is unavailable for plant acquisition, bound to iron and aluminium-oxy(hydr)oxides

in acid soils, or occurs in calcium-phosphate forms in neutral to alkaline soils (Barrow, 2016). Furthermore, high concentration of organic P (Po) was found in soil, which requires hydrolysis before being taken up by plants (Pierre and Parker, 1927; Richardson et al., 2009; Turner et al., 2005). However, root-rhizosphere interactions modify properties of the soil and can increase Pi available via following mechanisms: (1) releasing of H^+ or OH^- resulting a change of pH in the rhizosphere (Hinsinger et al., 2003; Lei et al., 2016; Liu et al., 2016b); (2) releasing of carboxylates (*e.g.* malate or citrate) (Bolan et al., 1994; Gahoonia et al., 2000), resulting a complexation of ions such as Ca, Al or Fe involved in P sorption (Jones, 1998); or (3) releasing of acid phosphatases (APases; EC 3.1.3.2), enzyme which catalyse the hydrolyse of Po into Pi (Lu et al., 2016; Robinson et al., 2012a).

Two parameters are frequently used to describe the ability of plants to grow under different P supplies: P use efficiency (PUE) and P acquisition efficiency (PAE) (Vance et al., 2003; Wang et al., 2010). The first, PUE, is related to the capacity of a plant to use P for growth, via remobilization of P pools (*e.g.* perennial or senescent organs, vacuole, RNA, phospholipids) or modification in metabolic pathways with less energy requirements (Rose et al., 2011; Rose and Wissuwa, 2012). PAE is defined as the capacity of plant to take up new P from the soil via different strategies, controlled by the functioning of phosphate transporters, the soil exploration and root-rhizosphere interactions to increase the concentration of Pi available for uptake (Lynch, 2011; Ramaekers et al., 2010).

The roles of scion/rootstock interactions in regulating plant responses to P supply are poorly understood. Leaves provides photosynthetic products for maintenance and construction of whole plant including the roots (Friend et al., 1994; Mooney, 1972). A large proportion of the root-translocated carbon is using for root growth and respiration, but a little fraction is destined to exudation (Van Veen et al., 1991). The root system provides water and nutrient form the soil to shoot (Richards, 1983). In grafted system, shoot and root functions are provided by two different genotypes, no necessarily of the same species. Shoot-to-root and root-to-shoot signalling are essential for both development of scion and rootstock. The aim of this study is to understand the role of both the rootstock and scion genotype in regulating traits associated with P nutrition in grapevine by comparing all possible grafted combinations of two grapevine genotypes grown under two contrasted P supplies. The traits studied were growth, root development/morphology, metabolite concentrations and rhizosphere modifications.

Materials and methods

Plant material and growing conditions

Two genotypes of grapevine (*Vitis spp.*) were studied: one rootstock genotype of American origin, *V. berlandieri x V. rupestris* cv. 1103 Paulsen (1103P) and one typical European scion genotype, *V. vinifera cv.* Pinot noir (PN). All four possible scion/rootstock combinations were micro-grafted using the cleft grafting system, i.e. 1103P_1103P, 1103P_PN, PN_1103P and PN_PN. Plants were cultivated *in vitro* on McCown Woody Plant Medium (Duchefa) supplemented with 30 g L^{-1} sucrose and 0.27 µM 1-naphthalene acetic acid, and with 0.4 % agar, in a growth chamber at 22 °C and subjected to a photoperiod of 16 h light/8 h dark with a light intensity of 55 µmol m^{-2} s^{-1}. Six-week-old plantlets were then acclimated to perlite-filled pots, irrigated with water for four weeks, in a growth chamber at 26 °C and subjected to a photoperiod of 16 h light/8 h dark with a light intensity of 145 µmol m^{-2} s^{-1}. Plants were then transferred into hydroponic culture; each pot contained 2 plants of the same combonation with 700 mL of HP nutrient solution (described below) for 4 d. Nutrient solutions were oxygenated to 6.5 mg of O_2 dissolved per liter. Finally, plants were subjected to two contrasting P treatments: 0.6 mM of P (HP) or 0.001 mM of P (LP). 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 46.25 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 (Syngenta Agro S.A.S., Guyancourt, France) (i.e., 31.3 µmol ethylenediamine-N,N'-bis (2-hydroxyphenylacetic acid) NaFe). Nutrient solutions were refreshed each week for the 28 d of the experiment.

Plant growth measurements

After 1 and 28 d of treatment (DOT), leaves, stems and roots of 5 and 15 plants respectively, per combination per P treatment were harvested, rinsed and weighed (fresh weight, FW), frozen in liquid N and kept at -80 °C for metabolite and transcript profiling. Shoot FW was calculated as leaf plus stem FW; and Plant FW was calculated as shoot plus root FW. After 28 DOT, relative growth rate (RGR), expressed in $g.g^{-1}.d^{-1}$, was calculated as follows:

$$RGR = \frac{Ln(Plant\ FW28) - Ln(Plant\ FW1)}{28 - 1}$$

Where *FW28* was the plant FW after 28 DOT and *PlantFW1* was the mean plant FW after 1 DOT.

Gas exchange measurements

After 14 and 28 DOT, CO_2 assimilation rate (A), transpiration rate (E), water vapour conductance (GH_2O) and intracellular CO_2 concentration (C_i) were measured on mature leaves of 3 plants per combination per condition, using a portable open-system infrared gas analyser (GFS 3000, WALZ). Three measurement were performed for each leaf. For each plant, the same mature leaf was used for measurements at 14 and 28 DOT.

Root system morphology analysis

After 28 DOT, five plants per combination per P treatment were used for root system morphology analysis. Images were captured using an Epson 1640XL scanner and Epson scan software. Images were analysed using *WinRHIZO* software (Regent Instruments Inc., 1996). Number of tips, root average diameter, length, surface and volume of total root system were measured. Roots were indexed by diameter into different size classes. The percentage of each root class was calculated based on total root length. Correlations between root surface and root FW were calculated for each combination.

Variation of the concentration of protons in nutrient solution

From 7 DOT, the volume and pH of nutrient solution in each pot was measured for five pots per combination per condition and in pots without plants three times per week. pH values were transformed into proton concentrations using buffering capacity of the nutrient solutions at the beginning of week. Buffer capacities of the two nutrient solutions were calculated by the progressive addition of HCL and measurement of pH change. Variation of protons in the solution was calculated by taking into account the change in pH of the solutions without plants and volume of the solution. The surface of root system was estimated from linear regression between root FW and root surface obtained with root system morphological analysis for each combination grown in the two P treatments.

Visualisation of the spatial distribution of root acidification was performed on roots excised after 28 DOT and placed on 0.4 % agar plates with 0.1 % (w/v) of the pH indicator bromocresol purple for 1 h. Images were captured using scanner Epson 1640XL and Epson scan software.

Activity of acid phosphatases released from the roots

After 28 DOT, five plants per combination per P treatment 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 and root surface area was calculated using from linear regression between root FW and root surface area described above. p-nitrophenyl (pNP) concentration was measured by spectrophotometer at 405 nm.

Metabolite profile of plant tissues

The root, stem and leaf samples from 28 DOT were ground using a *Retsch™ MM400* cooled with liquid N. 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, tartrate and fumarate) were identified and quantified by ion exchange chromatography ICS-5000 HPIC system (Thermo Scientific Dionex), using an isocratic gradient of KOH from 4 to 28 mM for 16 min, with a volume of sample injected of 20 µL. PAE was estimated using the extractable Pi content in whole plant by root FW.

Transcript level quantification

After 28 DOT, three pools of three root tips per plant (~15 mm in length) were harvested and immediately snap-frozen in liquid N. 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 (q) PCRs 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. Gene expression was calculated as normalized relative quantities (NRQs) as defined by Hellemans et al. (2007), with the reference genes ACTIN, GAPDH and SAND3' for normalization. Primer sequences are listed in Supplementary Table VIII.1.

Statistical analysis

All statistical analyses were performed using the software R (R Core Team, 2016). When assumptions for parametric tests were not respected, difference between combinations for a given P supply were analysed by a multiple comparison test after a Kruskal- Wallis test at $P < 0.05$, 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. P-treatment effects for each combination were analysed by a Wilcoxon non-parametric test at $P < 0.05$, with the Bonferroni correction. For each P supply, scion and rootstocks effects were analysed by a Wilcoxon non-parametric test at $P < 0.05$, with the Bonferroni correction. When assumptions for parametric test were respected, data were analysed using a two analysis of variance (ANOVA $P < 0.05$, with Tukey's Honest Significant Difference test), with combination and P supply, or scion and rootstock as factors.

Results

Growth and photosynthetic activity under non-limiting HP condition are mainly controlled by the scion genotype

Under non-limiting conditions (HP), the grapevine scion/rootstock combinations studied showed differences in growth (Figure VIII.1) and a significant effect of scion genotype was observed (Table VIII.1). In fact, combinations with 1103P as the scion had a higher plant biomass (leaf, stem and root biomass) from the beginning of experiment (Supplementary Table VIII.2 and Figure VIII.1A, C, D, E, F). The use of 1103P as scion increased whole plant FW 3-fold compared to PN after 28 DOT (Figure VIII.1A). Combinations also differed in their RGR with PN_1103P showing a lower RGR than the others (Figure VIII.1B). Combinations with 1103P as the scion had a higher shoot:root ratio (Figure VIII.1G). The scion also affected the allocation of biomass between the leaves and stems, with 1103P allocating more biomass to leaves than PN (Figure VIII.1H). However, rootstock genotype was also involved in regulating these growth parameters, using PN as the rootstock increased shoot:root ratio, leaf/stem ratio and RGR (Table VIII.1).

After 14 and 28 DOT under non-limiting conditions, CO_2 assimilation rate, transpiration rate, water vapour conductance and intracellular CO_2 concentration were measured on mature leaves (Table VIII.2). An effect of scion genotype was observed; 1103P had higher values for on CO_2 assimilation rate, transpiration rate, and water vapour conductance under HP supply than PN.

Low P supply affected some growth and gas exchange parameters depending of the scion

After 28 DOT, LP induced a decrease of RGR for combinations with 1103P as the scion, related to a decrease of plant, stem, leaf and shoot FW (Figure VIII.1A-E). RGR and plant FW of combinations with PN as scion were not affected by LP supply (Figure VIII.1B-C). However, a decrease of shoot FW related to stem FW was observed (Figure VIII.1D-E). Root FW of all combinations was not affected by LP supply and consequently, shoot:root biomass ratio was decreased (Figure VIII.1F-G). After 28 DOT, CO_2 assimilation rate, transpiration rate and water vapour conductance were decreased between 20 and 40 % for combinations with 1103P as scion in response to LP supply (Table VIII.2).

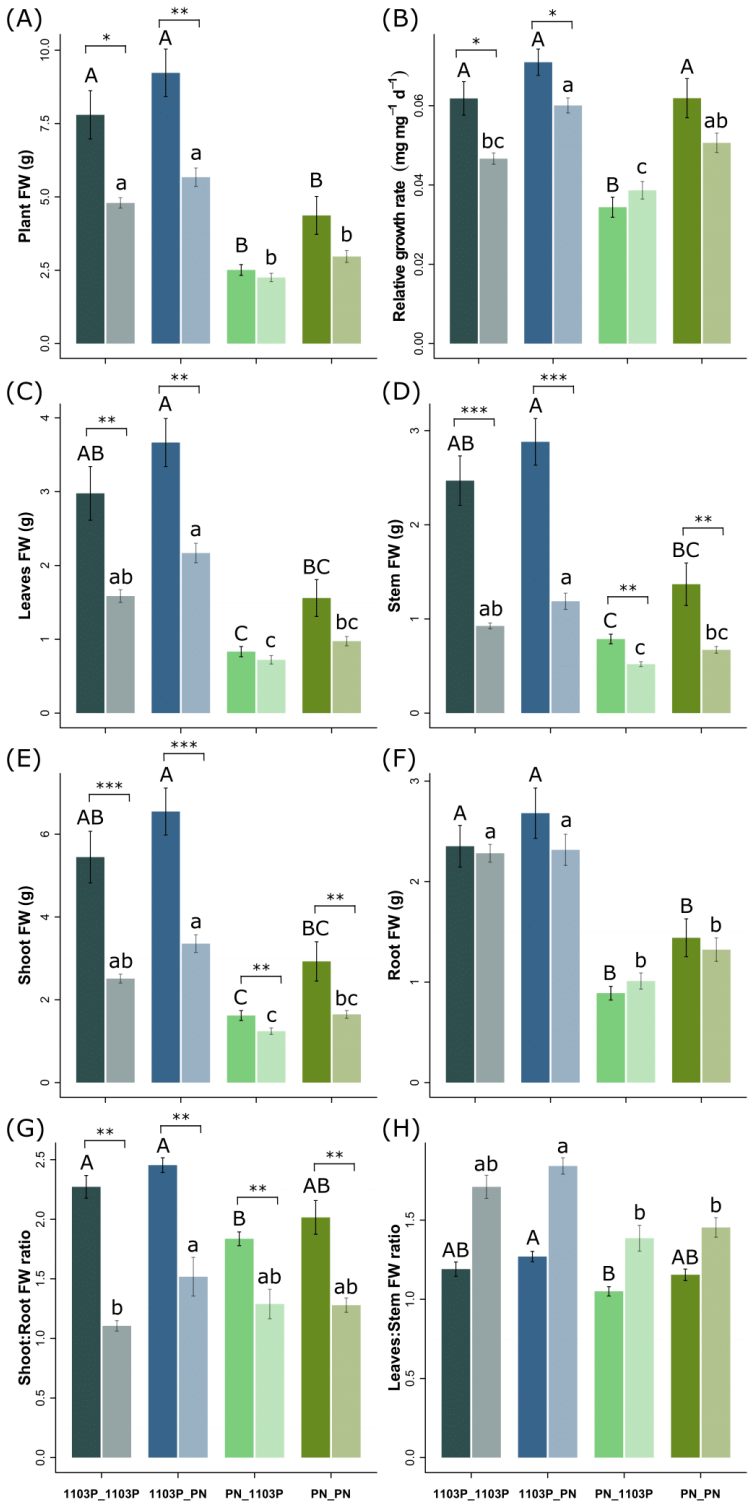


Figure VIII.1. (A) Whole plant fresh weight (FW); (B) relative growth rate; (C-F) leaf, stem, root and shoot FW; (G) shoot:root FW ratio and (H) leaves:stem FW ratio of hetero-grafted and homo-grafted combinations using 1103P and PN, cultivated in hydroponic culture under different P supplies, high P (HP, dark colours) or low P (LP, light colours), after 28 days of treatment. Means and standard errors shown (n=15). For each P-treatment, differences between combinations were analysed using a multiple comparison after Kruskal-Wallis tests at $P < 0.05$, uppercase letters indicate differences under HP supply while lowercase letters indicate differences under LP supply. For each combination, stars indicate an effect of P supply using Wilcoxon test at $P < 0.05$ with the Bonferroni correction (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

	HP		LP	
	Scion	Rootstock	Scion	Rootstock
Growth parameters				
Plant FW (g)	*		*	*
Shoot FW (g)	*		*	*
Leaves FW (g)	*		*	*
Stem FW (g)	*		*	
Root FW (g)	*		*	
Shoot:Root FW ratio	*	*		*
Leaves:Stem FW ratio	*	*	*	
RGR ($mg.mg^{-1}.d^{-1}$)	*	*	*	*
Gaz exchange				
A ($\mu mol\ m^{-2}\ s^{-1}$)	*			*
E ($mmol\ m^{-2}\ s^{-1}$)	*			*
GH_2O ($mmol\ m^{-2}\ s^{-1}$)	*			*
ci (ppm)				*
Root system development				
Total root length	***		***	
Total root surface	***		***	
Total root volume	***	*	***	
Root system morphology				
Total number of tips	***	*	***	
Tip density (cm^{-2})		***		**
Average diameter				
Root diameter distribution				
$3 < Ø$				
$2 < Ø < 3$		*		*
$1.5 < Ø < 2$		*		*
$1 < Ø < 1.5$				
$0.8 < Ø < 1$		*		
$0.6 < Ø < 0.8$				
$0.4 < Ø < 0.6$		*		
$0.2 < Ø < 0.4$				*
$Ø < 0.2$				
Rhizosphere interactions				
Variation of protons				
APases released				

Table VIII.1. Scion and rootstock effect on different parameters measured on plants cultivated under high P (HP) or low P (LP) supply for 28 days. For each P-treatment, scion and rootstock effect were analysed using a Kruskal-Wallis tests at $P < 0.05$, stars indicate significant differences (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

	1103P_1103P			1103P_PN			PN_1103P			PN_PN		
	HP	LP		HP	LP		HP	LP		HP	LP	
14 days of treatment												
A (µmol m^{-2} s^{-1})	4.56 ± 0.74 ^{A}	4.61 ± 0.32 ^{a}		3.34 ± 0.29 ^{A}	4.59 ± 0.38 ^{a}		2.51 ± 0.03 ^{A}	3.55 ± 0.39 ^{a}		3.51 ± 0.42 ^{A}	3.11 ± 0.61 ^{a}	
E (mmol m^{-2} s^{-1})	1.10 ± 0.07 ^{A}	1.34 ± 0.04 ^{a}		0.95 ± 0.06 ^{AB}	1.25 ± 0.13 ^{ab}		0.56 ± 0.06 ^{B}	0.85 ± 0.08 ^{b}		0.88 ± 0.11 ^{AB}	0.85 ± 0.13 ^{b}	
GH2O (mmol m^{-2} s^{-1})	81.01 ± 5.94 ^{A}	98.83 ± 3.73 ^{a}		64.01 ± 5.93 ^{AB}	89.12 ± 9.94 ^{ab}		39.91 ± 4.17 ^{B}	59.93 ± 5.74 ^{b}		62.69 ± 8.45 ^{AB}	60.79 ± 9.98 ^{ab}	
ci (ppm)	304.0 ± 12.02 ^{A}	319.3 ± 6.87 ^{a}		310.4 ± 1.43 ^{A}	305.5 ± 5.28 ^{a}		352.8 ± 18.16 ^{A}	298.1 ± 8.76 ^{a}	*	308.7 ± 14.49 ^{A}	309.2 ± 14.59 ^{a}	
28 days of treatment												
A (µmol m^{-2} s^{-1})	6.88 ± 0.19 ^{A}	3.65 ± 0.45 ^{a}	***	3.41 ± 0.34 ^{AB}	1.29 ± 0.12 ^{b}	***	1.11 ± 0.06 ^{C}	1.95 ± 0.24 ^{ab}	*	2.42 ± 0.15 ^{BC}	1.68 ± 0.12 ^{b}	*
E (mmol m^{-2} s^{-1})	1.64 ± 0.15 ^{A}	1.27 ± 0.11 ^{a}	*	0.97 ± 0.08 ^{AB}	0.59 ± 0.03 ^{b}	**	0.43 ± 0.03 ^{B}	0.58 ± 0.08 ^{b}		0.87 ± 0.18 ^{AB}	0.63 ± 0.01 ^{ab}	
GH2O (mmol m^{-2} s^{-1})	126 ± 13.07 ^{A}	95.2 ± 8.66 ^{a}	*	72.14 ± 5.91 ^{AB}	42.81 ± 1.86 ^{b}	***	31.63 ± 2.43 ^{B}	42.84 ± 6.01 ^{ab}		65.5 ± 12.22 ^{AB}	45.53 ± 1.51 ^{ab}	
ci (ppm)	301.4 ± 7.02 ^{A}	321.3 ± 8.99 ^{a}		318.6 ± 1.72 ^{AB}	341.8 ± 5.19 ^{a}		343.1 ± 6.85 ^{B}	314.3 ± 6.35 ^{a}	*	282.9 ± 16.06 ^{A}	334.5 ± 6.65 ^{a}	

Table VIII.2. CO_2 assimilation rate (A), transpiration rate (E), water vapour conductance (GH_2O) and intracellular CO_2 concentration (C_i) of hetero-grafted and homo-grafted combinations using 1103P and PN, cultivated in hydroponic culture under different P supplies, high P (HP) or low P (LP); after 14 and 28 days of treatment. Means and standard errors shown (n=9). For each P-treatment, differences between combinations were analysed using a multiple comparison after Kruskal-Wallis tests at $P < 0.05$, uppercase letters indicate differences under HP supply while lowercase letters indicate differences under LP supply. For each combination, stars indicate an effect of P supply using Wilcoxon test at $P < 0.05$ with the Bonferroni correction (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Root morphology were dependent on the rootstock genotype and were affected by low P supply

After 28 DOT, the root system morphology was analysed using *WinRHIZO* software. Length, surface area and volume of total root system were calculated, and differences between combinations were observed (Supplementary data VIII.3). The length, surface area and volume of the root system of combinations with 1103P as scion were higher compared to PN, in agreement with the root FW data (Figure VIII.1F and Table VIII.2). Total root system surface area was strongly correlated with root FW and not affected by P treatment. Consequently, the correlation between root FW and total root system surface for each genotype was calculated (Supplementary data VIII.4). Furthermore, LP supply did not affect the length and the surface area of the total root system but decreased the total root system volume (Supplementary data VIII.3). The number of tips per root system and their density were affected by the scion/rootstock combination (Figure VIII.2A-B). In fact, the number of tips is related to the root length, so the scion genotype 1103P conferred a higher number of tips (Table VIII.1). However, an effect of rootstock genotype was observed on tip density, while combinations with PN as the rootstock showed a higher tip density compared to 1103P (Figure VIII.2B). Finally, the average diameter of root, calculated on the whole root system was decreased for all combinations under LP supply (Figure VIII.2C).

During the analysis, roots were placed into nine classes depending of their diameter. The distribution of these different diameter classes in the root system was calculated using the cumulated root length of each of the classes. An effect of the rootstock genotype was observed on distribution of these different diameter classes (Table VIII.1 and Figure VIII.3). In fact, when PN was used as the rootstock, the root system had a higher proportion of roots with a diameter ranging between 1.5 and 3 mm, and between 0.4 and 0.6 mm. However, a significantly lower proportion of roots with a diameter ranging between 0.8 and 1 mm was observed for 1103P (Table VIII.1). Finally, LP supply affected the distribution of these different diameter classes with an increase of roots with a diameter ranging between 0.4 and 0.6 mm (Figure VIII.3).

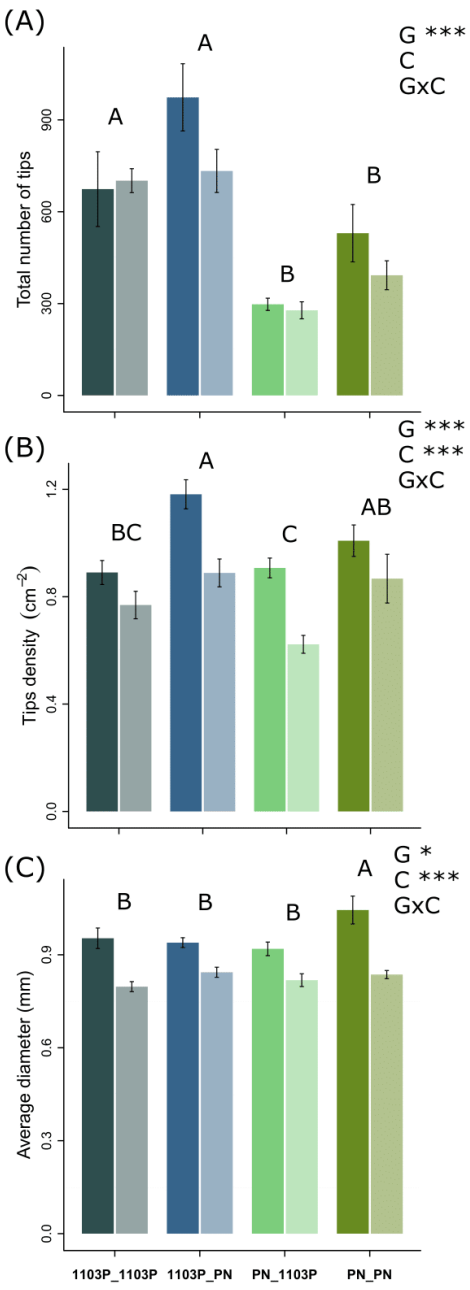


Figure VIII.2. (A) Number of tips, (B) tip density and (C) average diameter of total root system of hetero-grafted and homo-grafted combinations using 1103P and PN, cultivated in hydroponic culture under different P supplies, high P (HP, dark colours) or low P (LP, light colours); after 28 days of treatment. Means and standard errors shown (n=5). Data were analysed using a two-way ANOVA, with combinations (G) and P supply (C) as factors, stars indicate significant differences (* P < 0.05, ** P < 0.01, *** P < 0.001). Uppercase letters indicate differences between genotypes analysed using a Tukey test.

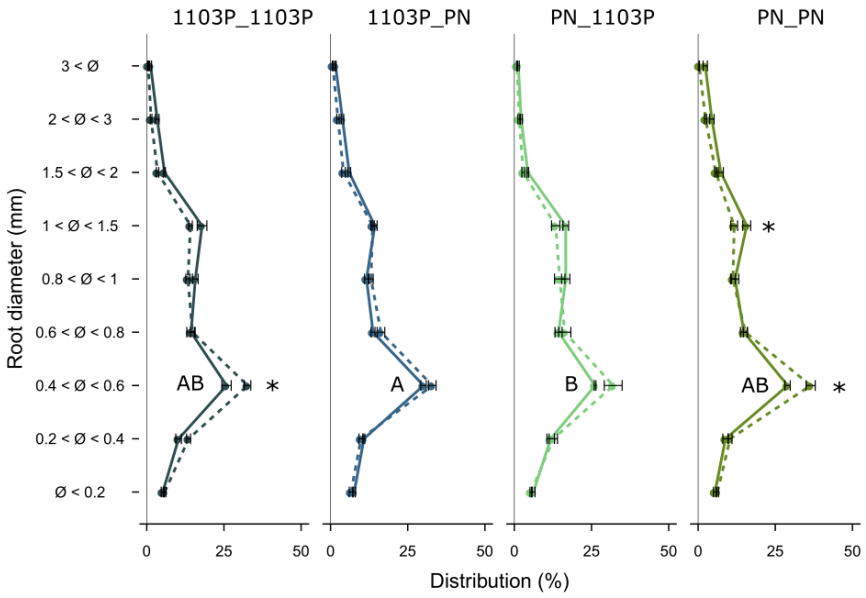


Figure VIII.3. Percentage of each root diameter class of the total length of root system of hetero-grafted and homo-grafted combinations using 1103P and PN, cultivated in hydroponic culture under different P supplies, high P (dark colours connected by solid lines) or low P (light colours connected by dashed lines); after 28 days of treatment. Means and standard errors shown (n=5). For each P-treatment, differences between combinations were analysed using a multiple comparison after Kruskal-Wallis tests at $P < 0.05$, uppercase letters indicate differences under HP supply. For each combination, stars indicate an effect of P supply using Wilcoxon test at $P < 0.05$ with the Bonferroni correction (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

pH of the nutrient solutions was affected by root functioning and depends of P supply

The buffering capacity of each nutrient solution was calculated using HCL 1 M within the pH range of 8 to 3 (Supplementary data VIII.5). HP solution had a higher buffering capacity, i.e. a variation of pH in this solution need a greater of addition of protons than in LP solution. Each week, nutrient solutions were refreshed, and the pH was adjusted to 5.8. The changes of pH of the two nutrient solutions without plants were measured and an increase of pH was observed for both solutions (Supplementary data VIII.6). pH measurements were done 2, 4 and 7 d after changing the nutrient solution from the second week. Taking account of the buffer capacity of the solution, the variation of pH without plants and the volume of solution in each pot, the flux of protons in the solution relative to the fresh solution was calculated and normalized by the estimated root surface area per pot (Figure VIII.4). Under HP, all the combinations alkalinised the nutrient solution. Conversely, under LP supply the concentration of

protons in the nutrient solution did not change for the first 18 DOT, from 18 DOT there was an increase in the content of protons to the nutrient solution. Significant differences between the two P treatments for each genotype were observed for all measurement points. Using bromocresol pH indicator, localization of acidification by root was observed and difference under P-treatment was shown (Supplementary data VIII.7). In fact, under HP supply acidification of rhizosphere was localized around the tips, while under LP supply acidification was localized all along the root.

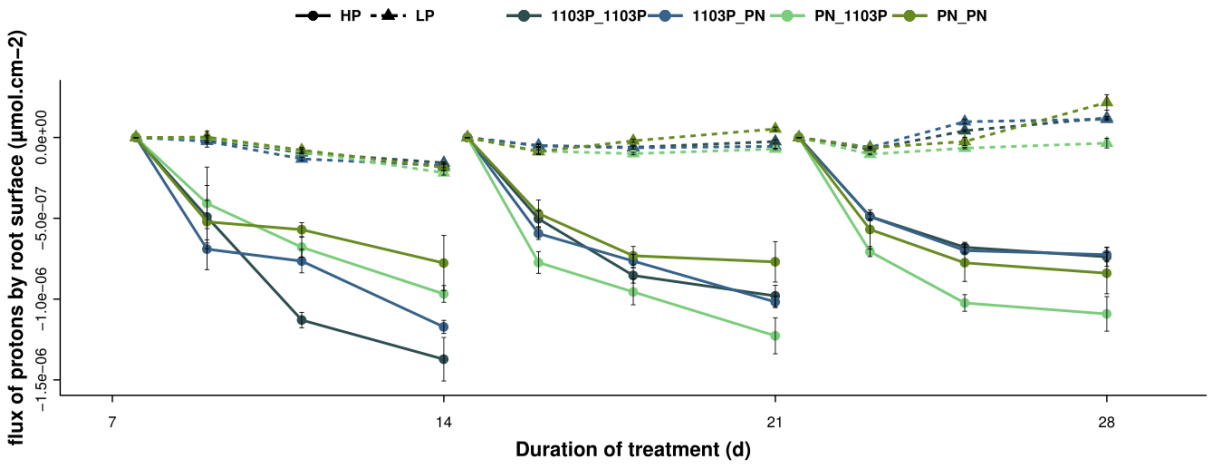


Figure VIII.4. Variation of proton content in the nutrient solution surrounding roots of hetero-grafted and homo-grafted combinations using 1103P and PN, cultivated in hydroponic culture under different P supplies, high P (dark colours connected by solid lines) or low P (light colours connected by dashed lines); during 28 days of treatment. Means and standard errors shown (n=5). For a given time point (except at the removing of nutrient solution), significant differences were observed between P supply using Wilcoxon test at $P < 0.05$ with the Bonferroni correction.

Activity of APases released by the roots increased under low P supply

The activity of APases released into the media by the roots was measured after 28 DOT (Figure VIII.5). The activity of APases sampled was increased approximatively two-fold for each combination under LP compared to HP; there was no significant effect of the genotype used as scion or rootstock (Table VIII.1).

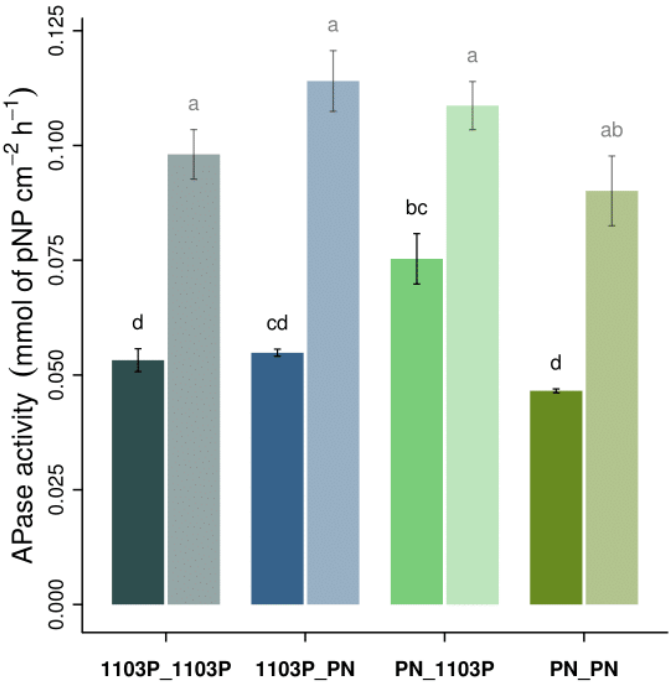


Figure VIII.5. Activity of acid phosphatases released by roots of hetero-grafted and homo-grafted combinations using 1103P and PN, cultivated in hydroponic culture under different P supplies, high P (HP, dark colours) or low P (LP, light colours); after 28 DOT. Means and standard errors shown (n=5). Letters indicate significant differences ($P < 0.05$) using a Tukey test after a two-way ANOVA, with combination and P supply as factors.

Metabolite profile of plants was affected by P supply and scion/rootstock genotype

After 28 DOT, the concentration of Pi, nitrate, sulphate and organic acids in leaves, stems and roots were analysed by ion exchange chromatography. Under HP supply, combinations showed differences in Pi content in the whole plant and PAE, when 1103P was used as the scion Pi content was increased, but PAE was less than PN (Figure VIII.6). Pi content was highly increased under HP compared to LP supply.

Nitrate concentration in roots, stems and leaves was not different under HP supply. However, an increase in nitrate concentration in stem and leaves was observed under LP supply when PN is used as scion (Figure VIII.7A and Table VIII.3). Sulphate concentration was higher in roots, stems and leaves when 1103P was used as rootstocks and was increased in response to LP supply in stems and roots (Figure VIII.7B and Table VIII.3).

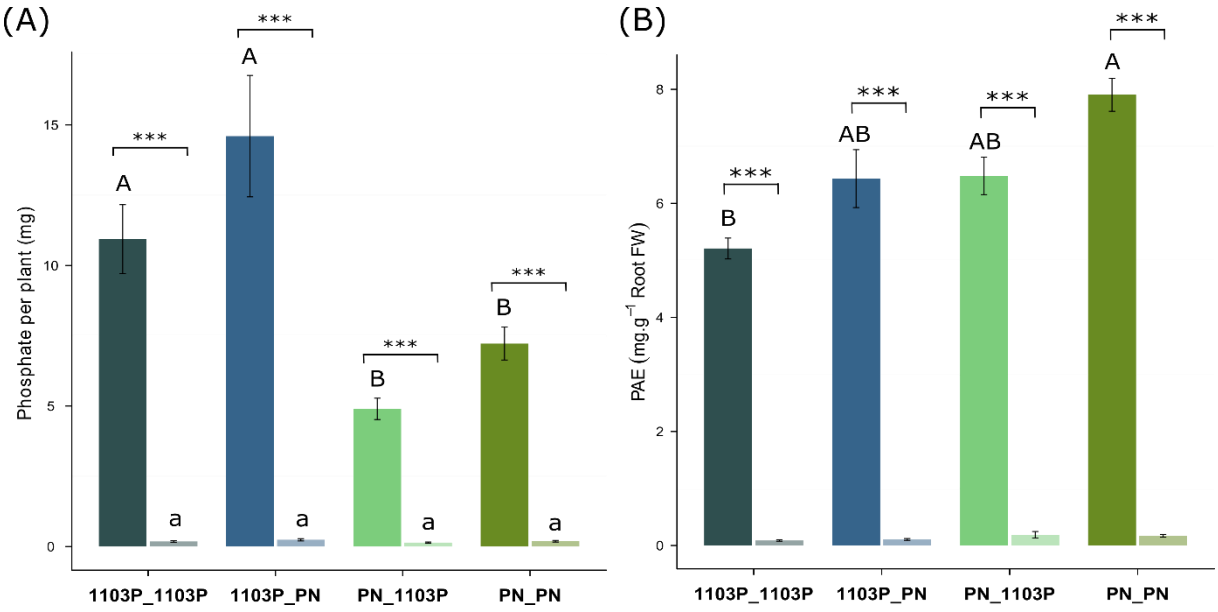


Figure VIII.6. (A) Phosphate content per plant and (B) phosphate acquisition efficiency (PAE) of hetero-grafted and homo-grafted combinations using 1103P and PN, cultivated in hydroponic culture under different P supplies, high P (HP, dark colours) or low P (LP, light colours); after 28 days of treatment. Means and standard errors shown (n=5). For each P-treatment, differences between combinations were analysed using a multiple comparison after Kruskal-Wallis tests at $P < 0.05$, uppercase letters indicate differences under HP supply while lowercase letters indicate differences under LP supply. For each combination, stars indicate an effect of P supply using Wilcoxon test at $P < 0.05$ with the Bonferroni correction (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

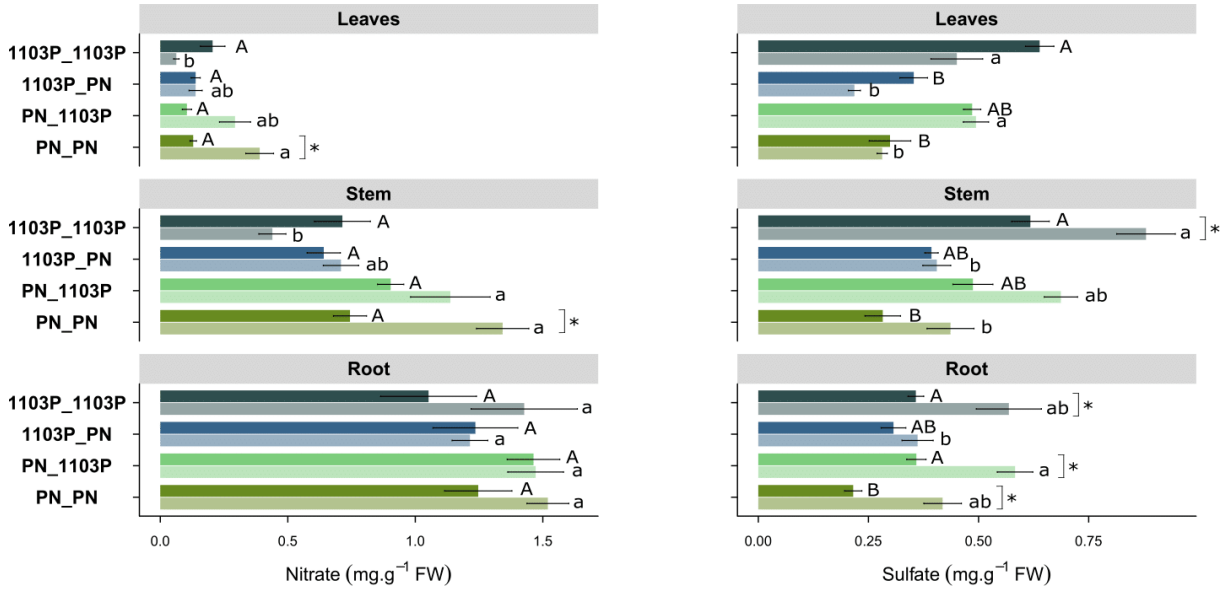


Figure VIII.7. Nitrate and sulphate concentration in leaves, stems and roots of hetero-grafted and homo-grafted combinations using 1103P and PN, cultivated in hydroponic culture under different P supplies, high P (HP, dark colours) or low P (LP, light colours); after 28 days of treatment. Means and standard errors shown (n=5). For each P-treatment, differences between combinations were analysed using a multiple comparison after Kruskal-Wallis tests at $P < 0.05$, uppercase letters indicate differences under HP supply while lowercase letters indicate differences under LP supply. For each combination, stars indicate an effect of P supply using Wilcoxon test at $P < 0.05$ with the Bonferroni correction (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Total organic acids concentration (the sum of malate, tartrate, citrate and fumarate concentrations) was not different between the different scion/rootstock combinations studied, but an increase was observed in leaves in response to LP supply (Figure VIII.8). Furthermore, total organic acids concentration shown an increase under LP supply in roots when 1103P was used as rootstock (Figure VIII.8). When 1103P was used as scion, the increase in total organic acids concentration in leaves was mainly explained by a higher concentration of malate (Figure VIII.9).

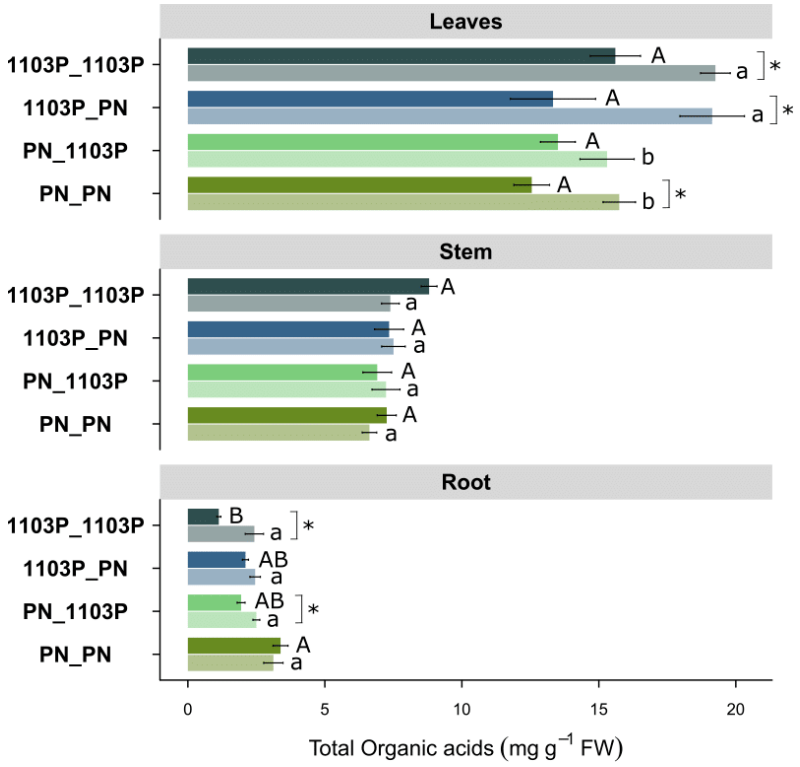


Figure VIII.8. Total organic acids (malate, tartrate, citrate and fumarate) concentration in leaves, stems and roots of hetero-grafted and homo-grafted combinations using 1103P and PN, cultivated in hydroponic culture under different P supplies, high P (HP, dark colours) or low P (LP, light colours); after 28 days of treatment. Means and standard errors shown (n=5). For each P-treatment, differences between combinations were analysed using a multiple comparison after Kruskal-Wallis tests at $P < 0.05$, uppercase letters indicate differences under HP supply while lowercase letters indicate differences under LP supply. For each combination, stars indicate an effect of P supply using Wilcoxon test at $P < 0.05$ with the Bonferroni correction (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

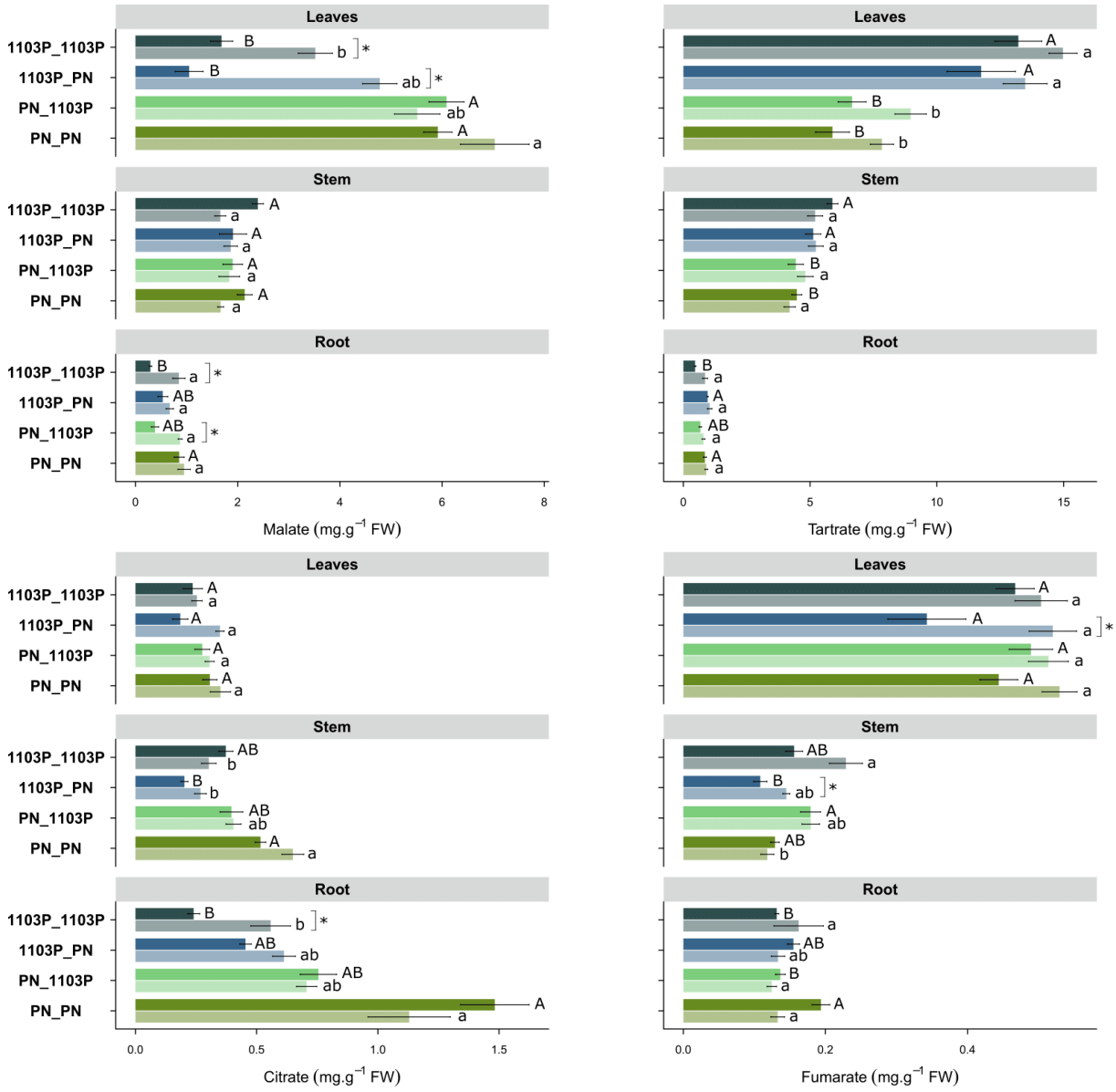


Figure VIII.9. Malate, tartrate, citrate and fumarate concentrations in leaves, stems and roots of hetero-grafted and homo-grafted combinations using 1103P and PN, cultivated in hydroponic culture under different P supplies, high P (HP, dark colours) or low P (LP, light colours); after 28 days of treatment. Means and standard errors shown (n=5). For each P-treatment, differences between combinations were analysed using a multiple comparison after Kruskal-Wallis tests at $P < 0.05$, uppercase letters indicate differences under HP supply while lowercase letters indicate differences under LP supply. For each combination, stars indicate an effect of P supply using Wilcoxon test at $P < 0.05$ with the Bonferroni correction (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

	HP		LP/HP		LP	
	Scion	Rootstock	Scion	Rootstock	Scion	Rootstock
PAE	*	*			*	
Leaf						
Nitrate			*	*	*	
Sulphate		*	*			*
Malate	*		*		*	
Tartrate	*		*		*	
Citrate	*	*		*		*
Fumarate				*		
OA			*		*	
Stem						
Nitrate			*	*	*	
Sulphate		*				*
Malate						
Tartrate	*				*	
Citrate	*			*	*	
Fumarate		*	*			*
OA						
Root						
Nitrate					*	
Sulphate		*	*			*
Malate		*		*	*	
Tartrate		*		*		*
Citrate	*	*	*		*	
Fumarate		*		*		
OA	*	*	*			

Table VIII.3. Scion and rootstock effect on different PAE, nitrate, sulphate and organic acids concentrations in leaves, stems and roots, on plants cultivated under high P (HP) or low P (LP) supply for 28 days. For each P-treatment, scion and rootstock effect were analysed using a Kruskal-Wallis tests at $P < 0.05$, stars indicate significant differences.

Gene expression

The transcript abundance of different genes involved in P nutrition were analysed in the roots after 28 DOT (Figure VIII.10). Scion/rootstocks combinations showed differences in expression of targeted genes under HP supply, and rootstocks and/or scion effect were analysed. When 1103P was used as rootstock, the expression of *PHT1.3a*, *PHO1d* and *PHO2* were increased. On the contrary, the expression of *SIZ1*, *PLDa*, *PAP10a*, *PAP10bc*, *PAP12c*, *PAP26b*, *HA6* and *HA9* was increased when PN was used as the rootstock. The scion genotype had a lower impact on the expression of these genes in the roots. In fact, only the expression of *PHT1.4e* was affected by the scion genotype, being increased when PN in used as the scion. The expression of most of the transcripts studied was increased for all scion/rootstock combinations in responses to LP supply, such as all *PHT1* family, *PHT2.1*, *PHO1a*, *SPX1/2*, *SPX3*, *PAP10a*, *PAP10bc* and *PAP12c*. However, other genes showed a contrasted response, such as *PHO2*, *PLDa* and *HA6* (encoding H^+-ATPase) with an effect of rootstock genotype. Finally, few transcripts were less abundant under low P supply such as *PHO1b*, *PHO1c* and *PAP26b*.

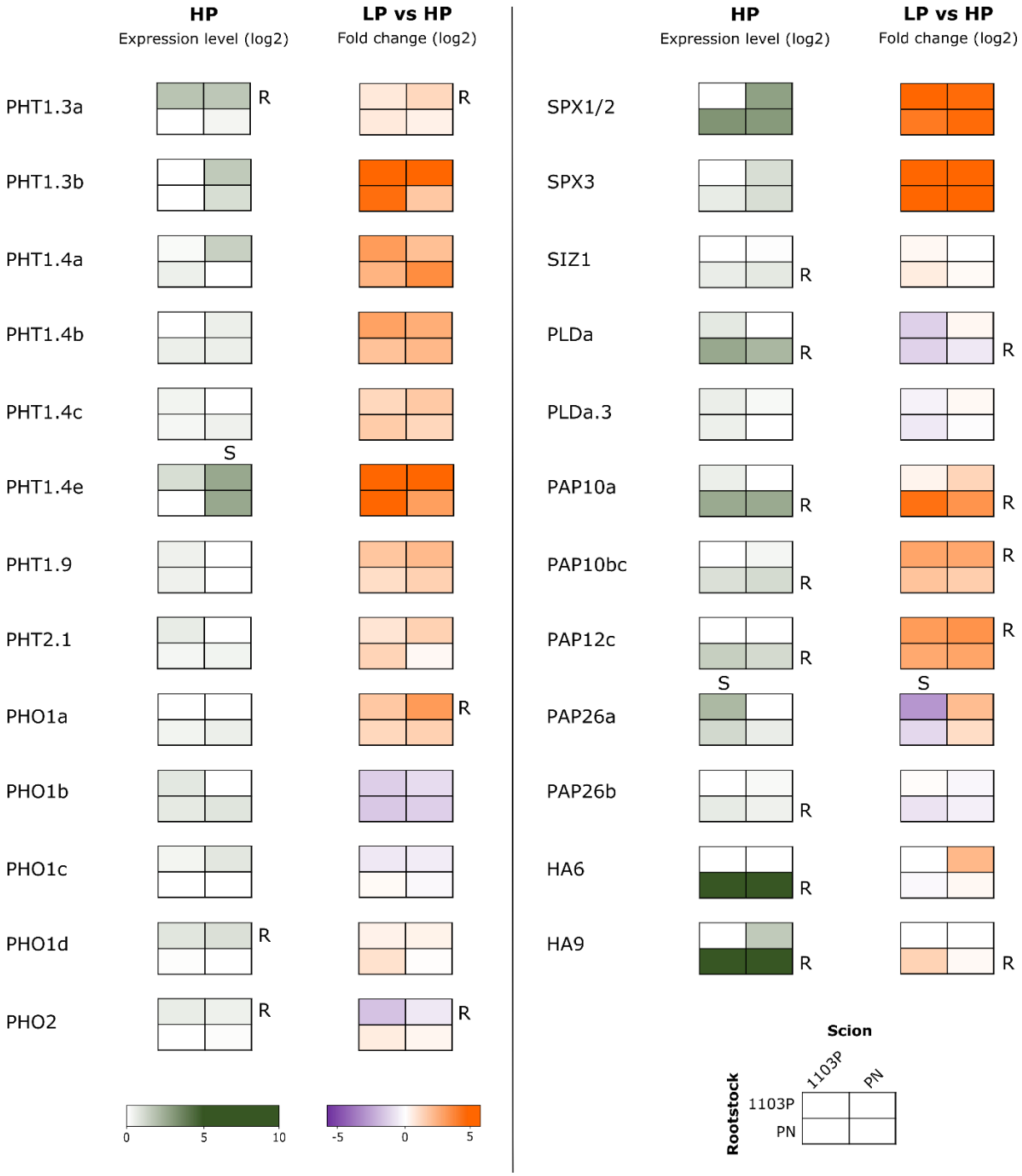


Figure VIII.10. Heatmaps of the abundance of transcripts for some genes involved in phosphate nutrition in roots of hetero-grafted and homo-grafted combinations using 1103P and PN, cultivated in hydroponic culture and their response to low P (LP) supply after 28 days of treatment. For transcript abundance under high P (HP) supply, green shade indicates the level of expression relative to the lowest value (n = 3). For transcript abundance changes in response to LP supply, the violet and orange shades indicate the extent of down- and up-regulation respectively (n = 3). Rootstock (R) and scion (S) effect were analysed using were analysed using a Kruskal-Wallis tests at $P < 0.05$, Differences were indicating by letters.

Discussion

Growth and photosynthetic activities are scion dependant under non-limiting HP conditions

Interaction between the CO_2 assimilation rate and Pi status in leaves is well known. Under non-limiting P supply, the CO_2 assimilation rate tend to a maximum value, suggesting that the photosynthetic activity is related to scion characteristics (Usuda and Shimogawara, 1991). *V. vinifera* cultivars are known to differ by their CO_2 assimilation rate (Keller, 2015). In our experiments under non-limiting conditions, when 1103P was the scion, CO_2 assimilation rate, transpiration rate and water vapour conductance was higher after 28 DOT, related to a higher plant biomass. These results are in agreement with the growth and photosynthetic activity of 1103P and PN cultivated in the same conditions as un-grafted cuttings (Chapter 2). Furthermore, rootstock genotype did not affect CO_2 assimilation rate, suggesting that scion control photosynthetic activity under non-limiting nutrient supply.

Growth and photosynthesis responses to low P supply are rootstock dependant

Phosphate deficiency reduces photosynthetic activity, CO_2 assimilation, chlorophyll concentration and rubisco activity (Bottrill et al., 1970; Lauer et al., 1989; Usuda and Shimogawara, 1991). In our study, LP supply caused a reduction of photosynthetic activity and the decrease of shoot and whole plant biomass with an effect of the rootstock genotype. These results suggest the rootstock can regulate photosynthetic and growth responses to P in grafted grapevines.

Under LP supply, all combinations shown a decrease of shoot FW, but did not change their root biomass, resulting a decrease of shoot:root FW ratio. A decrease of shoot:root FW ratio in a common response of pants to LP supply, allowing a greater surface of soil explored and a potential higher P acquisition (Hermans et al., 2006; Mollier and Pellerin, 1999). In fact, P-starved plants invested more carbon to form new roots and increase the P foraging capacity (Fernandez and Rubio, 2015).

Proton flux and activity of APases in the rhizosphere increase in response to LP supply

Under HP supply, a decrease of protons in the nutrient solution was observed for all combinations, certainly related to anion uptake such as phosphate and nitrate taken up by root using transporters functioning with the symport of protons (Marschner, 2011). On the contrary, the content of protons in the LP nutrient solution was unchanged until 19 DOT, when it increased; this absence of change in pH is probably due to the absence of Pi uptake and low P supply induced exudation of protons

frequently observed in many plant species. There were no differences in pH release between the different scion/rootstock combinations, this result is agreed with the previously chapter on un-grafted grapevines. Furthermore, the expression of *HA6* and *HA9*, encoding H^+-ATPase (Pii et al., 2014), were not correlated with the increase of proton content in response to LP supply, but were higher when PN was used as scion.

The activity of APases released into the media was increased approximatively two-fold for each scion/rootstock combination under LP compared to HP; there was no significant effect of the scion or rootstock genotype. Related to this increase of APase activity, the relative abundances of *PAP10a*, *PAP10bc* and *PAP12c* were increased in response to LP supply. *PAP10* and *PAP12* encode secreted PAP, known to be increased in Pi starvation response in different species such as Arabidopsis, tomato or rice (Tian et al., 2012). *PAP26a* and *PAP26b* did not shown variation of expression under LP supply compared HP non-limiting condition. This is in agreement with the literature and the previously chapter concerning un-grafted grapevines, as the expression of *PAP26* does not increase under LP supply, while the activity of the released APases does increase (Tran et al., 2010a; Tran et al., 2010b; Veljanovski et al., 2006).

pH of the rhizosphere and the Pi availability were also related to the releasing of organic acids. In fact, organic acid release contribute to an acidification of the rhizosphere but its contribution is not very clear and is various according to the species studied (Hoffland et al., 1989).

Phosphate content is dependent on rootstock acquisition and scion development

Under non-limited HP supply, the Pi content in plant is determined by its PAE, controlled by the genotype and environment interactions. 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 homografts compared to 1103P_1103P homografts, 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 defined as whole plant Pi divided by root FW. This could suggest that grafting itself, plant growth conditions and/or plant developmental stage alters PAE in grapevine and/or that the method used to calculate PAE affects the results.

PHT1 family is responsible for Pi acquisition to root from the soil (Nussaume et al., 2011). The level of expression in the root of the seven *PHT1* genes identified in the grapevine genome showed differences between the scion/rootstock combinations under HP supply. In fact, the expression of *PHT1.3a* and *PHT1.4e* showed a rootstock and scion effect respectively, indicating difference in regulation from genotypes. All genes of *PHT1* family were strongly up-regulated under LP supply, suggesting a higher capacity to take up Pi from the soil (Figure VIII.10). PHT1 activity results from a complex signalling pathway including transcriptional, post-transcriptional and post-traditional regulations. In fact, *PHT1* transcript levels are controlled by different key regulators such as PHR1, microRNA miR399 and SPX proteins (Duan et al., 2008; Rubio et al., 2001). Furthermore, post-transcriptional factors such as PHO2, a protein with a E2 ubiquitin conjugase domain, are involved in the degradation of PHT1 transporters (Bari et al., 2006). Our data shown differences in expression of PHO2 depending of rootstock genotype, suggesting differences in post-transcriptional regulation and consequently in PHT1 proteins quantities.

Metabolite profile of plant tissues is dependent on P supply

Sulphate and Pi interactions in plants are well known. Under Pi starvation, plants have strategies to enhance the use of Pi present in cells, such as, the remobilization of Pi from cellular compounds, particularly in phospholipids of membranes (Chen et al., 2011a). Phospholipids are substituted by galactolipids and/or sulpholipids allowing the plant to use the Pi released. In our study, an increase of sulphate concentration was observed in stem and root tissues, suggesting a remobilization of phospholipids from cellular membranes to increase PUE. In general, the concentration of sulphate was higher when the plants were grafted onto the rootstock 1103P than PN. Rootstock genotypes are known to alter sulphur concentration in leaves of its scion, while 1103P confers more sulphur than another rootstock *V. riparia* cv. RGM (Lecourt et al., 2015).

The degradation of phospholipids is catalysed by the phospholipase D family (PLD) and the increase of abundance of the transcripts encoding these proteins in root is a common response to LP supply (Cruz-Ramírez et al., 2006). Under non-limiting conditions, *PLDa* was more highly expressed when PN is the rootstock, which could suggest that there are intrinsic differences in the regulation of this gene between the two genotypes. The expression of two PLD proteins studied in this work, *PLDa* and *PLDa.3*, were not increased under LP supply in our study, but down regulated.

Root development is scion dependent, while root morphology is a characteristic of rootstock and is affected by low P supply

The influence of the scion on rootstock development is poorly understood. However, a few studies have reported an effect of the scion genotype on root system characteristics in grapevine and apple, such as, shoot:root ratio, root biomass or total root length (Harrison et al., 2014; Oslobeanu, 1978; Tandonnet et al., 2010).

In agreement with literature, our results showed an influence of the scion genotype on root length and biomass, certainly related to the greater photosynthetic activity and carbon supply to root growth. Whereas morphological traits, such as, tip density or the proportion of fine roots, was not affected by scion and is rootstock dependant. However, these morphological traits were affected by LP supply with an increase of the proportion of fine roots (from 0.4 to 0.6 mm in diameter), and consequently a decrease of average root diameter of total root system. Few studies have associated the decrease of root diameter with a higher specific root length, indicating the development of a root system with a lower demand of carbon per unit of root length (Pang et al., 2010; Zobel et al., 2007).

Higher tip density is an essential factor for P acquisition (Postma et al., 2014) and for many species the enhancement of emergence of lateral roots is a common response to LP supply (Peret et al., 2011; Peret et al., 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 grapevine, the adaptation of the root system to limited nutrient availability is poorly understood. Differences in branching between grapevine genotypes submitted to different N conditions was observed with an increase of lateral roots numbers in response to low N supply (Cochetel, 2016), suggesting that grapevine genotypes can adapt the branching of their root system under nutrient starvation. Finally, our results shown a decrease of tip density in response to LP supply for all combinations studied, without effect of scion or rootstock genotypes, in agreement with our previous study on un-grafted grapevine genotypes.

Conclusion

Scion and rootstock provide photosynthetic product and water/nutrient respectively. The balance between demand and translocation is controlled by shoot-to-root and root-to-shoot communication. The objective of this study was to determine the roles of both scion and rootstock on parameters involved in P acquisition in grafted grapevines. In this study, we showed that the scion, presumably via its capacity to assimilation and/or translocation of carbon, can modify the development of the root system. However, some parameters of root system architecture, such as fine roots proportion or lateral roots density, was not affected by scion genotype and still properties of rootstocks. All scion/rootstock combination shown the same induction of APase releasing and rhizosphere acidification to LP supply. However organic acids concentration in the different tissues was affected by a scion/rootstock combination, and the response to LP supply shown an effect on scion and rootstock genotype. Known signals shoot-to-root and root-to-shoot involved in P nutrition, such as hormones, sugars, metabolites and miRNA, suggesting regulation of P use and acquisition by both grafting partners. Scion effect on other parameters of root system must to studied (e.g. gravitropism or crown root emergence), to determine the extent of scion role on rootstock development and this capacity to explore and alter the soil to acquire nutrients.

Funding

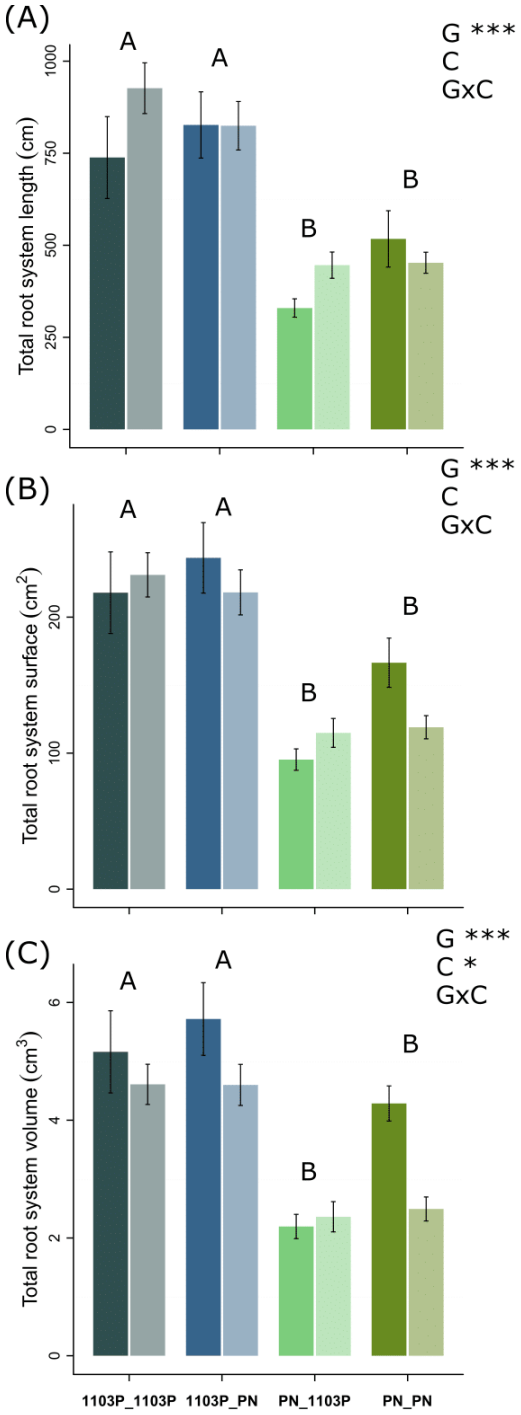
This study has been carried out with financial support from the French National Research Agency (ANR) in the frame of the Investments for the future Program, within the Cluster of Excellence COTE (ANR-10-LABX-45).

Gene name	Accession number	Forward primer	Reverse primer	Efficiency
SPX 1 / SPX 2	VIT_11s0016g05330	GATGGACAGGAAGGGTGTGG	TTTCCTTCAGAGCCCGCAAT	91
SPX 3	VIT_15s0048g00190	CAGGAACACAGTTGCAGCAC	TGGGGATGGGAGAGTGGAAT	91.5
PHT 1;3a	VIT_13s0067g03280	GGGCAATTGTGGCTTTGTCT	TGGTCCTCCCCAGAGAGTTG	96.5
PHT 1;3b	VIT_16s0050g02380	CCCCACTAAAGATAAGCCTGGG	TCCTACTAGGGCAACCCCAA	103.2
PHT 1;4a	VIT_05s0049g00920	TTGTGCTCGGGGTAGTCAAC	CCAGTCCTGGTAGAAGGGGA	90
PHT 1;4b	VIT_05s0049g00930	CCCACCTGGTATTGGAATGAGA	TAGTAGGAGGCTGCATGTCCA	85
PHT 1;4c	VIT_05s0049g00940	CCCGAGTCCAAGGGAAAGTC	GGAACAGTCCTAGCCTGCTG	99.1
PHT 1;4e	VIT_16s0050g02370	TTGGTGACGGAGACCAAAGG	GTGTGCTAGGCATCTGGGTT	94.5
PHT 1;9	VIT_18s0122g00780	CCCGTGAAACCAAGGGAAGA	GAATCCGCGATGAGTTTCGC	95.8
PHT 2;1	VIT_00s0291g00060	TGCGGCTTCCGTTGTTCTAT	TAAGTCCCCTTGCAAACCCC	100
PHO1a	VIT_01s0011g02520	CGGGCTATTTTTAGCTGCCC	ACAGCTCTGAACTTGCCTGC	90.9
PHO1b	VIT_01s0182g00150	GCTTGGAGATCATCCGTCGT	GACTTGAATGCGCGGTATGC	96.8
PHO1c	VIT_05s0049g01410	AGTTGCTTGGGTGGAGACAG	CCAGTGCCCACGTCTGATAA	91.9
PHO1d	VIT_14s0108g01130	CAGCATTGGTTGCAGTGGTT	AATGCCCTGTACTTCCCCAC	96.3
PHO2a	VIT_00s0265g00070	TTGTGCTGTGGAAGCAGGAT	GTGCAGGCAAACTCAAACCA	80.2
SIZ1	VIT_06s0004g03180	CAGTGGGGGTCATGCTGAAT	AGCAAAGAAGCAGTGTCAGC	91
PAP10a	VIT_03s0038g00220	AGCCAGTTTTGGACACGCTA	GTGGGTCCACAAACCTTCCA	96.9
PAP 10bc	VIT_03s0038g00230	TGGTTTTTCAACAGGTTCTGGC	AAGGTTGATAACATCATGACTGGG	96.3
PAP12c	VIT_18s0001g13340	GGACCTCTTCAGAAAAATCCTCT	ACCAAATGGGACAGTGAACAT	100.1
PAP26a	VIT_04s0008g06520	ACAAATGGCGATCGGTATCCT	AGACCTTCCTGATTCCCTCCA	107.2
PAP26b	VIT_11s0118g00240	TGGGCAAGTAAGCTGGGAAG	AAGGAGCAGTCCAGCCAATC	92.4
HA6	VIT_01s0011g01030	GGTTCAGCCTCTGTCACCG	TCCAAACCTGAAGGACATCAGTC	94.6
HA9	VIT_02s0012g00950	TAGGGCTTCCTGGGTTGAGA	GCGTCCTCCAACTGTCTCTG	86
PLDα-3	VIT_04s0008g05450	TCCAAGTCAACCAGAATGGCA	ACTCAGATACGGTGCCAAGG	90.8
PLDα	VIT_09s0002g06760	TCGCCGATGGCAAGTACTATG	GCCTTCTTCTTAAGCAACTCACC	96.7
ACTIN	VIT_04s0044g00580	CTTGCATCCCTCAGCACCTT	TCCTGTGGACAATGGATGGA	93.6
GAPDH	VIT_17s0000g10430	CCACAGACTTCATCGGTGACA	TTCTCGTTGAGGGCTATTCCA	91
SAND3'	VIT_06s0004g02820	TGCTGGGTTACCCCGGAGTTTGA	CAGACCCGGTTGCACGTCCG	89.9

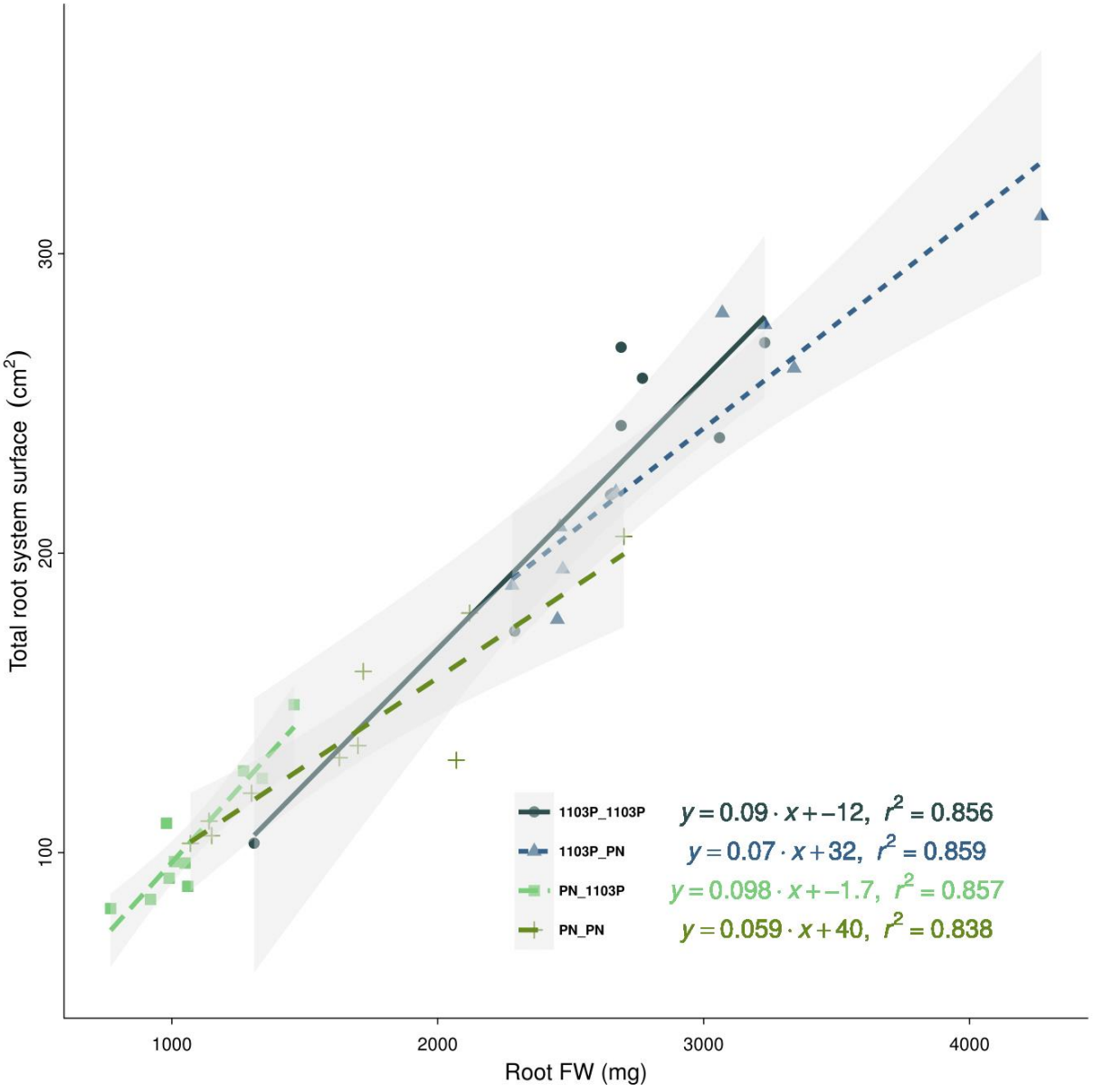
Supplementary table VIII.1. List of primers used for RT-qPCR experiments.

	1103P_1103P		1103P_PN		PN_1103P		PN_PN	
	HP	LP	HP	LP	HP	LP	HP	LP
1 DAT								
Plant FW (g)	1.39 ± 0.07 ^{A}	1.35 ± 0.10 ^{a}	1.31 ± 0.15 ^{AB}	1.11 ± 0.12 ^{ab}	0.97 ± 0.05 ^{AB}	0.78 ± 0.05 ^{b}	0.75 ± 0.04 ^{B}	0.74 ± 0.07 ^{b}
Shoot FW (g)	0.74 ± 0.04 ^{A}	0.81 ± 0.10 ^{a}	0.64 ± 0.07 ^{AB}	0.53 ± 0.04 ^{ab}	0.46 ± 0.03 ^{AB}	0.42 ± 0.03 ^{ab}	0.36 ± 0.03 ^{B}	0.35 ± 0.03 ^{b}
Leaves FW (g)	0.38 ± 0.01 ^{A}	0.44 ± 0.06 ^{a}	0.39 ± 0.04 ^{A}	0.32 ± 0.03 ^{ab}	0.18 ± 0.01 ^{B}	0.18 ± 0.01 ^{b}	0.19 ± 0.02 ^{B}	0.18 ± 0.01 ^{b}
Stem FW (g)	0.36 ± 0.02 ^{A}	0.37 ± 0.04 ^{a}	0.25 ± 0.03 ^{AB}	0.21 ± 0.02 ^{ab}	0.28 ± 0.03 ^{AB}	0.24 ± 0.03 ^{ab}	0.17 ± 0.02 ^{B}	0.17 ± 0.02 ^{b}
Root FW (g)	0.65 ± 0.04 ^{A}	0.54 ± 0.02 ^{a}	0.67 ± 0.08 ^{A}	0.58 ± 0.08 ^{a}	0.51 ± 0.02 ^{AB}	0.36 ± 0.02 ^{a}	0.39 ± 0.01 ^{B}	0.39 ± 0.04 ^{a}
Shoot:Root FW ratio	1.15 ± 0.04 ^{A}	1.49 ± 0.20 ^{a}	0.96 ± 0.07 ^{A}	0.95 ± 0.08 ^{a}	0.91 ± 0.06 ^{A}	1.16 ± 0.06 ^{a}	0.92 ± 0.07 ^{A}	0.91 ± 0.06 ^{a}
Leaves:Stem FW ratio	1.09 ± 0.05 ^{AB}	1.17 ± 0.05 ^{ab}	1.57 ± 0.07 ^{A}	1.56 ± 0.04 ^{a}	0.68 ± 0.08 ^{B}	0.81 ± 0.10 ^{b}	1.11 ± 0.04 ^{AB}	1.12 ± 0.07 ^{ab}

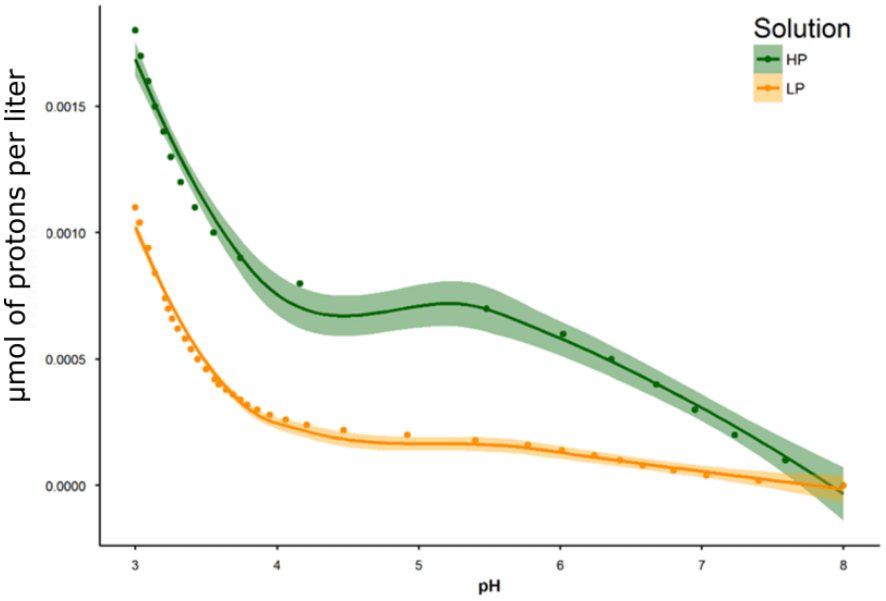
Supplementary table VIII.2. Whole plant, shoot, leaf, stem and root fresh weight (FW); shoot:root and leaves:stem FW ratio, number of leaves and relative growth rate of hetero-graft and homo-graft combinations using 1103P and PN, cultivated in hydroponic culture under different P supplies, high P or low P; after 1 days of treatment. Means and standard errors shown (n=5). For each P-treatment, combination differences were analysed using a multiple comparison after Kruskal-Wallis tests at $P < 0.05$, uppercase letters indicate differences under HP supply while lowercase letters indicate differences under LP supply. For each combination, no effect of P supply was observed using Wilcoxon test at $P < 0.05$ with the Bonferroni correction.



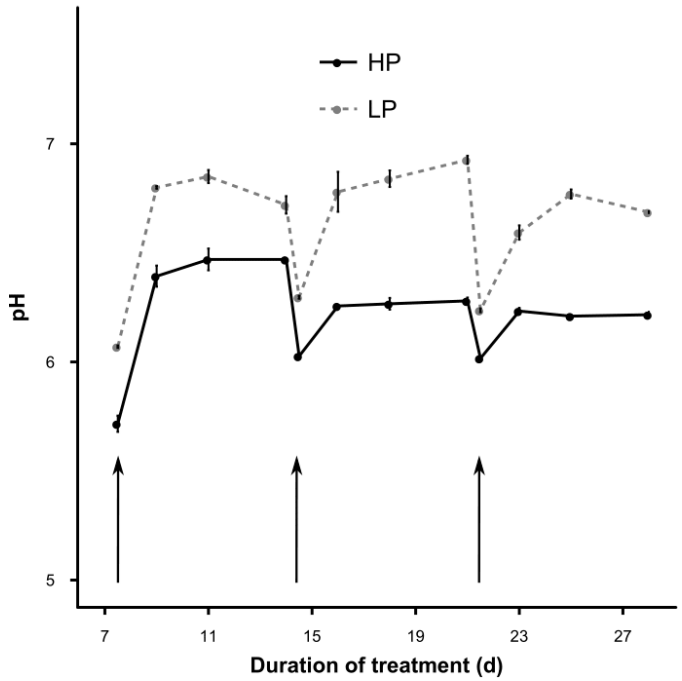
Supplementary Figure VIII.1. (A) Length, (B) surface and (C) volume of total root system of hetero-grafted and homo-grafted combinations using 1103P and PN, cultivated in hydroponic culture under different P supplies, high P (HP, dark colours) or low P (LP, light colours); after 28 days of treatment. Means and standard errors shown (n=5). Data were analysed using a two-way ANOVA, with combination (G) and P supply (C) as factors (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Uppercase letters indicate differences between combinations analysed using a Tukey test.



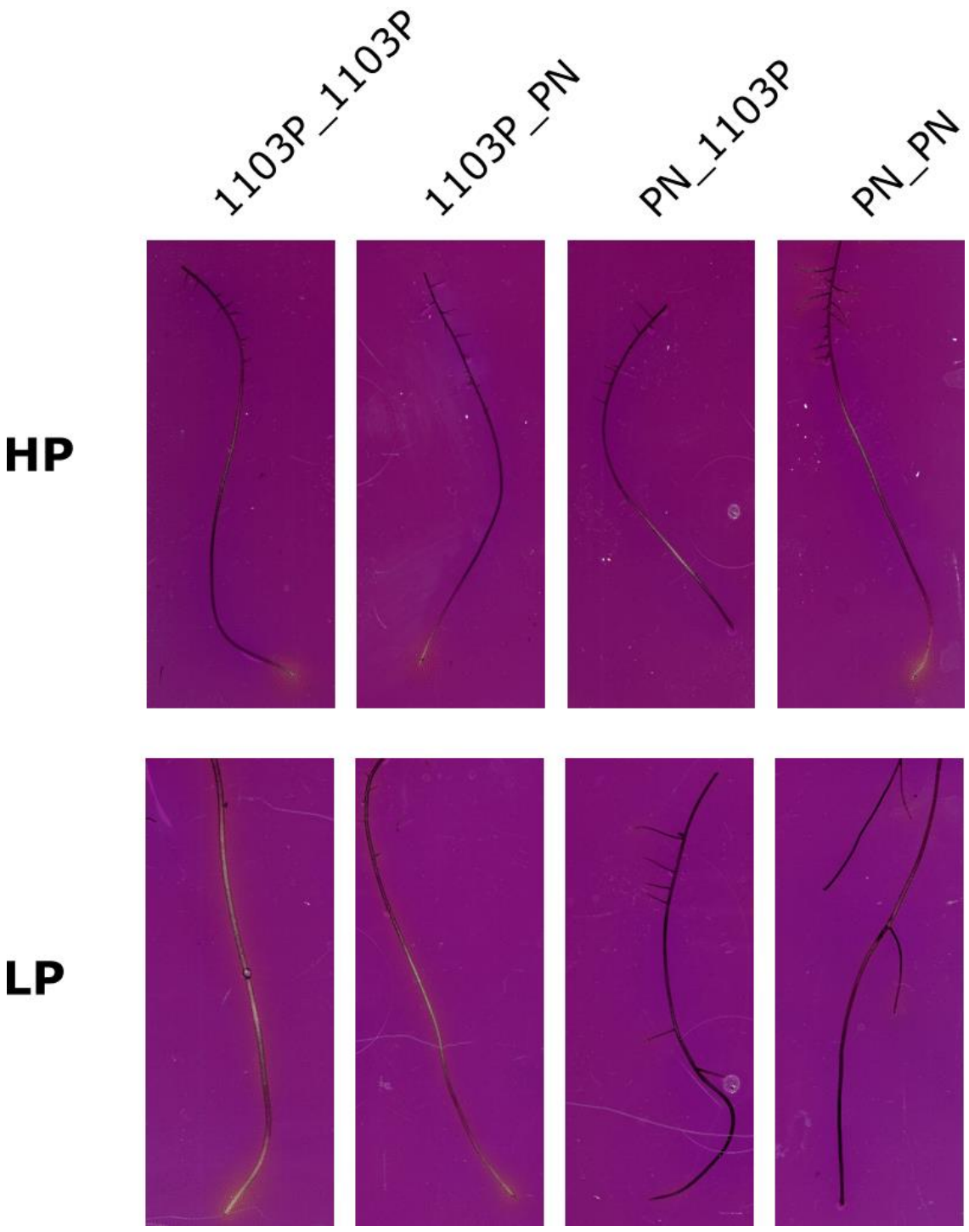
Supplementary Figure VIII.2. Linear regression between fresh weight and surface of total root system of hetero-grafted and homo-grafted combinations using 1103P and PN, cultivated in hydroponic culture under different P supplies, high P or low P; after 28 days of treatment.



Supplementary Figure VIII.3. Buffering capacities of the two nutrient solutions containing two different P concentrations, 600 µM (HP, green) or 1 µM (LP, orange), calculated by the progressive addition of HCL and measurement of pH change.



Supplementary Figure VIII.4. pH changes of nutrient solutions containing two different P concentrations, high P (HP, black circles connected by solid lines) or low P (LP, grey circles connected by dashed lines), without plants. Arrows represent removing and adjusting pH of nutrient solutions.



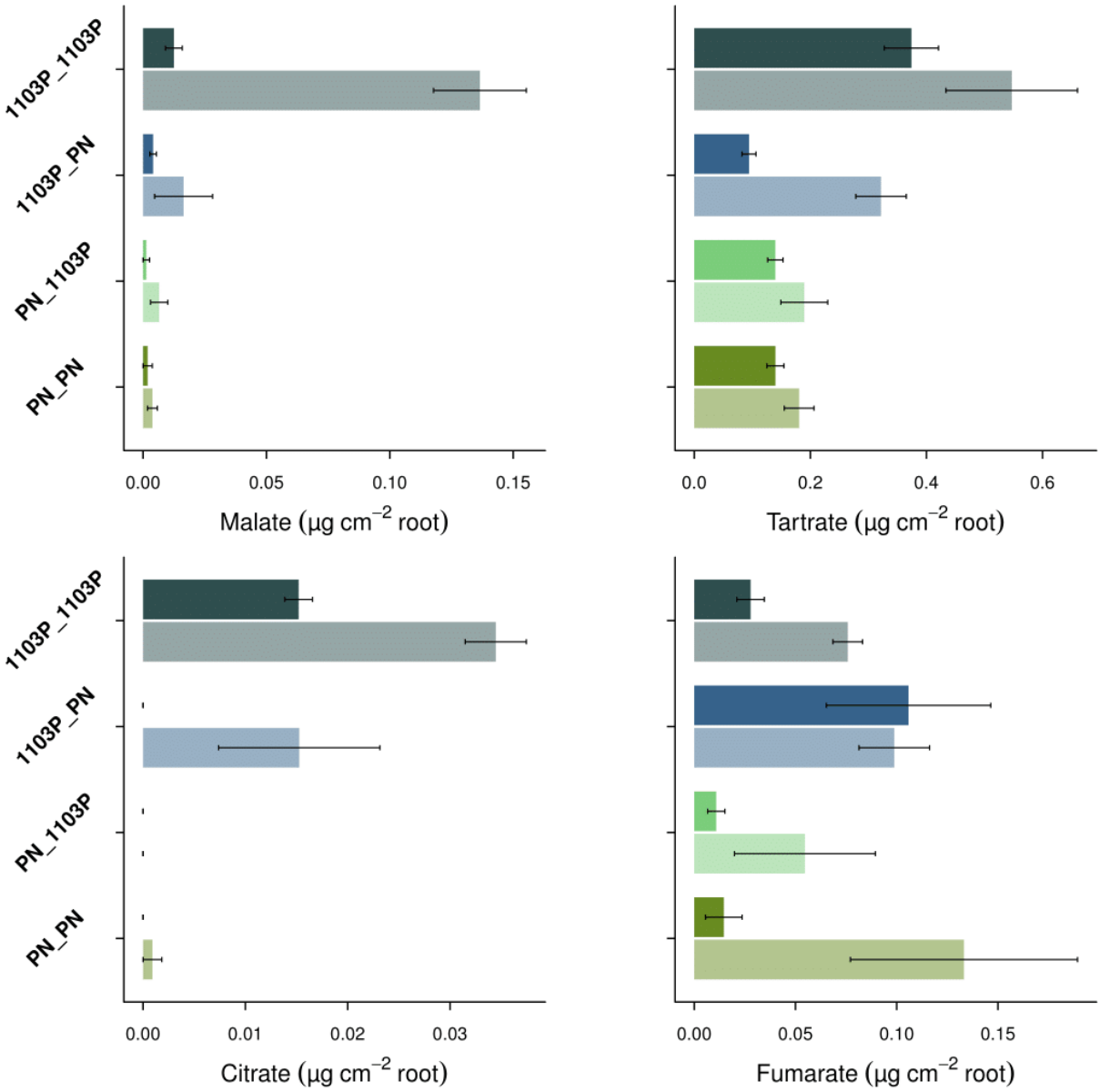
Supplementary Figure VIII.5. The rhizosphere acidification by excised roots of hetero-grafted and homo-grafted combinations using 1103P and PN, cultivated in hydroponic culture under different P supplies, high P (HP) or low P (LP); after 28 days of treatment, using bromocresol purple pH indicator. Yellow indicate a decrease of pH.

Perspectives

Ce chapitre met en avant l'interaction du greffon et du porte greffe sur les caractéristiques de la nutrition phosphatée. L'effet du fonctionnement du greffon et plus précisément de son activité photosynthétique sur le développement racinaire est mis en évidence. Les paramètres étudiés sur les porte-greffes modèles tels que 1103P et RGM peuvent donc être modulés par le greffon utilisé et modifier leurs caractéristiques notamment en conditions minérales non limitantes. Cependant le rôle dominant du porte-greffe en réponse à la faible disponibilité en P est mis en évidence, révélant des propriétés génotypiques non influencées par le greffon.

Evidemment ce chapitre soulève de nouvelles questions sur le contrôle de la nutrition phosphatée chez la Vigne. Tout d'abord l'influence du greffon sur le système racinaire du porte-greffe devrait être mieux caractérisée afin de comprendre les paramètres dépendant de l'apport carboné du greffon et ceux restant des caractéristiques propres aux porte-greffes. De plus, peu d'informations permettent de déterminer si l'influence du greffon est dépendante du génotype de porte-greffe. La compréhension de l'interaction entre greffon et porte-greffe est donc indispensable afin de définir la réponse de la Vigne à la faible disponibilité en P.

L'identification et la quantification des acides organiques exsudés dans la rhizosphère ont été effectuées au cours de cette expérimentation. Les exsudats racinaires ont été prélevés à l'aide de la totalité du système racinaire, comme décrit page 120. Cependant, les résultats (Supplementary Figure VII.6) sont difficilement interprétables comme discuté dans le chapitre précédent. Une étude plus approfondie de la caractérisation des exsudats racinaires est nécessaire afin d'identifier leur régulation en réponse à la disponibilité à P.



Supplementary Figure VIII.6. Acides malique, tartrique, citrique et fumarique exsudés par les racines des différentes combinaisons de greffes, avec PN et 1103P, cultivées en hydroponie avec différentes disponibilités en P, 600 µM (couleurs foncées) ou 1 µM (couleurs claires); après 28 jours de traitement. Les moyennes et erreurs standards sont calculées sur n=5. Les acides organiques ont été récoltés à partir de tout le système racinaire transféré dans 40 ml d'eau ultrapure pendant 6h.

CHAPITRE 4

Réponse à la faible disponibilité en phosphore en fonction du porte-greffe et du greffon : approche transcriptomique

En collaboration avec Dr. Noé Cochetel

Introduction

Several studies show that the transcriptome of plants responses considerably to Pi supply, in both the roots and the leaves (Lan et al., 2012; Misson et al., 2005; Morcuende et al., 2007; Wasaki et al., 2006; Wasaki et al., 2003; Woo et al., 2012). For example, recently (Wang et al., 2018) identified 9371 transcripts differentially expressed in response to LP supply for 10 d in roots of oat (*Avena sativa L.*). In the literature, genes involved in sensing, signalling and response to Pi starvation are called Pi-starvation-inducible genes (PSI) (Lan et al., 2018). Lan et al. (2018) performed a meta-analysis of previous published studies on the response of *A. thaliana* roots to P supply and identified a core of one hundred of PSI genes induced few hours or days after Pi starvation, independent of growth condition, experimental design and method of analysis. Among this list of PSI genes, several groups were represented such as genes related to lipid metabolism and galactolipid biosynthesis, transcription factors containing SPX domains, Pi transporters of the PHT family, protein kinases and intracellular secreted PAPs (Lan et al., 2018).

Despite the conservation of some transcriptomic responses to P supply, genotypic differences have been identified in maize which highlighted the role of detoxification of reactive oxygen species in tolerating LP conditions in short term responses (2 and 8 d, Du et al. (2016)) and the biosynthesis of secondary metabolites, ion transport and phytohormone regulation in long term responses (25 d, Sun et al. (2016)). In grapevine, the transcriptome of roots of different rootstock genotypes has been shown to respond differently to N (Cochetel et al., 2018) and water deficit (Corso et al., 2015; Yıldırım et al., 2018). To date, there have been no studies on the how scions modify the transcriptome of a rootstock; however a number of studies have done reciprocal experiments and have identified rootstock responsive transcripts in the scion (Cookson and Ollat, 2013; Jensen et al., 2010; Liu et al., 2016c; Prassinos et al., 2009). To understand the root responses to LP supply at the transcriptome level in grafted grapevine, RNA sequencing was used to investigate transcript abundance in the root tips of the plants used in the experiments described in chapter 3. The bioinformatic analysis was performed by Dr. Noé Cochetel, University of Nevada, Reno, USA. This approach allowed the identification of transcripts differentially expressed in response to P supply in grapevine roots, and how the transcript response differs between two different genotypes and whether it is affected by grafting with a non-self-scion.

This chapter aims to describe the results of a preliminary analysis of the RNA sequencing data, but an extensive investigation is in progress and will be completed in the near future.

Materials and methods

Plant material and growing conditions

Plants used in this experiment were submitted to the same growing conditions that are described in chapter 3. See plant material and growing conditions page 129.

RNA extraction and sequencing

After 27 h of treatment, three pools of three root tips per plant (~15 mm in length) were harvested and immediately snap-frozen in liquid N. 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).

RNA-Seq libraries were generated from 500 ng of total RNA using TruSeq Stranded mRNA LT Sample Preparation Kit (Illumina), according to manufacturer's instructions. Briefly, following purification with poly-T oligo attached magnetic beads, the mRNA was fragmented using divalent cations at 94°C for 2 min. The cleaved RNA fragments were copied into first strand cDNA using reverse transcriptase and random primers. Strand specificity was achieved by replacing dTTP with dUTP during second strand cDNA synthesis using DNA Polymerase I and RNase H. Following addition of a single 'A' base and subsequent ligation of the adapter on double stranded cDNA fragments, the products were purified and enriched with PCR (30 sec at 98°C; [10 sec at 98°C, 30 sec at 60°C, 30 sec at 72°C] x 12 cycles; 5 min at 72°C) to create the cDNA library. Surplus PCR primers were further removed by purification using AMPure XP beads (Beckman-Coulter) and the final cDNA libraries were checked for quality and quantified using capillary electrophoresis. These libraries were then sequenced on the Illumina Hiseq 4000 as paired-end 50 base reads following Illumina's instructions. The RNA-seq library construction and sequencing was realized by the platform "GenomEast" in Strasbourg, France.

Pre-processing of RNA-seq data

Adapter dimer reads were removed using DimerRemover (https://sourceforge.net/projects/dimerremover/) and quality of each sample was assessed with FastQC v0.11.2 (Andrews, 2014). Filtered reads were mapped onto the V1 grapevine genome (Jaillon et al., 2007) using Tophat v2.0.14 (Kim et al., 2013) and the bowtie2 v2.1.0 aligner (Langmead and Salzberg, 2012). Quantification of gene expression from uniquely aligned reads was performed using HTSeq v0.6.1 (Anders et al., 2015) and CRIBI V1 annotation (http://genomes.cribi.unipd.it/grape/).

Co-expression network analysis

A co-expression gene network was constructed using the WGCNA software package (v1.63) in R (Langfelder and Horvath, 2008; Langfelder and Horvath, 2012). Prior to this analysis, low-expressed genes were removed with a minimum threshold of 20 counts in all the libraries. A total of 22,131 genes satisfying the above threshold was obtained. Counts data were transformed using the function varianceStabilizingTransformation of the package DESeq2. The resulting set of counts was used for network construction and module detection using the function blockwiseModules. Briefly, an adjacency matrix was created by calculating the biweight mid-correlation raised to a power β of 11 (soft threshold estimated with the pickSoftThreshold function) and the maxPoutliers parameter set to 0.05. The subsequent Topological Overlap Matrix (TOM) was used for module detection using the DynamicTreecut algorithm with a minimal module size of 30 and a branch merge cut height of 0.25. The module eigengenes were used to evaluate the association between the resulting 26 modules and traits (P supply, Scion, Rootstock).

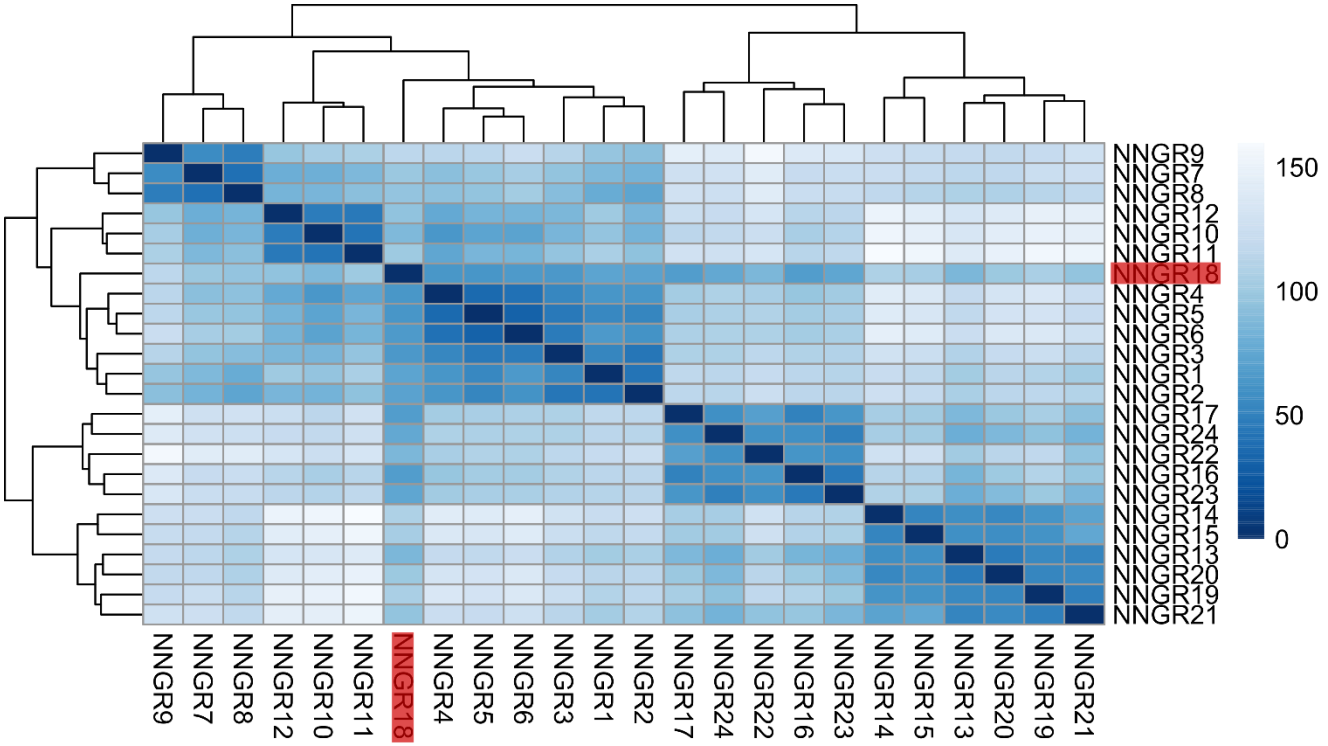
Differential expression analysis and functional categories enrichment

The R package DESeq2 (Love et al., 2014) was used to identify differentially expressed genes using the following threshold: False Discovery Rate (FDR) adjusted p-value <0.01. Gene Ontology (GO) enrichment was performed using the R package topGO (Alexa and Rahnenfuhrer, 2016). Enriched functional categories with an FDR adjusted p-value > 0.01 after Fisher's test were filtered out.

Results

Samples validation

Before the expression analysis, a sample control quality was performed. The sample NNGR18 corresponding to 1103P_PN cultivated in LP, was very different from two other biological replicates, NNGR16 and NNGR17, and did not clustering with them (Figure IX.1). As a consequence, the sample NNGR18 was removed for the following analysis.



ID	Combination	Condition
NNGR1	1103P_1103P	HP
NNGR2	1103P_1103P	HP
NNGR3	1103P_1103P	HP
NNGR4	1103P_1103P	LP
NNGR5	1103P_1103P	LP
NNGR6	1103P_1103P	LP
NNGR7	PN_1103P	HP
NNGR8	PN_1103P	HP
NNGR9	PN_1103P	HP
NNGR10	PN_1103P	LP
NNGR11	PN_1103P	LP
NNGR12	PN_1103P	LP
NNGR13	1103P_PN	HP
NNGR14	1103P_PN	HP
NNGR15	1103P_PN	HP
NNGR16	1103P_PN	LP
NNGR17	1103P_PN	LP
NNGR18	1103P_PN	LP
NNGR19	PN_PN	HP
NNGR20	PN_PN	HP
NNGR21	PN_PN	HP
NNGR22	PN_PN	LP
NNGR23	PN_PN	LP
NNGR24	PN_PN	LP

Figure IX.1. Sample distance and clustering of transcriptome profile of root tips of hetero-grafted and homo-grafted combinations using 1103P and PN**,** cultivated in hydroponic culture under different P supplies, high P (HP) or low P (LP); after 27 hours of treatment.

Transcriptome was mainly affected by P supply and rootstock genotypes

Principal component (PC) analysis of transcriptome in the tip roots was performed (Figure IX.2). The first two principle components, PC1 and PC2, explained 58 and 29 % of total variability respectively. Principle component 1 separated the scion/rootstock combinations in function of the rootstock genotype, while PC2 separated is in function of P supply. Considering each scion/rootstock combination and P treatment, individual samples grouped closely together. In general, there was a small effect of the scion on the root transcriptome, except when 1103P was the rootstock under HP supply.

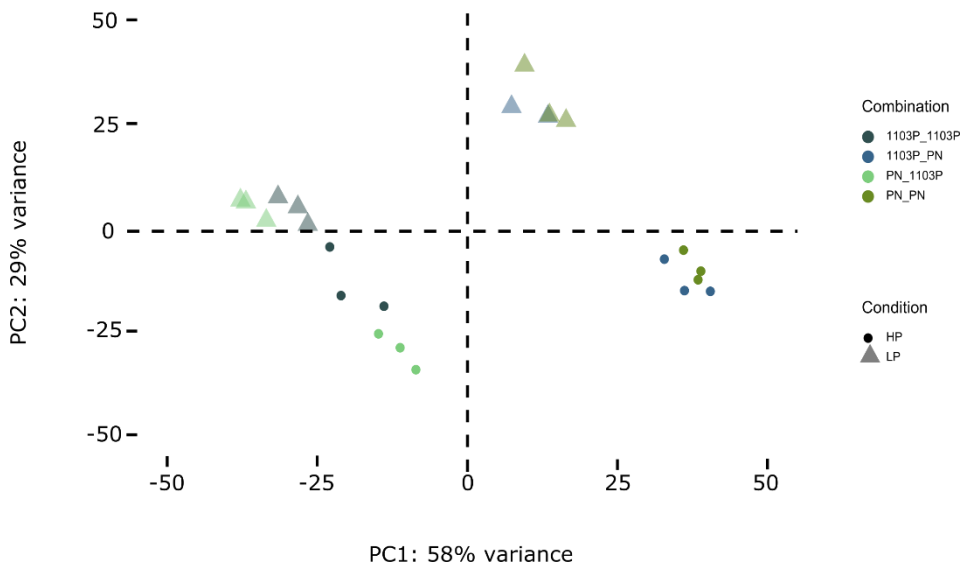


Figure IX.2. Principal component (PC) analysis of transcriptome in the tip roots of hetero-grafted and homo-grafted combinations using 1103P and PN, cultivated in hydroponic culture under different P supplies, high P (HP, circle in dark colours) or low P (LP, triangle in light colours); after 27 h of treatment.

Clusters of genes depending of P-supply or the rootstock genotypes were identified

A gene co-expression network analysis was performed to investigate clusters of highly correlated genes (assigned to co-expression modules). 26 modules (including the grey module containing the unconnected genes) were found and analysed for their correlation with P-treatment, scion or rootstock genotype. Three modules (brown, grey60, purple) were highly correlated to P supply (with a correlation coefficient >0.80 and a P-value <0.01), five modules (pink, turquoise, midnight blue, tan and yellow) were correlated to the rootstock genotype, while none to the scion genotypes (Figure IX.3).

Considering Pi supply, the module "purple" showed the higher correlation with HP supply, while the module "grey60" showed the higher correlation with LP supply (Figure IX.4). Many functional categories were found to be related to P supply described in Figure IX.4, mainly linked to biotic stress in response to LP supply.

Genes involved in Pi starvation were found up-regulated in LP supply such as VIT_02s0025g05110 (MATE efflux family protein), positively and negatively correlated with the module "grey60" and "purple" respectively. Multidrug and toxic compound extrusion (MATE) are involving in the release of low molecular weight metabolites including organic acids, and genes encoding MATE are known to be induced in LP supply (Omote et al., 2006; Valentinuzzi et al., 2015). Other genes showed a down-regulation under LP supply and negatively correlated with the 'grey60' module, such as VIT_06s0004g00030 (GIBBERELLIC ACID METHYLTRANSFERASE 2) and VIT_19s0140g00120 (GIBBERELLIN 2-BETA-DIOXYGENASE) involved in gibberellins pathway, known to be repressed by Pi limitation (Devaiah et al., 2009). Finally, VIT_01s0182g00140 (EXS (ERD1/XPR1/SYG1) family protein)) shown a positive correlation with the module 'purple', so a higher expression under HP supply. VIT_01s0182g00140 encodes a homologue protein of PHO1;H8, known to be down-regulated in root of A. thaliana under LP supply (Wang et al., 2004).

Considering the rootstock genotype, the module "turquoise" showed the higher correlation with 1103P, while the module "yellow" showed the higher correlation with PN (Figure IX.5). These modules contained functional categories correlated to one rootstock genotype without the influence of the P supply or the scion genotype. For 1103P, many highly connected genes were related to carbohydrate metabolism, while highly connected genes with PN were involved in biotic stress (Figure IX.5).

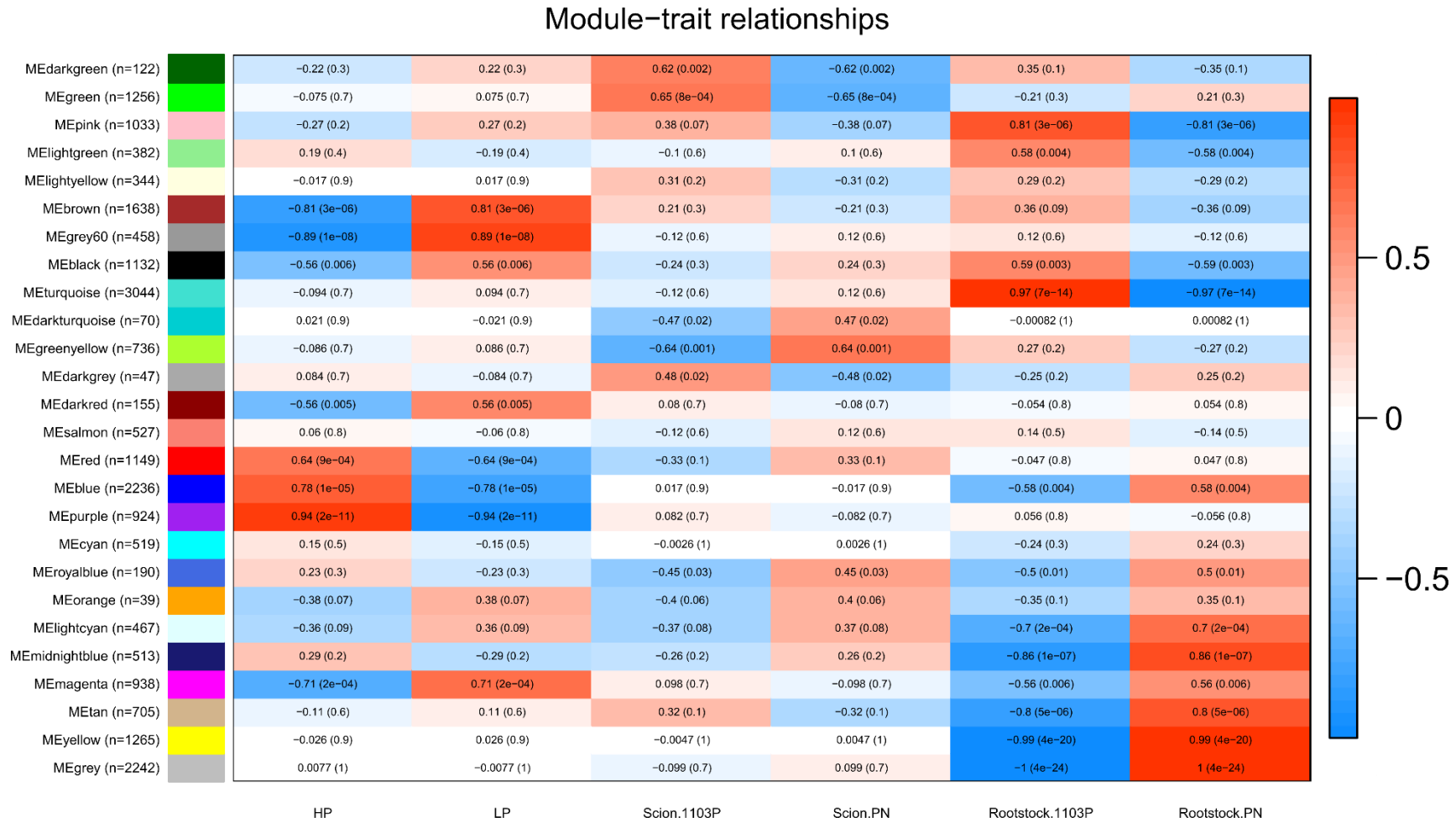


Figure IX.3. Module-trait relationships. Columns correspond to factor studied and their association with each module eigengene is done by a correlation coefficient and its P-value. A positive correlation coefficient between the factors was represented with the red colour, while a negative correlation with the blue colour. For each module, the number of contigs was available in the left panel.

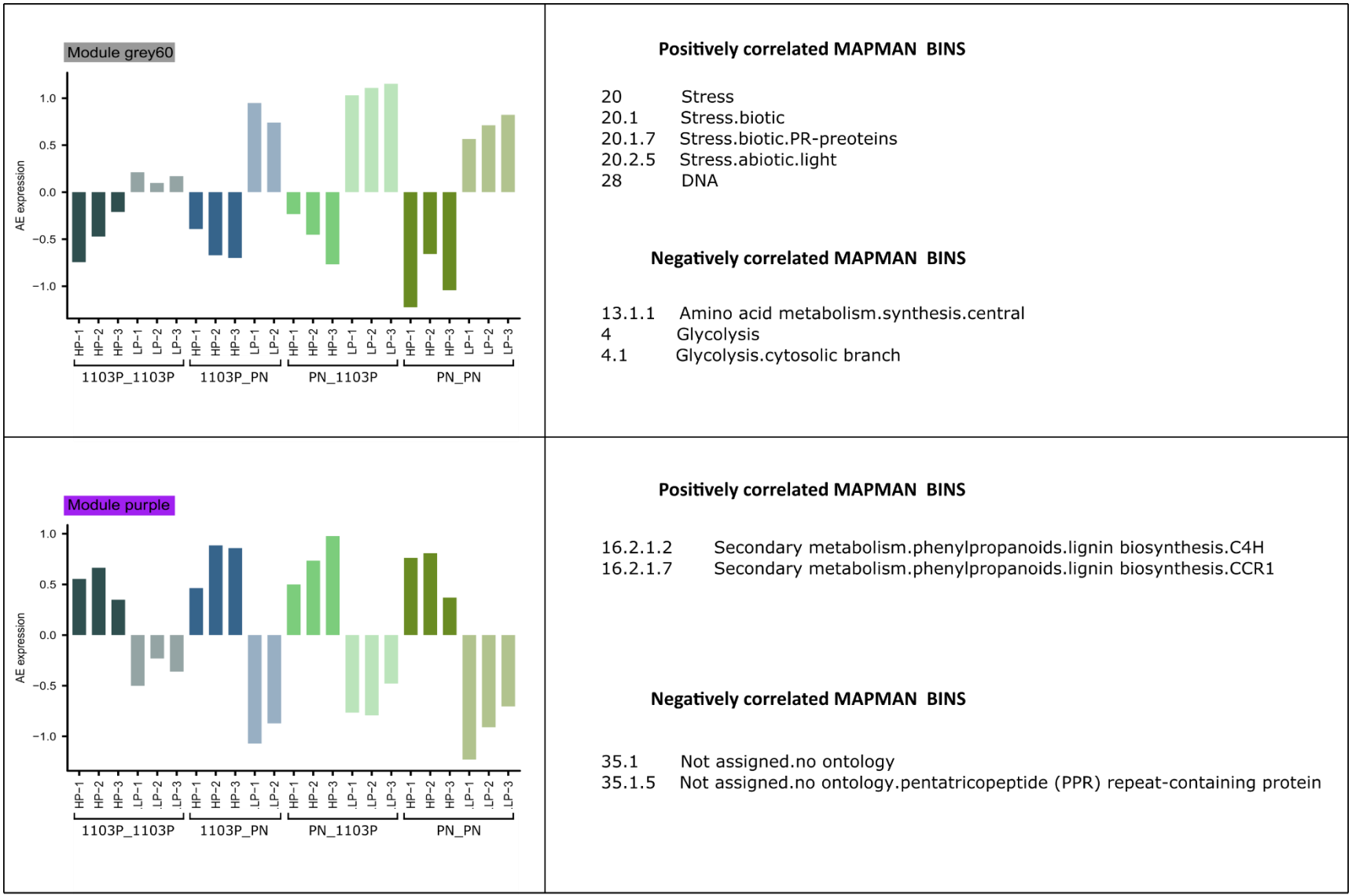
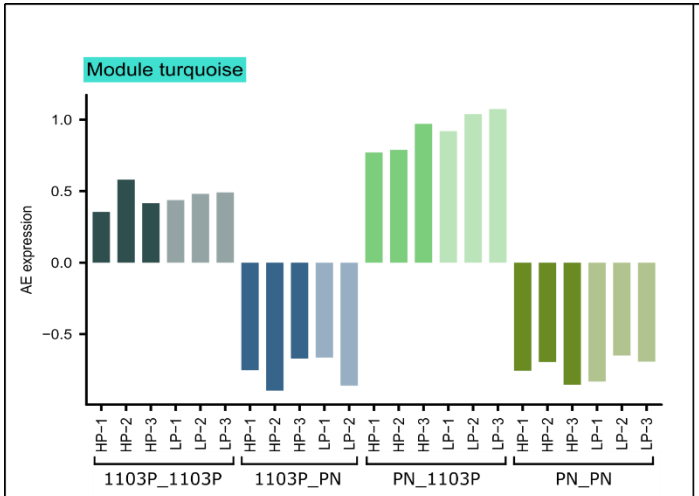


Figure IX.4. Average eigengene expression for selected modules ‘grey60’ and ‘purple’ correlated to P supply. In the right panel, major functional categories linked with the module were available.

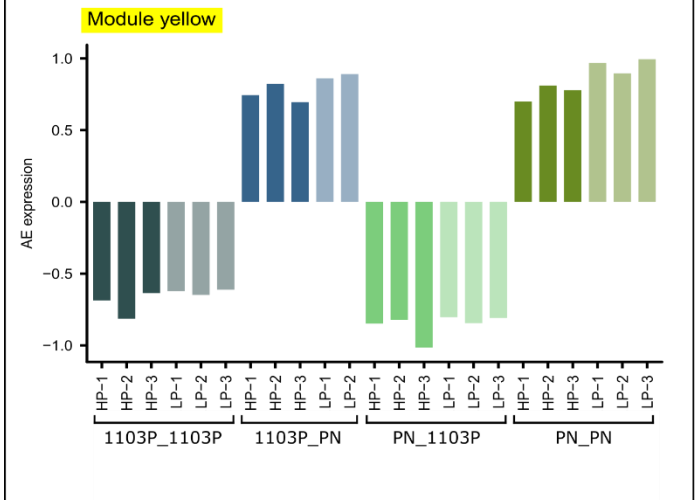


Positively correlated MAPMAN BINS

2.2.2	Major CHO metabolism.degradation.starch
2.2.2.1	Major CHO metabolism.degradation.starch.starch cleavage
26	Misc
26.3.1	Misc.gluco-, galacto- and mannosidases.alpha-galactosidase
27.1	RNA.processing
27.1.2	RNA.processing.RNA helicase

Negatively correlated MAPMAN BINS

20	Stress
23.3.2	Nucleotide metabolism.salvage.nucleoside kinases
23.3.2.1	Nucleotide metabolism.salvage.nucleoside kinases.adenosine kinase
26.3.4	Misc.gluco-, galacto- and mannosidases.endoglucanase
27.1.2	RNA.processing.RNA helicase
28.1	DNA.synthesis/chromatin structure



Positively correlated MAPMAN BINS

20	Stress
20.1	Stress.biotic
20.1.7	Stress.biotic.PR-proteins
27.1.2	RNA.processing.RNA helicase
28.1	DNA.synthesis/chromatin structure

Negatively correlated MAPMAN BINS

26.3.1	Misc.gluco-, galacto- and mannosidases.alpha-galactosidase

Figure IX.5. Average eigengene expression for selected modules ‘turquoise’ and ‘yellow’ correlated to the rootstock genotype. In the right panel, major functional categories linked with the module were available.

The scion has an influence on the response to LP supply in grafted grapevine

The gene co-expression network analysis did not identify genes modules correlated with the scion genotype (with a correlation coefficient >0.80 and a P-value <0.01). However, the analysis of the differentially expressed genes (DEGs) in root tips in response to LP supply, identified transcripts responding P supply specific to scion/rootstock combinations (Figure IX.6). These results suggest a high effect of scion on rootstock transcriptome; and on the differential regulation of gene expression in root tips in responses to LP supply. Due to the role of the shoot in the systemic response to LP supply, genotypic properties of the scion could be involved in the sensing and the signalling. In fact, shoot regulates root responses in LP supply, via different signalling pathways such as sucrose, phytohormones, miRNA etc. (Amtmann et al., 2005; Hammond and White, 2008; Pant et al., 2008).

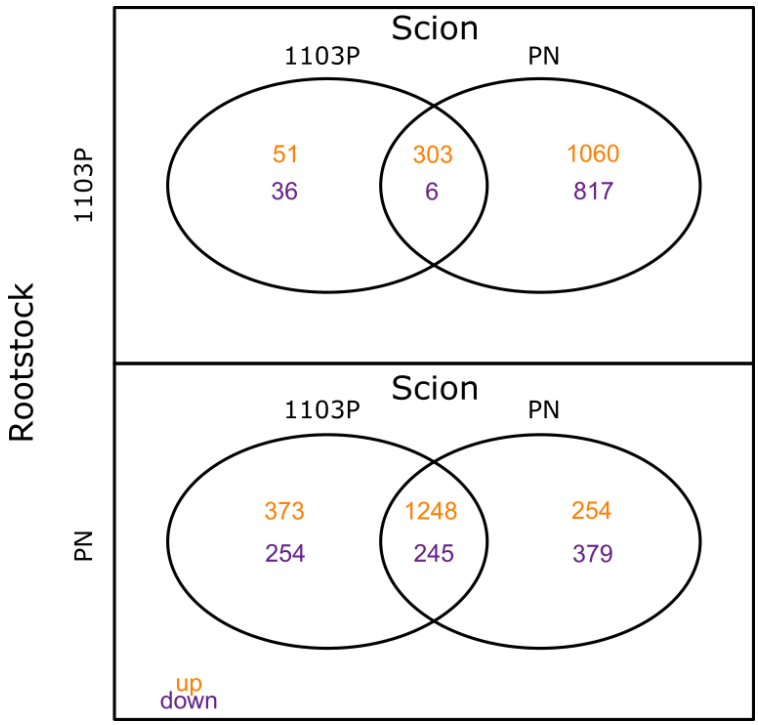


Figure IX.6. Analysis of the differentially expressed genes (DEGs) in root tips in response to LP supply, of hetero-grafted and homo-grafted combinations using 1103P and PN, cultivated in hydroponic culture after 27 hours of treatment. Violet and orange indicate the down- and up-regulation respectively of DEGs in response to LP supply.

Conclusion and perspectives

To understand transcriptomic regulation of grafted grapevine in responses to LP supply, RNA sequencing was performed. Preliminary analysis shown a large variation on the differential regulation of gene expression in root tips between the scion/rootstock combinations studied. The interpretation of these results needs more time. Furthermore, effect of both scion and rootstock was observed suggesting a genotype specific interaction between the two grafted partners.

DISCUSSION GENERALE

ET PERSPECTIVES

Ce travail de thèse a consisté à mettre en évidence les mécanismes impliqués dans la nutrition phosphatée de la Vigne et plus spécifiquement à comprendre pourquoi les porte-greffes issus de *V. rupestris* ou *V. berlandieri* conférent des quantités plus importantes de P à leur greffon que les porte-greffes issus de *V. riparia*. Pour cela, différents paramètres de la nutrition phosphatée ont été étudiés dans un premier temps sur trois génotypes cultivés en boutures simples, dont deux porte-greffes, RGM (*V. riparia* cv. Riparia Gloire de Montpellier) et 1103P (*V. rupestris* x *V. berlandieri* cv. 1103P Paulsen), et un cépage d'origine Eurasienne, PN (*V. vinifera* cv. Pinot noir). La caractérisation de ces génotypes a débuté par l'étude de leur capacité à utiliser les réserves accumulées l'année précédente, à acquérir du Pi depuis le sol, et leurs contributions relatives lors de la reprise végétative (Chapitre 1). La seconde partie de l'étude a consisté à comprendre les mécanismes liés à la PAE plus forte de 1103P par rapport à RGM, via l'étude de la morphologie et le fonctionnement de leur système racinaire (Chapitre 2). Dans un deuxième temps, l'impact du greffage et plus précisément du greffon sur la morphologie et le fonctionnement racinaire dans le cadre de la nutrition phosphatée a été étudié (Chapitre 3 et 4). L'ensemble de ces travaux permettent d'avancer des hypothèses sur l'origine des variations des teneurs en P dans le greffon induites par le porte-greffe.

Régulation de l'acquisition du Pi par les transporteurs PHT1

Chez la plante, l'acquisition en Pi externe est assurée par les co-transporteurs de la famille PHT1 (Ullrich-Eberius et al., 1984). Ces transporteurs se retrouvent principalement au niveau des membranes plasmiques (Chen et al., 2011b). Cette famille multigénique a fait l'objet de nombreuses études sur un grand nombre d'espèces végétales. De nombreux de gènes PHT1 ont été identifiés, dont 9 chez *A. thaliana* (Nussaume et al., 2011; Poirier and Bucher, 2002), 13 chez le riz (*Oryza sativa*) (Liu et al., 2011), 8 chez la tomate (*Solanum lycopersicum*) (Chen et al., 2014), ou encore 12 chez le peuplier (*Populus trichocarpa*) (Loth-Pereda et al., 2011). Cependant les transporteurs PHT1 diffèrent par leur localisation au sein des divers organes de la plante et leur activité (Nussaume et al., 2011). En effet, il a été démontré l'existence de transporteurs à forte ou faible affinité en Pi, présentant des caractéristiques distinctes (Dunlop et al., 1997). Le fonctionnement optimal des transporteurs à faible affinité est assuré lors de concentrations externes en Pi de l'ordre de 50 à 300 µM, alors que celui des transporteurs à forte affinité est associé à des concentrations de l'ordre de 1 à 12 µM (Dunlop et al., 1997; Ullrich-Eberius et al., 1984). L'induction des transporteurs à forte affinité est un des mécanismes majeurs dans la réponse à la faible disponibilité en P (Bucher and Fabianska, 2016). La régulation transcriptionnelle des PHT1 est également différente suivant les transporteurs étudiés. En effet,

l'expression de certains *PHT1* est induite au niveau racinaire en réponse à la faible disponibilité en P alors que d'autres sont réprimées (Teng et al., 2013; Teng et al., 2017).

Les travaux présentés dans ce manuscrit montrent que 1103P possède une efficience d'acquisition en Pi plus forte que RGM. Cette différence peut provenir de l'activité des transporteurs PHT1 au niveau racinaire. Nous avons étudié l'expression de 7 gènes de la famille *PHT1* dans les racines, identifiés par homologie de séquence avec les gènes connus chez *A. thaliana*. L'ensemble des gènes *PHT1* étudié montre des profils d'expression différents suivant les génotypes et la disponibilité en Pi. Quatre d'entre eux, *PHT1;4a*, *PHT1;4b*, *PHT1;4c* et *PHT1;9*, sont fortement exprimés dans les tissus racinaires. *PHT1;4a* et *PHT1;4b* sont également plus exprimés chez 1103P comparés à RGM, pouvant participer à expliquer les différences d'acquisition en Pi observées entre les deux génotypes. De plus l'expression de ces deux gènes est induite en réponse à la faible disponibilité en P, montrant l'adaptation des génotypes étudiés. L'expression des gènes *PHT1;3b* et *PHT1;4e* est très faible chez les 3 génotypes cultivés en forte concentration en Pi (600 µM). Cependant, chez les deux porte-greffes étudiés, une très forte induction de leur expression est observée après 28 jours de culture à faible concentration en Pi (1µM). Nous pouvons supposer que ces deux transporteurs partagent des caractéristiques communes en termes de fonctionnement avec les transporteurs à forte affinité en Pi, identifiés chez les autres espèces végétales. Une étude plus approfondie est en cours, portant sur les séquences codants les transporteurs de la famille des PHT1 de la Vigne, via une approche phylogénétique incluant plusieurs espèces comme réalisée chez le peuplier par exemple (Loth-Pereda et al., 2011). Cette identification est nécessaire afin d'identifier plus précisément les transporteurs PHT1 présents chez la Vigne, leur rôle, ainsi que leur implication dans la réponse à la disponibilité en P.

Cependant, l'étude de l'expression des gènes *PHT1* ne prend pas en compte leur régulation post-transcriptionnelle et/ou post-traductionnelle. Ces dernières jouent un rôle très important dans la capacité racinaire à acquérir le Pi externe (Bayle et al., 2011). De nombreux régulateurs ont été identifiés chez *A. thaliana*, participant à la réponse de la plante à la faible disponibilité en Pi (Chiou et al., 2006). Un des exemples les plus connus concerne la régulation négative de transporteurs PHT1 via la protéine PHO2 comportant un domaine protéique E2 (ubiquitine conjugase), elle-même contrôlée par le micro ARN miRNA399 (Bari et al., 2006; Doerner, 2008). L'étude de l'expression des gènes *PHT1* ne permet donc pas à elle seule d'expliquer les différences d'efficience d'acquisition en Pi observées entre les 3 génotypes de Vigne étudiés, ne prenant pas en compte l'activité protéique.

Développement racinaire

Les variations de concentration en P observées chez le greffon en fonction des porte-greffes peuvent provenir de différences en termes de développement, de morphologie et d'architecture racinaire. Le système racinaire est responsable de l'acquisition des ressources hydrique et minérales pour l'ensemble de la plante. L'équilibre entre les organes sources (racines) et les organes puits (tiges, feuilles, fruits) est donc un paramètre important pour l'alimentation minérale de la plante. Les génotypes de porte-greffes de la Vigne montrent des différences en termes d'allocation de biomasse. 1103P montre une biomasse racinaire plus importante par rapport à sa biomasse aérienne, comparé à RGM. Ces différences sont en adéquation avec les résultats obtenus sur les mêmes génotypes cultivés en hydroponie (Cochetel et al., 2018). Ces résultats laissent supposer que 1103P possède une plus forte capacité à assurer l'alimentation minérale de sa partie aérienne. De plus, en réponse à la faible disponibilité en Pi, les génotypes montrent une allocation de croissance différente, favorisant le développement racinaire au détriment de la croissance aérienne. Cette adaptation est courante chez les plantes, permettant d'augmenter la surface de sol explorée et donc leur capacité à acquérir les minéraux (Hermans et al., 2006).

Les caractéristiques d'un système racinaire sont reliées à sa capacité à explorer le sol et à acquérir les nutriments. Cependant, suivant la mobilité des nutriments dans le sol, le système racinaire est plus ou moins adapté à les acquérir (Miguel et al., 2015; Postma et al., 2014). L'acquisition des nutriments dont la diffusion est élevée (e.g. NO_3^- ou SO_4^{2-}) est favorisée par un système racinaire dont la densité de ramification est faible (Zhan and Lynch, 2015), tandis que l'acquisition des nutriments dont la diffusion est faible (e.g. Pi, K^+, NH_4^+ ou Mn^{2+}) est avantagée par un système racinaire fortement ramifié (Jia et al., 2018). Concernant ces paramètres, les porte-greffes de la Vigne sont contrastés (Smart et al., 2006). Plus précisément, cultivés *in* vitro 1103P possède un système racinaire plus ramifié que RGM, évoquant une capacité supérieure à acquérir le Pi (Cochetel, 2016). L'analyse des systèmes racinaires effectuée dans ce manuscrit ne montre pas de différence en termes de nombre de ramification en fonction de la longueur total du système racinaire entre les génotypes étudiés cultivés en hydroponie. Cependant, cette analyse ne nous informe pas sur la densité de ramification des racines adventives ou sur la longueur des ramifications.

Afin de compléter l'analyse de l'architecture racinaire des génotypes étudiés, une expérimentation complémentaire en culture *in vitro* a été effectuée (Figures X.1 et X.2). Les résultats montrent que 1103P possède un système racinaire supérieur en termes de longueur totale par rapport à RGM (Figure X.2A), mais surtout que le nombre de ramification par racine adventive est supérieure et donc que 1103P serait plus apte à acquérir le Pi du sol (Figure X.2D). Les paramètres racinaires étudiés au cours

de cette étude montrent également des variations en fonction de la teneur en Pi (Figure X.1). En effet, en réponse à l'absence de P dans le milieu, les génotypes montrent une augmentation de la longueur totale de leur système racinaire (Figure X.2A), expliquée par une augmentation du nombre de racines latérales par racine adventive (Figure X.2D). L'émergence de racines latérales en réponse à la faible disponibilité en P est une adaptation fréquemment observée chez les plantes (Peret et al., 2014; Zhu and Lynch, 2004). L'augmentation du nombre de racines latérales est un avantage dans l'acquisition du Pi, car cela augmente la surface de sol explorée et la surface d'absorption racinaire (Xie and Yu, 2003; Zhu et al., 2005). Au cours de cette expérimentation, la disponibilité en P n'a pas affecté la vitesse de croissance des racines adventives (Figure X.2C). Dans la littérature, l'influence de la teneur en P sur la croissance des racines primaires. En effet, chez certains génotypes la faible teneur en P induit une réduction de croissance, alors que d'autres maintiennent leur croissance (Chevalier et al., 2003).

Cette expérimentation a également permis de mettre en avant des différences en termes de gravitropisme racinaire entre 1103P et RGM (Figures X.1 et X.2B). L'architecture racinaire de 1103P reflète un système dit « plongeant », alors que celui de RGM est caractéristique d'un système « traçant ». Ces observations sont en accord avec les connaissances sur l'architecture racinaire des porte-greffes de la Vigne (Guillon, 1905; Smart et al., 2006). Le gravitropisme est un facteur très important pour l'acquisition du Pi. Le P étant distribué de façon hétérogène dans le sol et suivant un gradient descendant avec la profondeur ; un système racinaire traçant est normalement un avantage pour l'acquisition du Pi (Ge et al., 2000; Lynch and Brown, 2001). De plus, le gravitropisme est affecté par la disponibilité en P, mais cette réponse dépend du génotype étudié (Bonser et al., 1996). Dans notre cas, le gravitropisme n'a pas été influencé par la teneur en P (Figure X.2B), cependant l'expérimentation se déroule sur un temps relativement court (9 jours), avec l'utilisation de boutures impliquant des réserves internes disponibles pour assurer la croissance racinaire durant les premiers stades de développement.

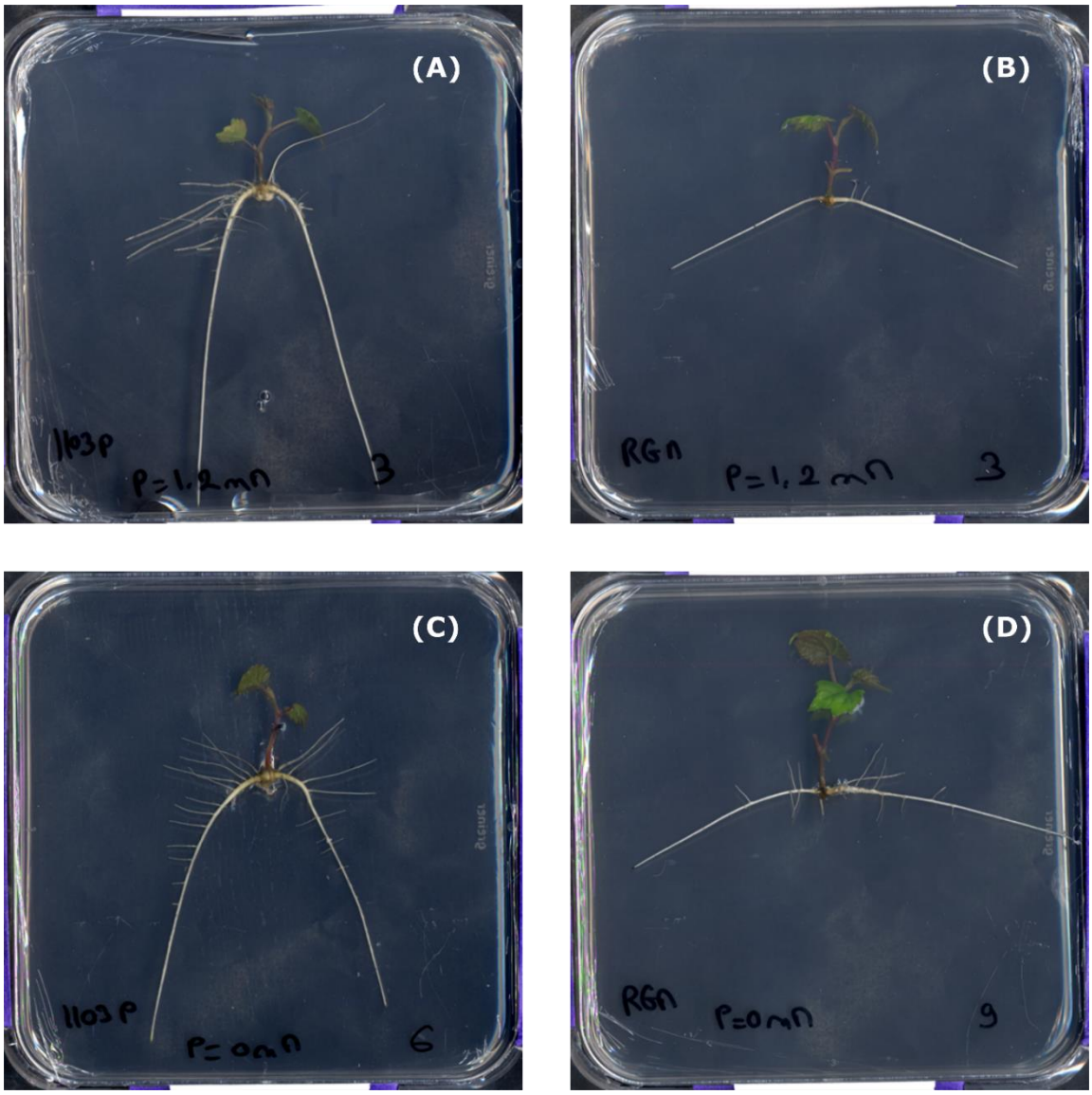


Figure X.1. Caractérisation de l'architecture racinaire sur boites de pétri de boutures simples de 1103P (A-C) et RGM (B-D), cultivées pendant 9 jours respectivement en présence (A-B) ou en absence de P (C-D). Les boutures ont été multipliées en culture *in vitro* sur un milieu nutritif commercial (*MCCOWN WOOD PLANT MEDIUM INCULDING VITAMINS © – M0220*), avec 0,5% d'AGAR, 30 g/L de saccharose et 0,27 µM d'acide 1-Naphtalène acétique (ANA), pH 5,8. Au début de l'enracinement, les boutures ont été placées pendant 9 jours en boites de pétri (12x12 cm), contenant 40 ml de milieu nutritif reprenant la composition de *MCCOWN WOOD PLANT MEDIUM INCULDING VITAMINS © – M0220,* complet ou dépourvu de KH_2PO_4, avec 0,5% d'AGAR et 30 g/L de saccharose, pH 5,8

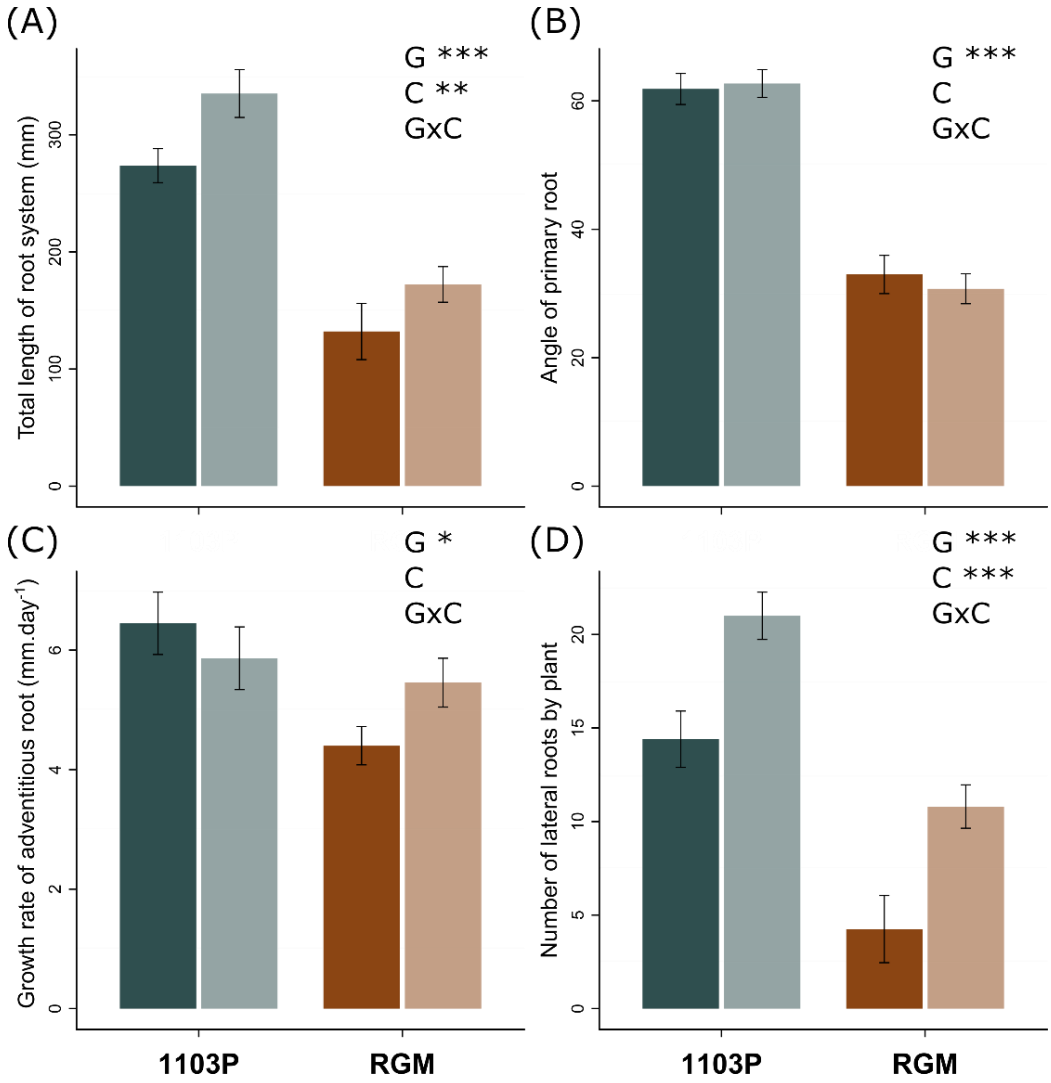


Figure X.2. Longueur totale du système racinaire (A), angle des racinaires adventives (B), vitesse de croissance des racines adventives (C) et nombre de racines latérales par plante (D) de boutures simples de 1103P et RGM, cultivées pendant 9 jours en présence (couleurs sombre) ou en absence de P (couleurs claires). Les conditions de culture sont indiquées dans la légende de la Figure X.1 (page 178). Les valeurs représentent les moyennes et les erreurs standards (n=5). Les données ont été analysées par un test ANOVA à 2 facteurs (G = Génotype, C = Teneur en P), $p < 0.05$ *, $p < 0.01$ **, $p < 0.001$ ***

L'analyse des systèmes racinaires effectuée en chapitre 3, montre des différences génotypiques et des modifications morphologiques en réponse à la faible disponibilité en P. Tout d'abord on observe une diminution du diamètre moyen des racines. On peut donc supposer une augmentation de la croissance en longueur au détriment de la croissance radiale. Cette adaptation permet un développement moins couteux en termes énergétique et l'exploration d'une plus grande surface de sol à la recherche de zones riches en P (Pang et al., 2010; Zobel et al., 2007). On observe également une diminution du nombre de pointes racinaires par rapport à la longueur du système racinaire. Cela peut provenir de différents facteurs, tels qu'une longueur spécifique des racines plus importante (rejoignant la diminution de diamètre moyen) ou une diminution de l'émergence de racines latérales. L'apparition et le développement des racines latérales sont modulés en partie par la disponibilité en P (Peret et al., 2011; Peret et al., 2014). En réponse à la faible disponibilité en P, certains génotypes montrent une augmentation du nombre de racines latérales, associée à une inhibition de la croissance de la racine primaire. Cependant des réponses contrastées ont été observées suivant les génotypes étudiés, certains montrant une diminution du nombre et de la longueur des racines latérales (Borch et al., 1999; Williamson et al., 2001; Zhu and Lynch, 2004). Les résultats obtenus dans ce manuscrit montrent une adaptation à une faible disponibilité en Pi, distribué de façon homogène dans le milieu. Or la disponibilité Pi pourrait agir comme un signal local et modifier la plasticité racinaire localement. En effet, des études ont montrées une réponse localisée en termes d'activité méristématique au niveau racinaire, du développement des poils absorbants et de l'initiation de racines latérales en fonction de la disponibilité en Pi (Hammond and White, 2008; López-Bucio et al., 2003). De plus, la réponse à la disponibilité en Pi en termes d'architecture racinaire est connue pour être locale en fonction des concentrations externes, où le système racinaire se développe en priorité dans les zones du sol riches en Pi (Drew, 1975).

Chez les plantes pérennes, le système racinaire se compose de racines fines, responsables de l'acquisition de ressources hydriques et minérales, et de racines épaisses et lignifiées, permettant l'ancrage de la plante et de stocker les réserves carbonées et minérales (Eshel and Beeckman, 2013). L'étude du développement et de la plasticité des fines racines sur plantes pérennes est difficile du fait de leur rapide renouvellement (Artacho and Bonomelli, 2016). La production et la maintenance des fines racines impliquent un certains nombre de facteurs édaphique tels que l'eau, la température ou la teneur en N (Artacho and Bonomelli, 2016; Comas et al., 2005). Afin de comprendre plus précisément le rôle du développement et de l'architecture racinaire de la Vigne dans la nutrition phosphatée, l'étude de la reprise de croissance, du développement et de la maintenance des fines racines est à prendre en compte. Des expérimentations en rhizotrons ou en plein champ seraient à

envisager afin de mettre en évidence la prospection du sol et des ressources en P, par des Vignes plus âgées.

L'ensemble de ces résultats montre l'importance d'étudier le système racinaire des différents génotypes de porte-greffe sur une distribution hétérogène en P, impliquant plus fortement les différences de gravitropisme observées. Une approche d'analyse d'architecture racinaire en sol devrait donc être envisagée afin d'étudier la capacité des porte-greffes à explorer les différentes couches de sols, contrastées par leur teneur en P, et ainsi déterminer leur possible efficience d'acquisition en fonction des ressources explorées. Le système hydroponique utilisé au cours de ce travail de thèse n'a pas permis d'étudier l'initiation et le développement des poils absorbants, alors que ces derniers participent intensément à l'absorption des nutriments. De plus, ces structures spécialisées peuvent représenter plus de 70% de la surface racinaire en contact avec le sol et leur développement est fortement influencé par la disponibilité en Pi (Gilroy and Jones, 2000; Parker et al., 2000).

Exsudation racinaire

Une partie des expérimentations de ce travail de thèse a porté sur l'interaction racine-rhizosphère et plus précisément sur les exsudats racinaires modifiant la disponibilité en Pi externe. En réponse à la faible disponibilité en P, les génotypes étudiés montrent une acidification de la rhizosphère et une augmentation de l'activité des acides phosphatases exsudées. L'acidification de la rhizosphère par le système racinaire en réponse à la faible disponibilité en Pi a déjà été observée chez plusieurs espèces, avec cependant des différences d'intensité et de localisation le long de la racine (Liu et al., 2016a). Dans notre cas, une acidification au niveau des pointes racinaires a été observée en forte teneur en Pi, alors qu'une acidification tout au long de la racine a été observée en réponse à la faible disponibilité en Pi. Une augmentation de pH de la rhizosphère agit sur les complexes insolubles de Ca-Pi et augmente la disponibilité en Pi dans la solution du sol (Barrow, 2016). De plus, la forte augmentation de l'activité des acides phosphatases exsudées observée, permet de déduire que les génotypes étudiés ont une forte capacité à exploiter les ressources en Po présent dans le sol. Cependant l'efficacité de ces mécanismes permettant d'augmenter la disponibilité en Pi, dépend en grande partie de l'emplacement de ces exsudats racinaires à travers le sol (Lynch, 2011). En effet, les teneurs en Po sont très élevées dans les couches superficielles de sol et diminuent rapidement avec la profondeur (Laliberté et al., 2012). La localisation de l'exsudation des acides phosphatases va donc influencer leur potentiel d'action en fonction de la teneur en Po. Cet aspect est très important au vu des différences en termes de gravitropisme racinaire observées entre les porte-greffes de Vigne étudiés. Enfin nous

pourrions également étudier la localisation des APases à l'échelle racinaire, afin de déterminer si la sécrétion se fait tout au long de la racine ou bien reste localisée au niveau des pointes racinaires. L'utilisation de substrat tel que le BCIP (5-bromo-4-chloro-3'-indolyphosphate) permet de visualiser l'activité des phosphatases (Wang et al., 2011b).

L'exsudation de composés carboxylés tels que les acides organiques agissent également sur la disponibilité en Pi dans la rhizosphère. Au cours de cette thèse, l'identification des acides organiques exsudés par la Vigne en réponse à la faible disponibilité en Pi n'a pas pu être menée à terme. Plusieurs méthodes de prélèvement ont été utilisées, conduisant à des résultats contradictoires. Une comparaison plus poussée est nécessaire afin de pouvoir déterminer qualitativement les quantités d'acides organiques exsudés. La détermination de la zone d'exsudation des acides organiques est cruciale afin de pouvoir interpréter les résultats obtenus. En effet, cela permettrait de récupérer avec plus de précision les exsudats racinaires, en se focalisant sur une zone précise telle que les pointes racinaires par exemple. Cela permettrait également de définir l'impact de ces acides organiques exsudés en fonction de leur localisation dans le sol. De plus, des différences en termes de concentrations en acides organiques dans les tissus de la plante entre les génotypes étudiés, portent à croire que des différences d'exsudation pourraient être également observées. Ces différences ont déjà été observées chez plusieurs génotypes de porte-greffes de la Vigne étudiés pour leur tolérance à la carence en fer (Covarrubias and Rombolà, 2015; Jiménez et al., 2007; Ollat et al., 2003a).

Afin de déterminer l'impact et la localisation de ces exsudations racinaires sur la disponibilité en Pi et sa potentielle acquisition par la Vigne, des approches *in situ* pourraient être envisagées, ayant l'avantage d'inclure le pouvoir tampon du sol, ainsi que la distribution hétérogène du P. La récolte de la solution du sol à proximité des racines et/ou des pointes racinaires pourrait être effectuée à l'aide de « micro-ventouses » et ainsi déterminer les concentrations en acides organiques ou l'activité des APases, ainsi que la teneur en Pi assimilable (Liu et al., 2016b; Shen and Hoffland, 2007). Enfin, l'étude de l'évolution du pH de la rhizosphère en temps réel, en fonction des différents fragments racinaires considérés, de la répartition hétérogène du P et du pouvoir tampon du sol pourrait être effectuée à l'aide d'équipements spécifiques tels que les optodes (Blossfeld and Gansert, 2007; Blossfeld et al., 2013).

Rôle du greffon

Chez la plante greffée, les deux génotypes associés assurent deux rôles distincts, la partie aérienne et le système racinaire qui fournissent respectivement les produits issues de la photosynthèse et les ressources hydrique/minérales. Le greffon régule donc la croissance du porte-greffe via l'export de composés carbonés issus de la photosynthèse vers la partie racinaire (Mooney, 1972). En viticulture, l'impact du greffon sur le développement et le fonctionnement du porte-greffe a peu été étudié. Cependant, quelques études ont mis en avant l'influence du greffon sur certains paramètres de l'architecture racinaire, tels que le développement, la longueur et le gravitropisme (Oslobeanu, 1978; Tandonnet et al., 2010). Au cours de ce travail de thèse, le rôle du greffon sur le développement racinaire de son porte-greffe a été mis en avant et confirme les données retrouvées dans la littérature. Ce contrôle du développement racinaire peut provenir de l'activité photosynthétique du greffon et donc la quantité de produits carbonés alloués à la croissance racinaire. Cependant, des caractéristiques intrinsèques au porte-greffe semblent ne pas être affectées par le greffon, telles que le nombre de pointes racinaires sur la longueur totale du système racinaire (donc la densité de ramification ou la longueur racinaire spécifique), ou encore la distribution des classes de diamètres racinaires. Cela suppose que certaines caractéristiques du porte-greffe lui sont propres, tandis que d'autres sont soumises à l'influence du greffon.

Face aux stress environnementaux, les plantes répondent via des signaux longue distance ou systémiques, permettant de diffuser l'information depuis les racines vers les parties aériennes et vice-versa. Ces signaux systémiques peuvent être de nombreux composés tels que les hormones, les sucres, les nutriments, certains métabolites, les peptides, les protéines ou encore les microARN (Liu et al., 2009). Plus précisément, la partie aérienne va réguler l'acquisition en nutriments via une cascade de signalisation afin que l'absorption et la translocation depuis les racines soient en adéquations avec les besoins des feuilles et de leur activité métabolique (Lough and Lucas, 2006). La perception et la réponse à la faible disponibilité en P résultent de la signalisation locale au niveau racinaire mais également de la signalisation systémique provenant de la partie aérienne (Bari et al., 2006; Hammond and White, 2008; Hammond and White, 2011; Pant et al., 2008). La régulation systémique en réponse à la faible disponibilité en P n'est pas clairement établie. Cependant, l'implication de signaux carbonés et en particulier des sucres provenant des parties aériennes et alloués vers la racines, semble de plus en plus une évidence (Amtmann et al., 2005; Hammond and White, 2008). De plus, la régulation post-transcriptionnelle et post-traductionnelle des PHT1 au niveau racinaire serait également initiée depuis les parties aériennes de la plante, via la signalisation de microARN, en particulier miRNA399, inhibant

l'activité de PHO2 responsable de la dégradation des transporteurs PHT1 (Doerner, 2008; Pant et al., 2008).

Au cours de ce travail de thèse, une influence du greffon a été observée sur la réponse à la faible disponibilité en P, notamment sur la croissance de la plante, la régulation des concentrations en acides organiques présents dans les différents tissus, ainsi que sur l'expression de gènes impliqués dans la nutrition phosphaté au niveau racinaire. L'analyse du transcriptome racinaire de différentes combinaisons de greffes, présentée en chapitre 4, met en avant le rôle du greffon sur l'expression d'un grand nombre de gènes. Ce travail initié permettra de mettre en avant les régulations transcriptomiques de la plante en réponse à la disponibilité en P, incluant l'implication des phytohormones, le rôle du greffon et la réponse systémique de plante.

En viticulture, la très grande majorité des génotypes utilisés en tant que greffon appartiennent à l'espèce *V. vinifera*. Bien que le greffon influence le développement et le fonctionnement racinaire, aucune information ne nous permet d'affirmer que les différents cultivars de *V. vinifera* ont le même impact sur leur porte-greffe et que l'affinité greffon/porte-greffe ne joue pas un rôle sur l'ampleur des modifications que le porte-greffe subit. Une comparaison de l'effet de plusieurs génotypes de *V. vinifera* sur les caractéristiques racinaires du porte-greffe est nécessaire afin de déterminer le rôle de chacun des acteurs de la plante greffée dans la nutrition phosphatée et plus globalement dans la nutrition minérale de la Vigne.

Efficience d'utilisation et remobilisation interne du P

Les génotypes étudiés ont montré des différences en termes de PUE. Lorsque la disponibilité en Pi dans le sol est élevée, 1103P montre une PUE plus faible que RGM, résultant d'une PAE plus grande et/ou de l'apparition d'un autre facteur limitant pour la croissance, réduisant la pleine utilisation des ressources en P acquises. A l'inverse, l'absence de Pi dans le milieu provoque une forte augmentation de la PUE de 1103P, induisant des mécanismes de renouvellement du P vers les structures en croissance. L'étude menée dans le chapitre 1, montre que ces différences de PUE ne sont pas induites par un taux de renouvellement plus important depuis les parties pérennes de la plante. On peut donc supposer une remobilisation intra-cellulaire du P, liée à l'utilisation du Pi vacuolaire (Foyer and Spencer, 1986), la dégradation de structures cellulaires telles que les ARN ou les phospholipides (Dodds et al., 1996; Nakamura et al., 2005), ou encore l'utilisation de voies métaboliques moins coûteuses énergétiquement (Hammond et al., 2004; Vance et al., 2003).

L'étude de ces mécanismes permettrait de mettre en avant des régulations d'utilisation du P au sein de la plante, susceptibles d'être en partie responsables des variations de croissance observées en réponse à la disponibilité en P en fonction du porte-greffe (Grant and Matthews, 1996b). Une approche de biologie moléculaire pourrait être envisagée au niveau foliaire, afin de déterminer les différences en PUE observée. Plus précisément, l'analyse des régulations transcriptionnelles de gènes codant les RNAses, les phospholipases et les APases, pourrait mettre en évidence des différences génotypiques dans la capacité des porte-greffes à remobiliser leur Pi interne.

Les mycorhizes

A travers ce travail de thèse, des différences génotypiques en termes d'acquisition en Pi ont été mis en évidence. Cependant la nutrition phosphatée n'est pas seulement assurée par la plante, mais peut dans certains cas être effectuée en grande partie par les symbioses mycorhiziennes, basées sur l'association d'une plante hôte et des champignons proliférants dans le sol (Smith and Read, 2010). L'efficacité d'acquisition en Pi par les mycorhizes vient principalement de leur forte capacité à explorer le sol, ainsi que leur aptitude à atteindre des zones non accessibles par les racines à cause de leur diamètre élevé (Smith and Read, 2010). La Vigne est connue pour être fortement colonisée par des champignons mycorhiziens à arbuscules (Nappi et al., 1985; Trouvelot et al., 2015). Dans certains cas, 45 à 75% des fines racines peuvent être infectées par des symbioses mycorhiziennes (Karagiannidis and Nikolaou, 1999). De plus, l'application d'inoculum permet d'augmenter de 50% la concentration en Pi dans les feuilles de la Vigne, montrant leur forte implication de l'acquisition du Pi (Ozdemir et al., 2010). Cependant, l'influence de la symbiose mycorhizienne dans la nutrition phosphatée de la Vigne est difficile à définir. En effet, le taux de symbiose est très dépendant des conditions édaphiques et principalement des teneurs en P (Karagiannidis and Nikolaou, 1999). Enfin, la capacité des porte-greffes à effectuer des symbioses mycorhiziennes semble différente (Ozdemir et al., 2010). Les mécanismes impliqués dans ces régulations ne sont pas encore très bien définis chez la Vigne. Des différences d'exsudation de composés de type strigolactones par les porte-greffes en réponse à la disponibilité en N ou P sont observées (Cochetel et al., 2018), et pourraient y contribuer via leur implication dans la mise en place des symbioses mycorhiziennes (Parniske, 2008).

Les dispositifs expérimentaux mis en place afin d'analyser les propriétés intrinsèques des porte-greffes régulant l'acquisition et leur utilisation en P ne prennent pas en compte l'impact des symbioses mycorhiziennes. Afin d'acquérir une meilleure connaissance des mécanismes impliqués dans la nutrition phosphatée de *Vitis spp.*, il sera nécessaire d'étudier la contribution ainsi que la régulation des symbioses mycorhiziennes chez la Vigne.

CONCLUSION

L'utilisation de porte-greffes en viticulture fut nécessaire afin de préserver la production de raisins et de vins à partir de *V. vinifera* reconnu pour ses qualités organoleptiques. Cependant le greffage induit des modifications sur la physiologie et le développement du greffon. Plus spécifiquement, la nutrition hydrique et minérale est altérée par le génotype de porte-greffe utilisé. Comprendre les mécanismes régulant la nutrition minérale de la Vigne est essentiel dans un contexte de changement climatique afin d'optimiser les objectifs de production viticole, de limiter les intrants fertilisants, d'adapter le matériel végétal au terroir et de pratiquer une viticulture de précision.

A travers ce travail de thèse, des différences intrinsèques entre porte-greffes ont été mis en évidence, visant à apporter des éléments de réponse dans la régulation de la nutrition phosphatée de la Vigne. L'objectif était de comprendre pourquoi les porte-greffes issus de *V. berlandieri* et/ou *V. rupestris*, apportent des concentrations en P plus fortes à leur greffon que ceux issus de *V. riparia*. L'utilisation de 1103P comme modèle a permis de démontrer son efficacité dans l'acquisition du Pi, probablement reliée à l'activité de ses transporteurs, sa forte capacité à explorer le sol à la recherche de ressources minérales, ainsi que son taux de remobilisation depuis les parties pérennes plus élevé que *V. riparia* cv. RGM.

Cependant, ces nouvelles connaissances soulèvent de nouvelles interrogations sur la régulation de la nutrition phosphatée et plus particulièrement sur le rôle du greffon dans le développement et le fonctionnement du porte-greffe. Comme pour la majorité des éléments minéraux, la perception de la carence en P par la plante se fait de manière locale au niveau du système racinaire, mais également au niveau foliaire impliquant des réponses systémiques affectant la plante entière. Comprendre ces deux voies de signalisation est essentiel afin d'adapter au mieux le choix du porte-greffes, du greffon, mais aussi de la combinaison greffon/porte-greffe en fonction des conditions environnementales et édaphiques.

ANNEXES

Communications orales et écrites

Publications

1. Phosphorus acquisition efficiency and phosphorus remobilization mediate genotype-specific differences in shoot phosphorus content in grapevine. **A. Gautier**, S. J. Cookson, C. Hevin, P. Vivin, V. Lauvergeat and A. Mollier. *Tree Physiology, 2018*

2. Petiole phosphorus concentration is controlled by the rootstock genetic background in grapevine. **A. T. Gautier**, S. J. Cookson, L. Lagalle, N. Ollat and E. Marguerit. Submitted to *Australian Journal of Grape and Wine Research*

3. Merging genotypes: graft union formation and scion/rootstock interactions. **A. T. Gautier**, C. Chambaud, L. Brocard, N. Ollat, G. A. Gambetta, S. Delrot and S. J. Cookson. Submitted to *Journal of Experimental Botany*

Communications orales

1. How do grapevine rootstocks modify phosphorus concentration in the scion? **A. Gautier**, A. Mollier, C. Hevin, P. Vivin, V. Lauvergeat and S. J. Cookson: SEB's Annual Meeting; Florence, Italy, 2018.

2. In grafted grapevines, physiological, transcriptional and hormonal responses to nutrient availability are strongly influenced by the rootstock genetic background. N. Cochetel, **A. Gautier**, E. Météier, I. Merlin, C. Hévin, J-B. Pouvreau, P. Coutos-Thévenot, M. Hernould, Z. Dai, P. Vivin, P-F. Bert, F. Escudié, M. S. Muñoz, C. Klopp, S. Delrot, S. J. Cookson, N. Ollat, and V. Lauvergeat: XII International Conference on Grapevine Breeding and Genetics; Bordeaux, France, 2018.

3. Rootstock adaptations to low phosphorus availability in grapevine. **A. Gautier**, C. Hevin, P. Vivin, V. Lauvergeat, A. Mollier and S. J. Cookson: LabEx COTE day; ISVV, Bordeaux, France, 2017.

4. Grapevine rootstocks responses to different phosphorus supply. **A. Gautier**, C. Hevin, P. Vivin, V. Lauvergeat, A. Mollier and S. J. Cookson: Young scientists of the ISVV day; ISVV, Bordeaux, France, 2016.

Posters

1. How do grapevine rootstocks modify phosphorus concentration in the scion? **A. Gautier**, A. Mollier, C. Hevin, P. Vivin, V. Lauvergeat, N. Ollat and S. J. Cookson: XII International Conference on Grapevine Breeding and Genetics; Bordeaux, France, 2018. Young scientist best poster award

2. Screening and modelling the diversity of root system architecture in Vitis genotypes: new opportunity for rootstock selection? J.P. Tandonnet, L. Saubignac, **A. Gautier**, N. Ghoribi, E. Marguerit, S. J. Cookson, V. Lauvergeat, A. Mollier, L. Pagès, N. Ollat and P. Vivin: XII International Conference on Grapevine Breeding and Genetics; Bordeaux, France, 2018.

3. Grapevine adaptations to phosphorus availability. **A. Gautier**, C. Hevin, P. Vivin, V. Lauvergeat, A. Mollier and S. J. Cookson: Young scientists day INRA BAP; ISVV, Talence, Bordeaux, 2017.

Vulgarisation scientifique

1. Comment adapter la viticulture de demain ? **A. Gautier**, C. Hevin, P. Vivin, V. Lauvergeat, A. Mollier, N. Ollat and S. J. Cookson: Pint of Science festival; Bordeaux, France, 2018.

2. Rootstock effect on processes involved in phosphorus nutrition in grapevine. **A. Gautier**, C. Hevin, P. Vivin, V. Lauvergeat, A. Mollier and S. J. Cookson: Vintage's conferences, Museum of wine, Bordeaux, France, 2016.

Grapevine Adaptation to Phosphorus Availability

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Phosphorus (P) is one of six essential macronutrients required by plants. However, P is one of the most unavailable macronutrients in the soil because it is present in inaccessible forms such as organic P (Po) and in complexes with insoluble cations. Roots acquire P as inorganic phosphate (Pi) which usually accounts for 35% to 70% of total soil P [1]. Under P deficiency, plants alter P utilization and attempt to increase their capacity to take up P from the soil by the adaptations described in Figure 1 [2].

Grapevine is grown grafted onto American *Vitis spp*. These rootstocks are adapted to different soil types and are known to alter scion responses to nutrient supply and scion elemental composition, e.g. alter petiole P concentration [3]. In this study, we compare the response to P supply of two genotypes known to confer different petiole P concentrations; *Vitis riparia* cv. Riparia Gloire de Montpellier (RGM) confers low P content, while the *V. rupestris* × *V. berlandieri* hybrid cv. 1103 Paulsen (1103P) confers high P content [4,5].

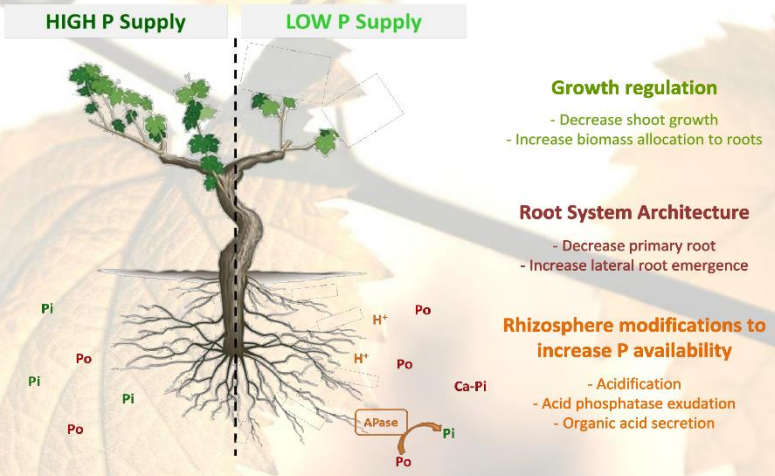


Figure 1. Mechanisms of adaptation of plants to low P supply include modifications of growth, root system architecture and the rhizosphere

Growth regulation

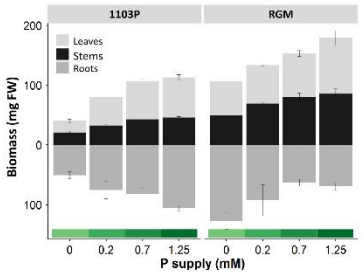


Figure 2. Plant biomass after 35 d of *in vitro* culture under four P supplies

The genotypes respond differently to P supply in terms of shoot/root biomass allocation (Figure 2)

Shoot/root biomass allocation is not affected by P supply in 1103P, but root growth is increased in RGM to increase the volume of soil explored

Root System Architecture (RSA)

There are intrinsic differences in RSA between the two genotypes, but the response to P supply is the same (Figure 3)

Under P deficiency, both genotypes increase their root surface to increase the volume of soil explored (Figure 3.A), this is due to an increase in lateral root emergence (Figure 3.B)

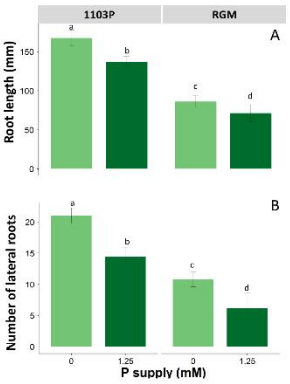


Figure 3. Root surface (A) and number of lateral roots (B) of plants grown in *in vitro* culture in vertical square petri dishes for 10 d under two P supplies

Rhizosphere modification

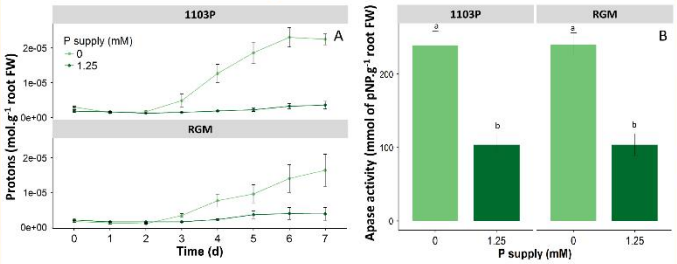
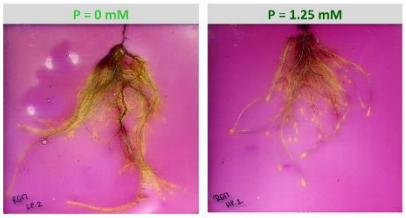


Figure 4. Root acidification of the media of plants cultivated in hydroponic culture under different P supplies (A); root acid phosphatase activity (APase) in the media over a period of 8 h after 7 d of growth under different P supplies (B)

Roots of both genotypes alter the rhizosphere in response to low P supply. Roots of 1103P acidify the growth media more than RGM (Figure 4.A). However, the induction of acid phosphatase exudation is the same for both genotypes (Figure 4.B)

The spatial distribution of acidification is restricted to just behind the root tips under high P supply and is throughout the root system under low P supply for both genotypes (Figure 5)

Figure 5. Root acidification of the media visualized with Bromocresol purple in agar plates after 7 d of growth under different P supplies



In this study we have characterized the response of two grapevine rootstocks to P supply. We show that they differ in their response to low P: the shoot/root ratio of RGM is reduced, whereas 1103P increases acidification of the rhizosphere. These results must be completed by P measurements to determine the P utilization efficiency of the different genotypes. We also plan to characterize the organic acids released by the roots as they have an important role in increasing the availability of P in the soil. In the experiments described above, the uptake of P has been separated from the interactions with the soil. In order to determine the efficiency of the adaptive mechanisms, experiments will be done using vineyard soil. Future work also includes the study of the responses of grafted grapevines to P supply.

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How do grapevine rootstocks modify phosphorus concentration in the scion?

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Grapevine is grown grafted onto American *Vitis spp*. These rootstocks are adapted to different soil types and are known to alter scion responses to nutrient supply and scion elemental composition. The parentage of rootstocks appears to alter scion nutrient concentration, in particular phosphorus (P) (Figure 0).

P concentration is determined by different parameters such as:

1. P Use or Acquisition Efficiencies (respectively PUE and PAE)
   Rate of P remobilization from perennial parts
2. Root system architecture
3. Plant-rhizosphere interactions to increase P availablility

How do 1103P (*V. berlandieri* & *V. rupestris*) confer higher scion P concentration than RGM (*V. riparia*) ?

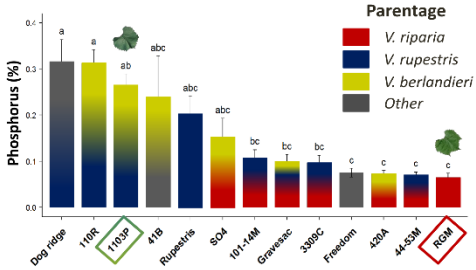


Figure 0. Petiole phosphorus concentration of Cabernet Sauvignon grafted on to 13 different rootstocks of different parentages at veraison (GreffAdapt, Bordeaux – France)

1 How do the genotypes use and take up P?

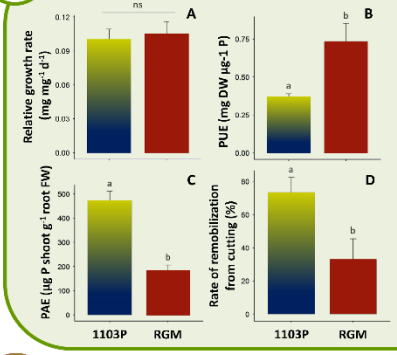
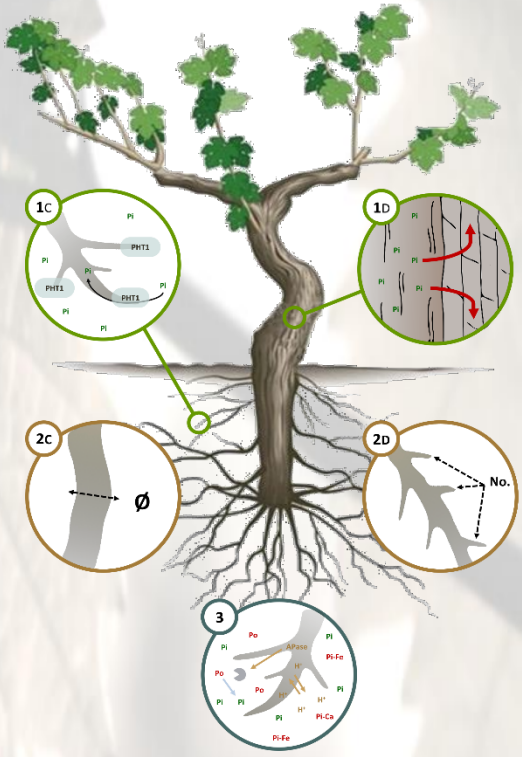


Figure 1: *Relative growth rate (A), PUE (B), PAE (C) and remobilization from cutting (D) of 1103P and RGM growing on sand with nutrient solution labelled with ^{32}P, 21 days after rooting. Means, standard deviations and results of t-test shown (n=5),*

1103P has a **lower P use** efficiency

1103P has a **higher** efficiency of **acquisition** and **remobilization** from woody parts

1103P > RGM



2 How do the genotypes explore the soil?

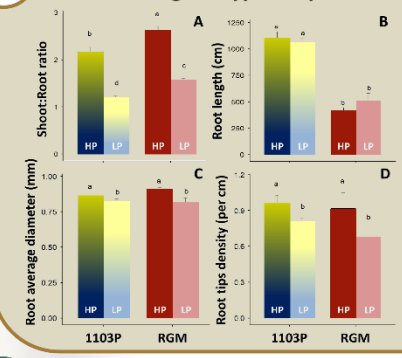


Figure 2: *Shoot:Root ratio (A), root length (B), average diameter (C) and tip density (D) of 1103P and RGM after 28 days of growth on hydroponic system with different P supply; 600 µM (HP) or 1 µM (LP)*

1103P has **a larger root system** and a higher capacity to explore the soil

1103P and **RGM adapt** their root system under low P supply

1103P > RGM

3 How do the genotypes affect the rhizosphere to increase P availability?

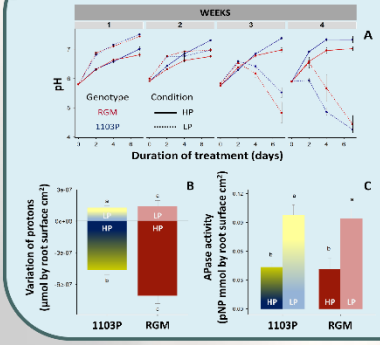


Figure 3: *pH evolution (A), variation in proton concentration (B) and activity of acid phosphatases (C) in nutrient solution of 1103P and RGM after 28 days of growth on hydroponic system with different P supply; 600 µM (HP) or 1 µM (LP)*

1103P and **RGM acidify** rhizosphere under low P supply

1103P and **RGM** release more **acid phosphatases** under low P supply

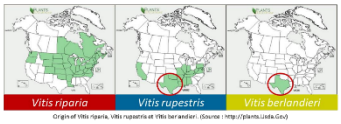
1103P = RGM

How to explain these differences between *V. berlandieri* & *V. rupestris* hybrids and *V. riparia*?

Species from different **geographic origin and adapted to** contrasting soil conditions

Soils of Texas are calcareous and often **deficient in P**

V. berlandieri & *V. rupestris* have acquired **mechanisms to maximize P uptake**?



Review - Merging genotypes: graft union formation and scion/rootstock interactions

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Abstract

Grafting has been utilized for at least the past 7000 years. However, historically grafting has been developed by growers without particular interest beyond the agronomical and ornamental effects, thus knowledge about grafting has remained largely empirical. Much of the commercial production of fruit, and increasingly vegetables, relies upon grafting with rootstocks to provide resistance to soil borne pathogens and abiotic stresses as well as to influence scion growth and performance. Although there is considerable agronomic knowledge about the use and selection of rootstocks for many species, we know little of the molecular mechanisms underlying rootstock adaptation to different soil environments and rootstock conferred modification of scion phenotypes. Furthermore, the processes involved in the formation of the graft union and graft compatibility are poorly understood despite over one hundred years of scientific study. In this review, we provide an overview of what is known about grafting and the mechanisms underlying rootstock/scion interactions. We highlight recent studies that have advanced our understanding of graft union formation and outline subjects for further development.

Keywords: graft compatibility, grafting, phloem, plasmodesmata, rootstock, scion, xylem.

Introduction

Grafting is one of the most ancient horticultural techniques originating prior to 7000 B.C. in China (Mudge et al., 2009) and yet still today most commercial perennial fruit production is dependent upon grafting with rootstocks. Although more recent, vegetable grafting is increasing especially in cucurbits and solanaceous crops (Bie et al., 2017). Using grafted plants provides flexibility, allowing growers to combine different scion and rootstock traits independently. During grafting, cut tissues from different genotypes are brought into contact so that the plants join together to form one composite organism. During this process two individuals are forced to interact, and their survival depends on the efficiency of this interaction. Integrating the tissues of two individuals implies that adult, differentiated tissues, engage a process during which they dedifferentiate and form new conducting structures (as reviewed by Pina *et al.* (2017)). The underlying mechanisms must be significantly different for the xylem vessels (dead cells) and the living sieve elements of the phloem. The mechanisms responsible for this integration remain poorly understood (as reviewed by Melnyk (2017a), Melnyk (2017b) and Pina *et al.* (2017)) and the ability to be grafted is not ubiquitous across taxa; dicotyledonous plants graft together easily, whereas grafting is not possible in monocots as they lack a vascular cambium.

Natural variation in the adaptation of different species or accessions to specific biotic and/or abiotic soil conditions has been exploited to generate many rootstocks (as reviewed by Warschefsky *et al.* (2016) and Colla et al. (2017)). One of the most famous examples comes from the world of wine. Nearly all wine grapes are different varieties of the same European *Vitis spp.*, *V. vinifera*. At the end of the 19th century a soil-dwelling insect pest, Phylloxera, was accidentally introduced to Europe from North America devastating European vineyards. Researchers at the time rapidly realised that the roots of American *Vitis spp.* provided a natural tolerance and began grafting *V. vinifera* onto American *Vitis spp.* and hybrids (Ollat et al., 2016). Today, despite the continuing importance of Phylloxera resistant rootstocks to viticulture, we know little of the molecular basis of this trait.

How do rootstocks modify scion phenotypes?

Rootstocks confer differences in salinity tolerance, drought tolerance, water use efficiency, scion vigour, scion architecture, mineral element composition and use efficiency, phenology, and fruit quality and yield in a wide range of species (Colla et al., 2017; Kumar et al., 2017; Warschefsky et al., 2016). Traditionally, rootstock conferred differences in scion phenotypes have been determined empirically with little or no attention paid to the underlying mechanisms. However, it is clear that there is a genetic control of rootstock conferred modifications of scion phenotypes, since the parentage of

a given rootstock is frequently observed to indicate its behaviour in the field (Cordeau, 1998; Pico et al., 2017) and the identification of quantitative trait loci (QTLs) for a variety of rootstock conferred traits (Asins et al., 2017; Asins et al., 2010; Asins et al., 2015; Bert et al., 2013; Estan et al., 2009; Fazio et al., 2014; Foster et al., 2015; Knabel et al., 2015; Marguerit et al., 2012; Raga et al., 2014; Rusholme Pilcher et al., 2008; Tandonnet et al., 2018).

One important example of the use of rootstocks to confer traits to the scion is the use of dwarfing rootstocks of the Malling series in commercial apple orchards (Hatton, 1917). Dwarfing has been extensively studied with these rootstocks and three QTL of dwarfing have been identified in populations containing the M9 dwarfing rootstock (Fazio et al., 2014; Foster et al., 2015; Harrison et al., 2016b; Rusholme Pilcher et al., 2008). Recently, the apple WRKY transcription factor family was targeted as candidate genes of dwarfing control in the M26 rootstock and MdWRKY9 was identified as a potential candidate based on its differential expression between different dwarfing and non-dwarfing rootstocks (Zheng et al., 2018). The overexpression of *MdWRKY9* repressed *MdDWF4*, which controls the rate limiting step in brassinosteroid synthesis, thereby reducing brassinosteroid production and triggering dwarfing (Zheng et al., 2018). However, the ability of these transgenic rootstocks to confer dwarfing to a wild type scion and whether MdWRKY9 is the underlying cause of one of the QTLs of M9 conferred dwarfing in apple are still unknown.

Rootstocks could influence scion phenotypes via a variety of mechanisms:

Rootstocks could differ in their functioning, i.e. their ability to capture soil resources (via differences in root system architecture, root functioning and root interactions with the rhizosphere) and transport them to the scion. For example, both grapevine and citrus rootstocks have different root architectures (Dumont et al., 2016; Sorgona et al., 2007) and different capacities to take up phosphate and remobilise phosphorus reserves (Gautier et al., 2018; Zambrosi et al., 2012). Similarly, higher root length is associated with higher stomatal conductance and transpiration in grafted grapevine under low and moderate water deficit (Peccoux et al., 2018). Furthermore, grapevine rootstocks differ in their pH exudation response to iron deficiency (Ollat et al., 2003a) and alter the microbiome of the soil (Marasco et al., 2018).

The graft interface itself could alter scion development directly; however, although frequently suggested in the literature, this seems unlikely in compatible grafts once the connections across the graft interface have been well established. In general, once established, the graft union offers little resistance to water movement (Adams et al., 2018; Clearwater et al., 2004; Nardini et al., 2006) and

there has been no clear evidence of the graft interface sequestering molecules despite being numerous suggestions in the literature (Gregory et al., 2013; Webster, 2004).

Rootstocks could differ in their regulation of shoot/root signalling in terms of both the concentration and fluxes of signalling molecules. A number of reviews have been devoted to the potential roles of long distance signalling molecules in regulating scion/rootstock interactions (Albacete et al., 2015; Goldschmidt, 2014; Venema et al., 2017). Grafting rootstocks that have been genetically modified to alter long distance signal molecules (such as hormones) can affect scion phenotypes. For example, the overexpression of isopentenyltransferase (IPT) a key enzyme of cytokinins biosynthesis, in tomato rootstocks increases cytokinin content of the scion and its resistance to salinity stress (Ghanem et al., 2011). Similarly, a recent study shows that methylation of the promoter of *IPT5B* is higher in roots of the dwarfing apple rootstock M9 compared to a high vigour rootstock, and that this is correlated with a reduction in *IPT5B* expression in the root and cytokinin content in the shoot. There are also numerous examples of certain signals being associated with rootstock conferred traits. For example, grapevine rootstocks can confer differences in shoot behaviour (e.g. shoot branching) consistent with differences in the biosynthesis of mobile signalling molecules (e.g. strigolactones) (Cochetel et al., 2018). In tomato grafts, growth and plant responses to the abiotic environment have been associated with modifications of the concentration of certain hormones e.g. under low potassium supply, shoot biomass is negatively correlated with the concentration of the ethylene precursor aminocyclopropane-1-carboxylic acid (Martinez-Andujar et al., 2016). As genetic resources are limited for most commercial crops, unequivocal experimental proof of the molecular mechanisms underlying genotypic variation in rootstock conferred traits is difficult to obtain.

In perennials crops, rootstocks and scions could differ in their perception of seasonal environmental signals related to dormancy; seasonal changes in climate have to be coordinated between two different species potentially adapted to different temperature regimes. There have been reports of rootstocks altering bud break, leaf senescence and the cessation of growth at the end of the growing season (Dong et al., 2008; Loureiro et al., 2016; Prassinos et al., 2009; Wang et al., 1994), but the mechanisms remain unknown. In kiwi, rootstocks differ in the development of root pressure in the spring and this was associated with the vigour conferred to the scion, with high vigour rootstocks more rapidly increasing root pressure (Clearwater et al., 2007). Similarly, if grafted plants consist of two individuals with different biological clocks and rhythms, it is possible that rootstocks can influence circadian rhythms of the scion, and vice versa.

How do scions alter rootstock phenotypes?

Rootstocks are known to alter a wide range of scion phenotypes, but little attention has been paid to scion effects on rootstock phenotypes despite the fact that such effects have long been recognized (Amos et al., 1930). The characterisation of scion effects on rootstock development has been largely limited to effects on root biomass or total root length (Amos et al., 1930; Harrison et al., 2016a; Tandonnet et al., 2010). There are numerous examples of shoot borne signals regulating root development in model species (Ko and Helariutta, 2017), e.g. metabolites, hormones, peptides, HY5 which regulates whole plant carbon and nitrogen status (Chen et al., 2016), microRNA 156 which regulates tuber formation in potato (Bhogale et al., 2014), and microRNA 399 which regulates phosphate uptake and translocation under phosphorus starvation conditions (Lin et al., 2014). Although studying how scions alter rootstock phenotypes is more of scientific than agronomic interest, future work in this area is a priority.

Future research directions

Our knowledge of the signals associated with rootstock modifications of scion phenotypes is growing rapidly and many QTLs regulating conferred traits have been identified. However, experiments designed to understand the genetic architecture of rootstock conferred traits have generally been restricted to the study of only one scion variety and have rarely included self-grafted controls. One exception is the study by Bert *et al.* (2013), in which tolerance to lime-induced iron deficiency in grafted rootstocks (with a unique scion) and un-grafted cuttings was compared. Bert *et al.* (2013) found that the genetic architecture of rootstock versus whole plant responses to iron deficiency were different, as such future research directions should address the roles of both the shoot and the root in regulating traits of interest in grafted plants.

The idea that the graft interface could sequester or physically alter the movement of signals between the scion and the rootstock originates from experiments on apple grafts without homo-grafted controls (Jones, 1974; Jones, 1976), new experiments are required to confirm this hypothesis.

Numerous small RNAs are found in the phloem sap (Buhtz et al., 2008) and are graft transmissible (as reviewed by Tamiru et al. (2018)) suggesting that they could modify scion/rootstock signalling. As interspecific grafting can modify DNA methylation patterns in the grafted partner (Wu et al., 2013); it is possible that epigenetic modifications underlie many rootstock conferred traits in crop species and will be of interest in the future.

What are the causes of graft incompatibility?

The commercial use of grafting depends upon the degree of graft compatibility, i.e. the ability of the assembled scion/rootstock to form and sustain a successful graft union. It is generally considered that graft incompatibility increases with the taxonomic distance but predicting compatibility is not always easy. Most intraspecific grafts and interspecific grafts (from within the same genus) are compatible, however interspecific graft incompatibility has been widely reported in fruit trees such as *Prunus spp.* (Pina et al., 2017). Intrafamilial grafts are rarely compatible, except within Solanaceae and Cucurbitaceae families in which compatibility between different genera is exploited in commercial grafting. Similarly, in Rosaceae certain cultivars of pear (*Pyrus communis*) are compatible with quince (*Cydonia oblonga*) rootstocks. Interfamilial grafts are almost always incompatible, however some may survive in the short-term (weeks) such as Arabidopsis/tomato grafts (Flaishman et al., 2008). In addition, short-term (weeks) survival of interfamilial grafts is used to graft-inoculate pathogens for scientific study (Aryan et al., 2016; Vigne et al., 2005). Graft incompatibility can express itself over various time frames from poor success soon after grafting to the dieback of grafted plants several years after planting in the field. This delayed dieback may be only in the appearance of incompatibility symptoms that have been progressing, unobserved, since shortly after the grafting was performed. Despite its importance in horticulture, little is known about the mechanisms that cause graft compatibility/incompatibility except for the special case of certain pear/quince grafts (Gur et al., 1968). Certain quince rootstocks contain prunasin, a cyanogenic glycoside, which can move into the pear scion where hydrolysis by β-glicosidases releases toxic cyanide that causes tissue necrosis and graft incompatibility.

There have been a small number of studies of the transcript or proteins accumulated at the graft interface during graft union formation to try to understand the molecular basis of graft incompatibility and differences between hetero- and homo-grafting (Chen et al., 2017; Cookson et al., 2014; Prassinos et al., 2009; Ren et al., 2018; Wang et al., 2016). Generally these studies have lacked appropriate controls (such as homo-grafts, and cut, but un-grafted scions and rootstocks) and/or sufficient sampling to accurately identify transcripts or proteins involved. This analysis is particularly complicated in perennial crops; grafting typically occurs when the wood is dormant during the winter months as such the graft union develops at the same time as the spring reactivation of the cambium (Cookson et al., 2013). The activation of the cambium may be different between the different genotypes studied; requiring that changes in transcription expression are studied over time so that transcript expression profiles associated with differences in the reactivation of the cambium can be separated from those associated with incompatibility responses. Furthermore, no attempts have been made so far to assign

the transcripts harvested from the mixture of cells at the graft interface to either of the grafting partners, although theoretically possible if there is sufficient variation between the scion and rootstock genotype and long reads are used. A similar technique determined the parental origin of transcripts identified in RNA sequencing data from allopolyploid species (Peralta et al., 2013).

How does the graft union form?

The process of graft union formation begins with formation of a necrotic layer, followed by adhesion of the two grafted partners, callus cell formation and the establishment of a functional vascular system; this has been extensively studied in hypocotyl grafts of Arabidopsis (Box 1). The role of different hormones in graft union formation and wound healing has been recently reviewed by Nanda and Melnyk (2018). Although auxins have been used to improve grafting success in viticulture since 1934 (according to Fallot (1970)), the precise role of auxins in phloem reconnection during graft union formation was only identified recently (Melnyk et al., 2015). In perennial crops, grafting is traditionally performed on over-wintering woody tissues in the spring whereas commercial vegetable grafting is generally done on hypocotyls or stems of actively growing plants soon after germination (Box 2) suggesting that the signalling processes involved may be different.

The development of the graft interface has been of scientific interest for nearly 100 years; the first classical microscopy studies being published in the 1920s (Bailey, 1923). More recently 3D imaging techniques have improved our understanding of the graft union in perennial crops, but with limited resolution (Bahar et al., 2010; Milien et al., 2012). Recently, significant progress has been made in understanding the early stages of vascular reconnection in hypocotyl grafts of Arabidopsis. Melnyk *et al.* (2015) showed that the scion and rootstock adhere 1-2 days after grafting, and the use of fluorescent dyes and proteins demonstrated that the phloem reconnects 3-4 days after grafting (which coincides the resumption of root growth). The xylem reconnects 6-7 days after grafting (Box 1). This study was restricted to the first week after grafting, so we still have no knowledge of the organisation of the limited secondary growth of grafted hypocotyls and how the newly formed xylem and phloem develop. In a subsequent paper, Melnyk *et al.* (2018) described in detail the genes differentially expressed during the time course of graft union formation and highlighted the up-regulation of many genes associated with vascular regeneration (Box 1).

There are many difficulties associated with studying cellular developments at the graft interface of woody perennial species. Namely the tissues are large, very hard and the identification of the exact location of the graft interface is impossible if the scion and rootstock have morphologically indistinct

callus cells. Furthermore, using assays similar to those described by Melnyk *et al.* (2015) to quantify the function of xylem and phloem across the graft interface of woody grafts of perennial crops is technically challenging because plants are grafted before bud break, so there are no leaves to drive transpiration and movement of labelled molecules.

In addition to the connection of vascular tissues across the graft interface, the connection of cell-to-cell contact via plasmodesmata is presumably important to the function of grafted plants. Plasmodesmata are small membrane channels of about 30 nm in diameter that pass through the plant cell wall, and provide membrane and cytosolic continuity between most cells of the plant. They are composed of a central element originating from the endoplasmic reticulum, the desmotubule. The unequivocal presence of plasmodesmata at the scion/rootstock interface has only been demonstrated in one study, in which the scion and rootstock could be identified using electron microscopy thanks so histological differences between the two genotypes (Kollmann and Glockmann, 1985). Many questions remain concerning the formation of plasmodesmata at the graft interface: 1) are they essential for grafting success and/or long term plant survival? 2) How do they form across the pre-existing cell walls of the scion and rootstock? 3) Where does the endoplasmic reticulum of the desmotubule come, from the scion, from the rootstock or from both grafted partners? Different models of plasmodesmata formation across the graft union are outlined in Box 3, in the first (A), the scion and rootstock coordinate to form a new plasmodesmata by thinning the cell wall and tethering endoplasmic reticulum to it, this is followed by the fusion of the endoplasmic reticulum from both grafting partners, cell wall thickening and the formation of a mature plasmodesmata. This would result in the formation of a desmotubule that originates from both grafted partners. In the second model (B), plasmodesmata formation is initiated by only one of the grafted partners (the rootstock in the example shown), the endoplasmic reticulum of the rootstock invaginates into the cell wall and new plasmodesmata are formed with desmotubules originating from only one grafted partner.

The unprecedented work of Stegemann and Bock (2009) and Fuentes *et al.* (2014) have shown that entire chloroplast and nuclear genomes can be horizontally transferred across the graft union (Box 1). The authors suggest two mechanisms for this process (1) fusion of neighbouring cells at the graft site, or (2) migration of nuclei from cell-to-cell through plasmodesmata in a cytomixis-like process. To date, we do not know the mechanism of organelle exchange at the graft interface (a model for this process is shown in Box 3C), or when or in which cell type(s) it occurs. However, given that these exchanges appear to be fairly frequent, cell fusion or migration of nuclei may actually be an essential component of the graft union formation process.

Future research directions

We still have little understanding of how the physical connections across the graft interface are formed, how the vascular tissue is integrated, how plasmodesmata are formed and how organelles are exchanged; future research should address these fundamental questions using fluorescent markers and correlative light-electron microscopy techniques.

In addition, to expand the range of rootstocks compatible with existing scion varieties, we need to advance the identification of the molecular elements underlying graft union formation and graft incompatibility. A clear understanding of the transcript or protein accumulation profiles at the graft interface associated with graft incompatibility is still lacking. Although it is logistically challenging to graft hundreds of different scion/rootstock combinations with a sufficient number of repetitions to accurately quantify graft compatibility, QTL studies of graft compatibility should be done in the future.

Conclusion

Despite thousands of years of agronomic use, science is just beginning to reveal the mechanisms underlying graft union formation and scion/rootstock interactions. Understanding how rootstocks can modify scion phenotypes will be valuable in aiding plant adaptation to climate change via the creation of new rootstocks. The increasing commercial use of grafted vegetables has renewed scientific interest in grafting because it holds the promise of providing sustainable solutions to numerous agronomic challenges.

Box 1 Key developments in understanding the graft union formation

• Auxin response genes are essential for phloem connection, but less so for xylem in Arabidopsis hypocotyl grafting.

Melnyk *et al.* (2015) showed that in Arabidopsis hypocotyl grafts, auxin accumulated above the graft interface just after cutting and that auxin is key to forming vascular connections between the scion and rootstock. By grafting green fluorescent protein labelled scions onto rootstocks defective in auxin signalling, they demonstrated that movement of green fluorescent protein from the scion to the rootstock was delayed up to 2-fold. However, transport assays from rootstock to scion showed that xylem connection is not significantly impaired.

• Genes are asymmetrically expressed between the scion and the rootstock around the graft interface of Arabidopsis hypocotyl grafts

Melnyk *et al.* (2018) characterised the genome wide gene expression changes induced during hypocotyl grafting in Arabidopsis (at 0, 6, 12, 24, 48, 72, 120, 168 and 240 h after grafting). The authors observed that gene expression response was very different between the scion and rootstock, and that much of the asymmetric gene expression differences were driven by the accumulation of carbon in the scion, and the carbon limitation in the rootstock (until the phloem reconnected). Interestingly, many genes associated with vascular formation were up-regulated in grafted tissues in comparison to the cut and separated tissues before the formation of functional vascular connections, indicating that a recognition mechanism was activated.

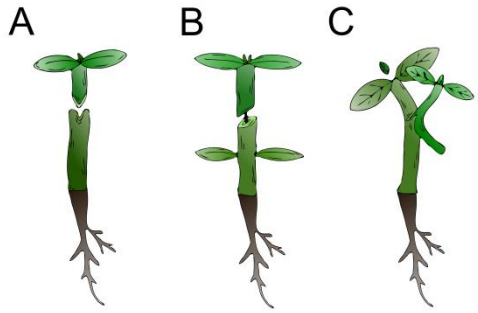
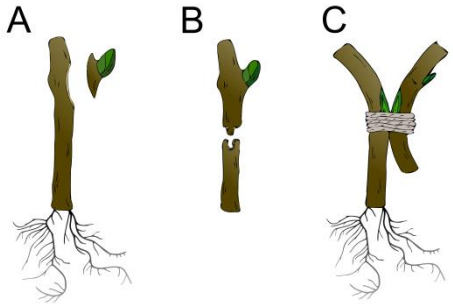
• Grafted partners can exchange nuclear and plastid genetic material in tobacco grafts

By grafting two transgenic tobacco lines, one carrying a kanamycin resistance gene and the yellow fluorescent protein gene in its nuclear genome, and the other one a spectinomycin resistance gene and the green fluorescent protein gene in its chloroplast genome, Stegemann and Bock (2009) could select double resistant cells from callus cells at the graft interface on selective media. Double resistant lines could not be obtained from tissues far from the graft interface suggesting that these genome transfer events were restricted to the graft interface. In a second paper (Fuentes *et al.* 2014), the same group showed in a similar fashion that not only chloroplast genomes were exchanged between the scion and rootstock at the graft interface, but nuclear genomes were exchanged and that allopolyploid cells could be selected for in the callus tissues.

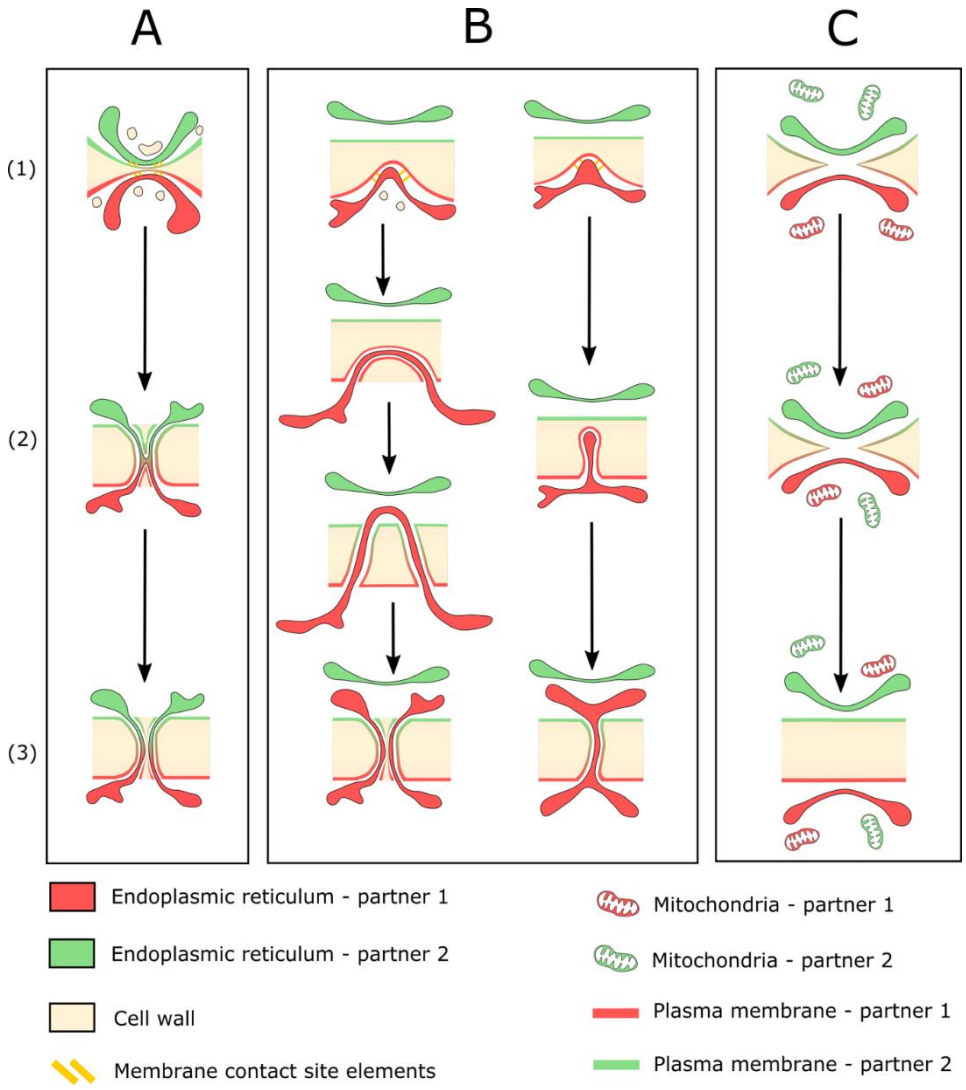
• Mitochondria are able to move from cell-to-cell through graft junction in tobacco grafts

To demonstrate the transfer of mitochondria at the graft interface, Gurdon *et al.* (2016) grafted two tobacco species, *Nicotiana tabacum* which has male sterile flowers due to cytoplasmic male sterility carried by mitochondria and *Nicotiana sylvestris* which has fertile flowers. As in Stegemann and Bock (2009) and Fuentes *et al.* (2014) papers, thanks to resistant genes carried in the nucleus and the chloroplast, they selected and regenerated hybrid lines from the callus cells at the graft interface. The regenerated plants were chimeric, showing three types of flowers: sterile, fertile and an intermediate phenotype suggesting that a mitochondrial transfer also occurs at the graft interface.

Box 2 Comparison of grafting in herbaceous versus woody plants

Herbaceous grafting	**Woody grafting**
 Diagrams of (A) hypocotyl grafting, (B) stem grafting and (C) approach grafting of herbaceous tissues	 Diagrams of (A) chip budding, (B) omega table-top grafting and (C) approach grafting of woody tissues
Tissues active and rapidly growing when grafted	Tissues dormant when grafted
Leaves and/or cotyledons photosynthesizing and transpiring when grafted	Buds dormant when grafted
Carbon accumulates in the scion, whereas the rootstock is carbon starved until phloem reconnects	Large supplies of starch in dormant wood of scion and rootstock
Root growth depends upon phloem connections with scion	Bud break and adventitious root formation largely independent of graft union development
Limited secondary growth	Considerable secondary growth

Box 3 Models of *de novo* plasmodesmata biogenesis and organelle transfer events at the graft interface



(A) Model of spatio-temporal coordination between both partners for plasmodesmata biogenesis. (1) Tethering of endoplasmic reticulum to plasma membrane at both sides of the graft interface and thinning of the cell wall (2) endoplasmic reticulum and plasma membrane fusions leading to the formation of branched plasmodesmata and thickening of cell wall through callose deposition (3) maturation from branched into twin plasmodesmata. (B) Biogenesis of plasmodesmata initiated by only one partner, (1) tethering of endoplasmic reticulum to plasma membrane at one side of the graft interface with thinning of the cell wall, (2) invagination of endoplasmic reticulum into the cell wall, (3) new twin or simple plasmodesmata are formed. (C) Organelles transfer events at the graft interface, (1) cell wall opening at the graft interface, (2) organelles transfers, and (3) pore closing.

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