

Research Paper

Identification of genes involved in almond scion tree architecture influenced by rootstock genotype using transcriptome analysis

Álvaro Montesinos^{a,b,c}, María José Rubio-Cabetas^{a,b}, Jérôme Grimplet^{a,b,*}

^a Departamento de Ciencia Vegetal, Gobierno de Aragón, Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA), Avda. Montañana 930, Zaragoza 50059, Spain

^b Instituto Agroalimentario de Aragón—IA2 (CITA-Universidad de Zaragoza), Calle Miguel Servet 177, Zaragoza 50013, Spain

^c Centro de Biotecnología y Genómica de Plantas, Universidad Politécnica de Madrid, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Madrid, Spain

ARTICLE INFO

Keywords:

Scion-rootstock interaction

Vigor

Hormonal regulation

Prunus dulcis

Tree shape

Transcriptomics

ABSTRACT

The emergence of almond (*Prunus amygdalus* (L.) Batsch, syn *P. dulcis* (Mill.)) intensive and semi-intensive cropping systems has created a necessity for new almond cultivars with vigor and shape adapted to these new circumstances. Hence, it is important to unravel which mechanisms are behind the regulation of the tree three-dimensional structure, or tree architecture, and what factors may play a role, like the choice of rootstock. In this study, we have analyzed the rootstock influence on the scion transcriptome, regarding the biological processes that control almond tree architecture. Three commercial almond cultivars were grafted onto three hybrid rootstocks known to confer different architecture to the scion, resulting in nine combinations, whose gene expression in shoot tips was analyzed via RNA-Seq. We report that differences in tree architecture phenotype are correlated with differential expression of genes involved in hormonal and molecular responses associated with the regulation of apical dominance, branch formation, plant growth, cell wall formation, or nitrogen assimilation. These results highlight the importance of the rootstock choice in selecting a desirable scion architecture and in establishing almond orchards.

1. Introduction

Rootstocks are widely used in numerous fruit and nut orchards (Warschefsky et al., 2016). Their use allows to confer traits of agro-nomical interest to the cultivars and to independently select favorable traits for scion and rootstock. The rootstock influences the scion phenotype for multiple characters, such as tree vigor, yield, flowering time, or fruit quality (Aloni et al., 2010; Martínez-Ballesta et al., 2010; Albacete et al., 2015; Foster et al., 2015; Warschefsky et al., 2016; Font i Forcada et al., 2020; Reig et al., 2022). Almond (*Prunus amygdalus* (L.) Batsch, syn *P. dulcis* (Mill.)) cultivars are graft-compatible with both almond and peach (*P. persica* (L.) Batsch) rootstocks and their inter-specific hybrids (Felipe, 2009; Rubio-Cabetas et al., 2017) are widely used in almond orchards, which almost exclusively consist of these scion/rootstock combinations.

Among these rootstock effects on the cultivar in various fruit tree species, researchers focused predominantly on scion vigor. Analysis in

both apple (*Malus × domestica*) and *Prunus* species have determined a correlation between rootstock and vigor-related parameters such as scion height or trunk diameter (Tworkoski and Miller, 2007; Tworkoski and Fazio, 2015; Yahmed et al., 2016; Scalisi et al., 2018; Balducci et al., 2019; Lordan et al., 2019; Narandžić and Ljubojević, 2022). Although the effect on other traits related to tree architecture like shoot production and development has been reported in apple cultivars, the interaction is less clear (Tworkoski and Miller, 2007; Seleznyova et al., 2008; Van Hooijdonk et al., 2010).

Apical dominance is a crucial regulator of tree architecture. It defines the capacity exerted by the shoot apical meristem (SAM) to repress lateral bud outgrowth, redistributing resources towards the elongation of the main axis (Hollender and Dardick, 2015; Wang et al., 2018a). Numerous factors are behind the regulation of apical dominance and bud outgrowth with auxins acting as the core regulator, which are predominantly transported throughout the axis by specific efflux and influx carriers, promoting apical dominance (Cho and Cho, 2013;

* Corresponding author at: Departamento de Ciencia Vegetal, Gobierno de Aragón, Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA), Avda. Montañana 930, Zaragoza 50059, Spain.

E-mail address: jgrimplet@cita-aragon.es (J. Grimplet).

<https://doi.org/10.1016/j.scienta.2023.112628>

Received 19 June 2023; Received in revised form 27 October 2023; Accepted 29 October 2023

Available online 3 November 2023

0304-4238/© 2023 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

Adamowski and Friml, 2015). Beside, auxin facilitates graft formation, and elevated levels in the rootstock promote callus and vascular cell development, proving that upward transport also happens at least over short distances (Zhai et al., 2021). The exact mechanism by which auxins repress bud outgrowth is yet under scrutiny, but strigolactones (SLs) are proven to act as auxin secondary messengers, inhibiting bud outgrowth (Dun et al., 2012; Shinohara et al., 2013; Bennett et al., 2016; Dierck et al., 2016a; Waldie and Leyser, 2018). Cytokinins (CKs) have the opposite effect, promoting bud outgrowth and shoot branching (Dun et al., 2012; Dierck et al., 2016b; Waldie and Leyser, 2018). Other hormones like gibberellic acid (GA) or brassinosteroids (BRs) are also involved in shoot development, but their effects are less characterized (Lo et al., 2008; Sun, 2010; Wei and Li, 2016). Sugars have been also described as an important regulator of bud outgrowth, promoting the formation of branches when there is high availability (Stokes et al., 2013; Mason et al., 2014). External stimuli such as light perception also control shoot development via photoreceptors phyA and phyB (Casal, 2012; Reddy and Finlayson, 2014; Holalu and Finlayson, 2017).

Tree vigor is mainly controlled by the hormonal response and nutrient availability. GA and BRs are involved in its regulation, primarily promoting cell elongation, although they have been described to stimulate cell proliferation too (Busov et al., 2008; Yamaguchi, 2008; Fridman and Savaldi-Goldstein, 2013). GA activity in cell elongation affects numerous aspects of plant growth, like seed germination, stem elongation, and flower development (White and Rivin, 2000; Ogawa et al., 2003; Griffiths et al., 2006; Gallego-Bartolomé et al., 2011). GA acts by connecting external clues such as light perception with molecular regulation of these processes (Alabadi et al., 2008; Filo et al., 2015). Furthermore, deficiencies in GA have been observed to affect tree vigor in several crops like poplar, apple, or peach (Hollender and Dardick, 2015; Hollender et al., 2016). CKs and auxins control plant vigor as well, regulating cell proliferation and cell elongation (Busov et al., 2008; Depuydt and Hardtke, 2011; Ma et al., 2016). Nutrient availability is crucial for plant development, especially nitrogen availability. Hormone synthesis and transport are tightly controlled by nitrogen supply (Krouk et al., 2011). Hence, nitrate acts as a signaling molecule, regulating gene expression, and controlling several developmental processes like root formation, shoot development, or flowering (Wang et al., 2018b).

In recent years, flowering has been linked with tree architecture. Hormones regulating tree architecture, like auxin or GA, are also part of flowering control, providing a possible crossroad between these developmental processes (Srikanth and Schmid, 2011). Studies in *Arabidopsis* and woody plants such as apple have proven that important flowering regulators like FLC or FT are involved in shoot development (Pin and Nilsson, 2012; Huang et al., 2013; Foster et al., 2014).

Characterization of all these processes affecting tree architecture using a collection of different rootstocks could help to a better understanding of how they influence scion phenotype. In a previous experiment (Montesinos et al., 2021), we grafted several almond cultivars onto various hybrid rootstocks observing that the rootstock influences parameters related to tree architecture like the number of shoots or shoot distribution through the trunk. Although several molecular processes have been linked to the regulation of tree architecture, little to none is known about the influence exerted by the rootstock genotype on the processes that are behind these changes in the scion. To unravel a cohesive view of the molecular mechanisms behind rootstock impact on the cultivar architecture, the transcriptome of nine scion/rootstock combinations, whose effect on scion traits was evaluated in a previous experiment, is here presented in a comparative analysis.

2. Materials and methods

2.1. Plant materials and growth conditions

In this study, a subset of nine scion/rootstock combinations from a previous trial with thirty combinations was chosen (Montesinos et al.,

2021), comprising three almond cultivars of agronomic interests which were grafted onto three different commercial rootstocks. The following combinations were selected after analyzing rootstock influence on scion architecture: ‘Densipac’ (Rootpac® 20), ‘Nanopac’ (Rootpac® 40), and Garnem® (GN15) as rootstocks, and ‘Isabelona’ (syn. ‘Belona’), ‘Diamar’ (syn. ‘Mardía’) and ‘Lauranne’ as cultivars. All rootstocks are hybrids from different origins. Garnem® is an almond × peach (*P. amygdalus* (L.) Batsch, syn *P. dulcis* (Mill.) × *P. persica* (L.) Batsch) hybrid rootstock, while the others come from the commercial Rootpac® series: Rootpac® 40 (*P. amygdalus* (L.) Batsch, syn *P. dulcis* (Mill.) × *P. persica* (L.) Batsch) and Rootpac® 20 (*P. cerasifera* × *P. besseyi*). Grafted plants were supplied by the Agromillora Iberia S.L. nursery in 2018 (Barcelona, Spain). Trees were planted in October 2018 at the Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA) experimental orchard El Vedado Bajo el Horno (Zuera, Zaragoza, 41°51'46.5"N 0°39'09.2" W). Trees were planted in an open field as a single axis and supported by a wooden stick. Trees were then left without pruning so that, they could express their natural growth habit unaltered. Conventional orchard practices were used for weed, pest, and disease control and drip irrigation. The soil type was calcareous with a pH of around 7–8.

2.2. Data collection

Seven descriptors of tree architecture were measured in three trees per scion/rootstock combination. These parameters, previously depicted in Montesinos et al. (2021), belong to three different categories: (i) vigor, (ii) branch quantity, and (iii) branch distribution. (i) Length: trunk length; IN_L: mean length of trunk internodes. (ii) Nb_B: number of primary branches; BbyIN: proportion of branches per number of internodes; Nb_LB: number of long branches (> 200 mm); B_NbAS: number of secondary branches per primary branch. (iii) Dist_B: distribution of branches through the trunk.

2.3. RNA-Seq analysis

Samples from the nine combinations mentioned were collected in a single morning (between 10 am and 11 am) from shoot tips of two-year-old branches from three different individuals per combination during the summer of 2020. Plants were at stage 75 on the BBCH scale. RNA extraction was performed from these samples using the CTAB method described previously (Meisel et al., 2005) with some modifications (Chang et al., 1993; Salzman et al., 1999; Zeng and Yang, 2002). Stranded mRNA-Seq analysis was carried out at Centro Nacional de Análisis Genómico (CNAG-CRG) in Barcelona, Spain. Sequencing was performed by an Illumina NovaSeq 6000 System - with > 30 M PE reads per sample and a read length of 2 × 50 bp. FASTQ files were converted with FASTQ Groomer (Galaxy Version 1.1.1) (Blankenberg et al., 2010). Adapter sequences were removed by processing the reads sequences of the twenty-seven individual datasets with Trimmomatic (Galaxy Version 0.38.0) (Bolger et al., 2014). RNA-Seq data alignment was carried out by HISAT2 (Galaxy Version 2.2.1), with a maximum intron length of 20,000 bp, (Kim et al., 2015) on the *P. dulcis* ‘Texas’ Genome v2.0 (Alioto et al., 2020). Duplicated molecules were located and mate-pairs were confirmed using the MarkDuplicates (Galaxy Version 2.18.2.2) and FixMateInformation (Galaxy Version 2.18.2.1) Picard tools respectively (<http://broadinstitute.github.io/picard>). featureCounts (Galaxy Version 2.0.1+galaxy2) was used to measure gene expression (Liao et al., 2014) using the gene annotation *P. dulcis* ‘Texas’ Genome v2.0 containing 27,044 genes (<https://www.rosaceae.org/analysis/295>). Differential analysis of count data was performed by edgeR (Galaxy Version 3.36.0) with default settings (Robinson et al., 2009). Genes with a corrected p-value below 0.05 and a log₂FC above 1 or below −1 were considered differentially expressed. All procedures were carried out using the Galaxy platform. Recent reports have shown that RNA-Seq methods are robust enough and validation by qPCR would confirm its results for a vast majority of transcripts. Non-concordant results between both techniques

were observed in less than 2 % (Everaert et al., 2017; Coenye, 2021) appearing typically in short and low-expressed genes. As these data are related to the Fragments Per Kilobase of transcript per Million (FPKM), differentially expressed genes were filtered to assure that they belong to the 98 % transcripts with the highest FPKM.

2.4. RNA-Seq data structural and functional analysis

Principal component analysis (PCA) was carried out using the R stats package with default parameters on the gene expression values for all the genes in the nine combinations. Distance between genes was measured using its correspondent function from the R stats package. Hierarchical clustering and correlation networks were performed using the WGCNA package (Langfelder and Horvath, 2008). GO enrichment was carried out using the tool GOEnrichment (<https://github.com/-DanFaria/GOEnrichment>) with a p-value cut-off < 0.1 and Benjamin-Hochberg correction.

3. Results and discussion

3.1. Rootstock influence on scion architecture correlates with differences in gene expression

The phenotypic effect of the rootstock on the nine different scion/rootstock combinations was measured using seven architecture parameters (Supplementary Data 1), which had been previously proven to be affected by the rootstock (Montesinos et al., 2021). PCA (Principal Component Analysis) was carried out using the phenotypic data collected for the nine scion/rootstock combinations (Fig. 1a). The first two components explained more than two-thirds of the variability, with the first component explaining 55.1 %, and the second 22.22 %. For two of the cultivars, ‘Isabelona’ and ‘Lauranne’, we observed a stronger influence of the cultivar than the rootstock since combinations involving these cultivars can be observed indistinctly clustering together on each side of Fig. 1a. ‘Isabelona’ combinations present a strong apical dominance phenotype while those with ‘Lauranne’ as scion display numerous branching and high vigor (Fig. 2; Supplementary Data 1). The effect of the rootstock in aerial traits in these two cultivars seems to be limited. However, we observed more diversity between individuals for the ‘Isabelona’/Rootpac® 40 combination (Fig. 1a). Contrarily to these two cultivars, ‘Diamar’ seemed more affected by the rootstock genotype. When grafted onto Rootpac® 20, which is a dwarfing rootstock, the plants showed high apical dominance and reduced branching similar to the plants carrying ‘Isabelona’ as the scion (Fig. 2; Supplementary Data 1). On the contrary, when grafted onto the vigor-inducing rootstock Garnem®, ‘Diamar’ combinations clustered with the ‘Lauranne’

combinations. Although Rootpac® 40 is a more vigor-inducing rootstock compared to Rootpac® 20, it does not reduce apical dominance at the same level as Garnem®. Therefore, ‘Diamar’/Rootpac® 40 combinations are between ‘Isabelona’ and ‘Lauranne’ combinations, but closest to the former (Fig. 1a).

A second PCA was carried out, using the expression for each gene as variables for the nine combinations (Fig. 1b). The first two components explained 40 % of the variability, with 24.28 % and 10.76 % of the variability respectively. Data related to each cultivar grouped. As for phenotypic data, combinations involving both ‘Lauranne’ and ‘Isabelona’ did not present marked differential distribution linked to the rootstock genotype (Fig. 1b). As observed for the phenotypic data, ‘Diamar’ combinations presented a contrasted position in the PCA. Individuals grafted onto Rootpac® 20 were separated from individuals grafted onto Garnem® and Rootpac® 40 (Fig. 1b). Therefore, the absence of a rootstock effect in ‘Isabelona’ and ‘Lauranne’ combinations seems to be linked to a lack of differential gene expression under these conditions.

RNA-Seq data were then subjected to a hierarchical clustering analysis (Fig. 2). Data samples were separated according to the scion genotype, which was expected as the samples were taken from this part of the plant, showing the global gene expression variation between each genotype. Since these clusters depend on the complete gene expression profile and not only on the genes that may affect tree architecture, other processes not linked to the phenotype might also affect these results. It is not the objective of this study to draw conclusions on comparing varieties between each other since in these comparisons it is hard to separate the “cultivar effect” from the “rootstock effect”. On the other hand, there is very limited research on the molecular influence of the rootstock genotype in a single cultivar. For the comparisons intra-cultivar, combinations with ‘Lauranne’ and ‘Isabelona’ as cultivars were clustered in one group each with no effect of the rootstocks and no clear gene expression differences when comparing the whole transcriptomes. In ‘Diamar’ we observed a clear separation of samples grafted onto Rootpac® 20 from the others. Transcriptomics data do not allow to clear separation of Garnem® and Rootpac® 40 unlike what was observed with the phenotypic data where ‘Diamar’/Rootpac® 40 presented an intermediate phenotype between ‘Diamar’/Rootpac® 20, which displayed low vigor and strong apical dominance, and ‘Diamar’/Garnem® (Fig. 2).

Overall in the ‘Diamar’ case, the phenotypic profile of architecture characters is in accordance with the observed data for gene expression in shoot tips, which allows us to assume that the differentially expressed gene in this tissue might be related to differential architecture.

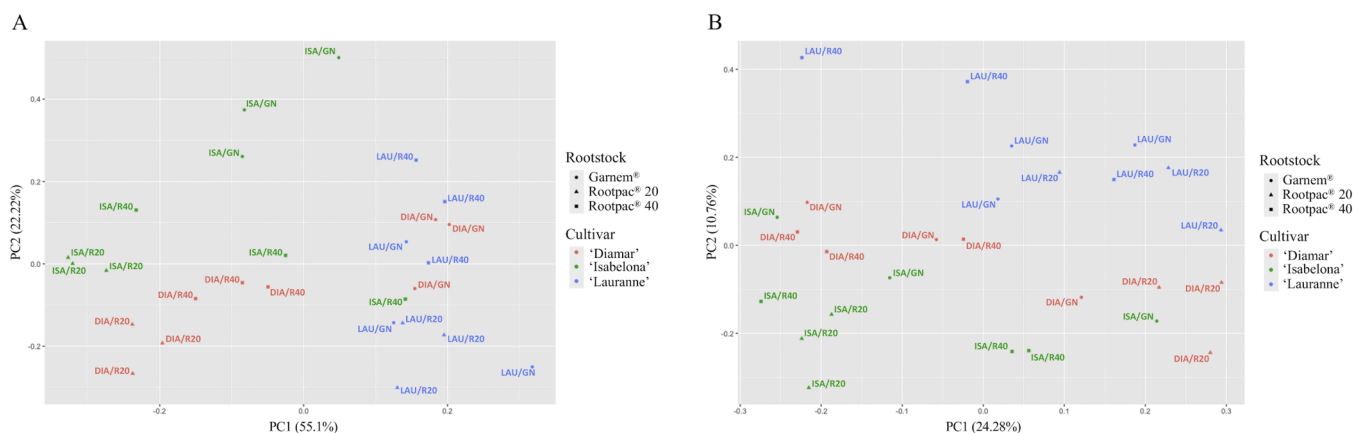


Fig. 1. Principal component analysis (PCA) of the nine scion/rootstock combinations. A. PCA of the phenotypic data. B. PCA of the global expression profile data. DIA/GN: ‘Diamar’/Garnem®; DIA/R20: ‘Diamar’/Rootpac® 20; DIA/R40: ‘Diamar’/Rootpac® 40; ISA/GN: ‘Isabelona’/Garnem®; ISA/R20: ‘Isabelona’/Rootpac® 20; ISA/R40: ‘Isabelona’/Rootpac® 40; LAU/GN: ‘Lauranne’/Garnem®; LAU/R20: ‘Lauranne’/Rootpac® 20; DIA/R40: ‘Lauranne’/Rootpac® 40.

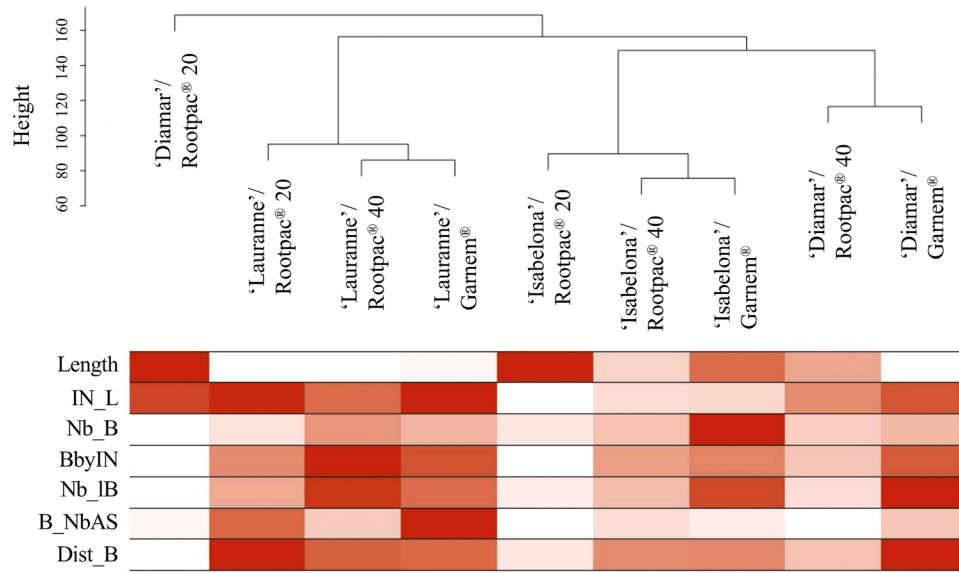


Fig. 2. Hierarchical clustering of the global transcriptome of the nine scion/rootstock combinations. The intensity of color in the heatmap below the clustering represents the values for each phenotypic trait. Length: trunk length; IN_L: mean length of trunk internodes; Nb_B: number of primary branches; BbyIN: proportion of branches per number of internodes; Nb_IB: number of long branches (> 200 mm); B_NbAS: number of secondary branches per primary branch; Dist_B: distribution of branches through the trunk.

3.2. Rootstock differentially affects metabolism genes in 'Diamar' combinations

When comparing the same cultivar grafted onto different rootstocks, 'Lauranne' and 'Isabelona' combinations did not show any DEGs in any comparison (Supplementary Data 2, 3). As it was previously stated, the reduced rootstock effect on the scion architecture correlates with this absence of differences in gene expression. In the same way that we observed the impact of the rootstock on the scion phenotype, we did observe DEGs in 'Diamar' combinations (Supplementary Data 4). In these comparisons, DEGs were only observed when compared to individuals grafted onto the dwarfing rootstock Rootpac® 20, while we did not observe DEGs between Garnem® and Rootpac® 40. Both are hybrid almond × peach rootstocks which are described to confer vigor to the scion. This similar influence on phenotype exerted by the rootstock genotype would explain the lack of differences in gene expression observed here. We observed 311 DEGs more expressed with both vigor-inducing rootstocks than with Rootpac® 20 and 118 more expressed in Rootpac® 20. A total of 667 DEGs were found more expressed specifically with Rootpac® 40 than with Rootpac® 20 and 305 more with

Rootpac® 20 than with Rootpac® 40. A total of 354 DEGs were detected comparing Garnem® with Rootpac® 20, with 297 DEGs more expressed with Garnem® and 52 with Rootpac® 20 (Fig. 3).

To characterize the biological processes and molecular functions associated with these DEGs, a GOenrichment analysis was carried out (Fig. 4). Since the majority of DEGs appeared more expressed in combinations with the vigor-inducing rootstocks Garnem® and Rootpac® 40, we focused on these genes. When analyzing molecular function terms (Fig. 4a), we observed an enrichment of those related to “catalytic activity” in Garnem® and Rootpac® 40 combinations, especially in the “oxidoreductase activity” category. In both combinations, 'Diamar' presented more vigor than when grafted onto Rootpac® 20, and the enrichment of DEGs belonging to these GO categories is probably due to higher metabolic activity in the shoot tips of these combinations, which are growing more actively. The term “transmembrane transport” was enriched in individuals grafted onto Garnem® (Fig. 4b). This might be due to the more active transport of nutrients or hormones linked to active growth (Park et al., 2017; Wang et al., 2018b). In individuals grafted onto Rootpac® 40, we observed an enrichment of DEGs belonging to the terms related to cell division. It is maybe linked to

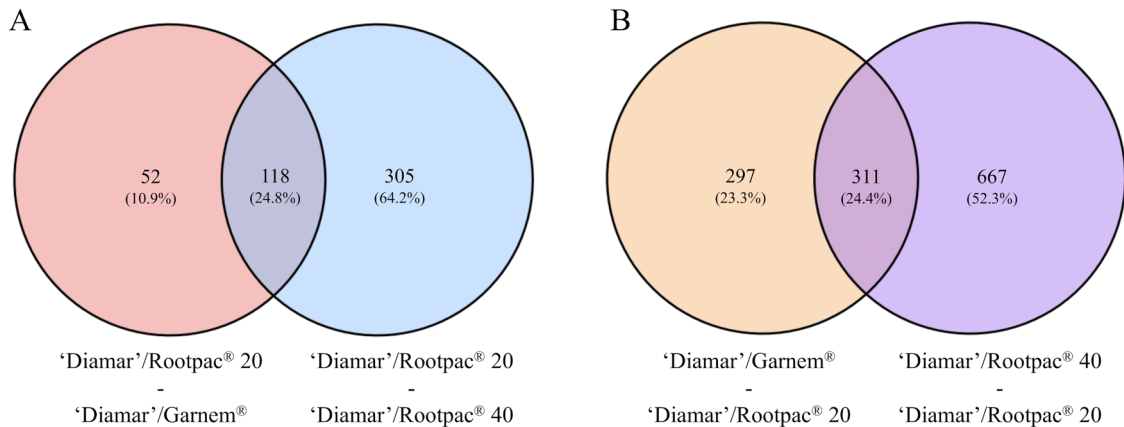


Fig. 3. Venn diagrams of differentially expressed genes (DEGs) in combinations with 'Diamar' as the scion. A. DEGs more expressed in combinations with Rootpac® 20 than with Rootpac® 40 or Garnem®. B. DEGs less expressed in combinations with Rootpac® 20 than with Rootpac® 40 or Garnem®.

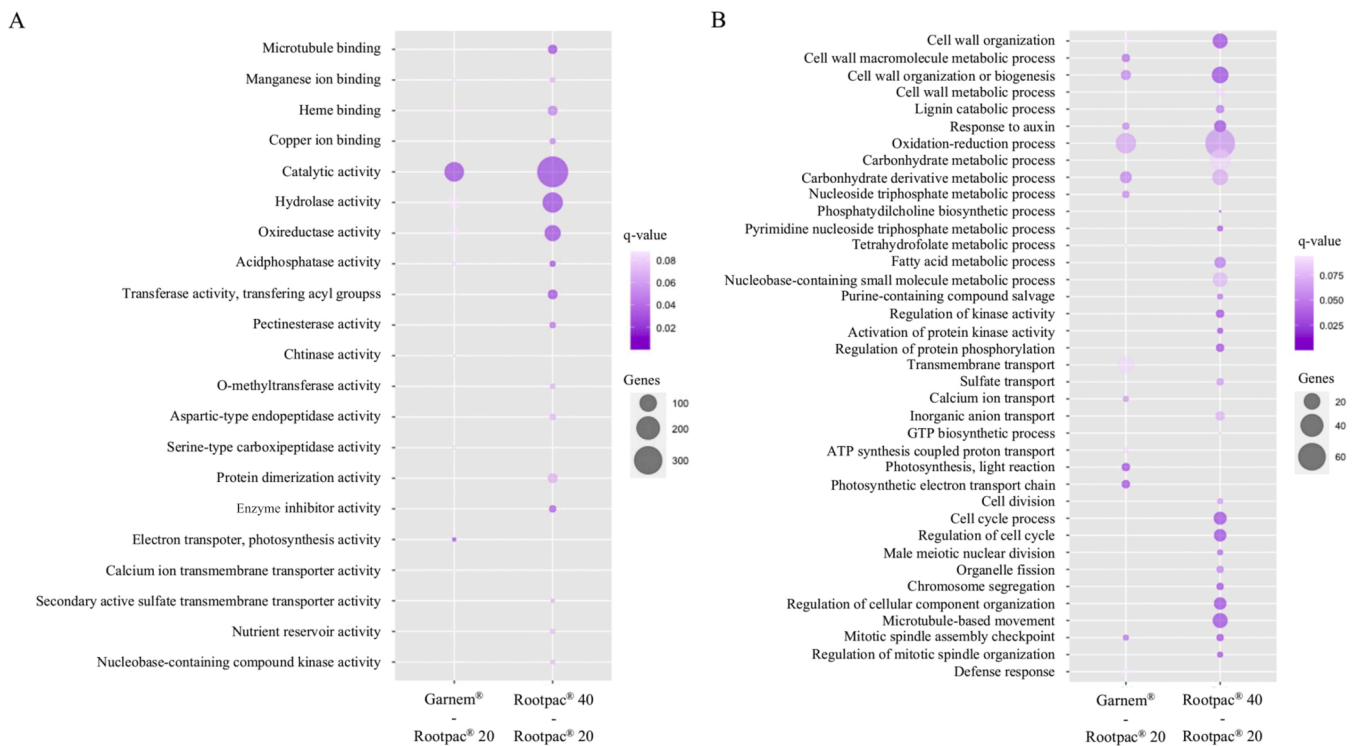


Fig. 4. GOenrichment of differentially expressed genes (DEGs) in combinations with ‘Diamar’ as the scion. A. Molecular function terms for ‘Diamar’ combinations in which DEGs were less expressed in combinations with Rootpac® 20 than with Rootpac® 40 or Garnem®. B. Biological process terms for ‘Diamar’ combinations in which DEGs were less expressed in combinations with Rootpac® 20 than with Rootpac® 40 or Garnem®.

promoting cell proliferation, or to cell elongation, which could therefore lead to its more vigorous phenotype (Sablowski, 2016).

For terms representing biological processes (Fig. 4b), we detected an enrichment of DEGs from the term “photosynthesis” in Garnem® combinations. The overrepresentation of these genes might be due to a higher photosynthetic rate and carbon assimilation that could be linked to the higher vigor displayed by ‘Diamar’ when grafted onto Garnem®. DEGs characterized by the term “carbohydrate derivative metabolic process” were enriched in individuals grafted onto Garnem®. While a more active metabolism is expected in scions grafted onto a vigor-conferring rootstock, like Garnem®; sugars are also an important regulator of branching, and the enrichments of DEGs associated with their pathways may be related to the low apical dominance and numerous branching observed in the ‘Diamar’/Garnem® combination (Mason et al., 2014; Barbier et al., 2015). In both the ‘Diamar’/Garnem® and the ‘Diamar’/Rootpac® 40 combinations, terms associated with “cell wall organization” were enriched (Fig. 4b). Similar terms were enriched in previous transcriptomic analysis characterizing rootstock effect in grapevine and citrus (Cochetel et al., 2017; Liu et al., 2017b). Regulation and reorganization of the cell wall are crucial to allow plant growth, which explains why DEGs related to these processes are upregulated when individuals are grafted onto rootstocks that favor more active growth, like Rootpac® 40 and Garnem® (Cosgrove, 2016; Vaahtera et al., 2019). Several terms associated with cell cycle and cell division are enriched in the ‘Diamar’/Rootpac® 40 combination (Fig. 4b). This reinforces the notion that growth is upregulated in individuals grafted onto Rootpac® 40 compared to those grafted onto Rootpac® 20.

In general, we observed an enrichment of terms linked to molecular functions and biological processes in vigor-inducing rootstocks that characterize a more active metabolism, likely due to a more active cell division. Since differences in gene expression are only detected when comparing combinations with vigorous rootstocks to the ‘Diamar’/Rootpac® 20 and not between them, it seems that we are looking at a

regulation of these processes that explained the low vigor conferred by Rootpac® 20 to the scion.

3.3. DEGs associated with promoting apical dominance were upregulated in ‘Diamar’/Rootpac® 20

Multiple genes likely associated with establishing apical dominance and inhibiting bud outgrowth were upregulated in ‘Diamar’ individuals grafted onto Rootpac® 20. Auxin is the main regulator of these processes, being synthesized in apical leaves and transported through the axis (Barbier et al., 2019). *NF-YA10* (Prudul26A005445), which negatively regulates lateral root density and is likely involved in the regulation of the auxin-signaling regulatory pathway (Sorin et al., 2014; Zhang et al., 2017), was highly expressed in the ‘Diamar’/Rootpac® 20 combination (Table 1). In *Arabidopsis*, *NF-YA10* is highly expressed in mature leaves in the expression atlas (Klepikova et al., 2016), while in grapevine (*Vitis vinifera*), its orthologue is over-expressed in woody stems and swelling buds (Fasoli et al., 2012). When grafted onto Rootpac® 20, scions present a phenotype with reduced branching and longer branches. Hence, *NF-YA10* expression in these shoot tips may be part of a regulation process in the formation of branches promoting cell growth or might be a marker of more mature tissues with reduced replication, but its involvement in the auxin pathway is uncertain. *CKX7* (Prudul26A024231) was upregulated in ‘Diamar’/Rootpac® 20 (Table 1). Cytokinin oxidase/dehydrogenase enzymes negatively regulate CKs by inactivating them (Köllmer et al., 2014). Silencing of family members in rice leads to increase branching (Yeh et al., 2015), thus its highly expression, when grafted onto Rootpac® 20, may be related to its reduced branching phenotype. Another regulator of CKs, *PAN* (Prudul26A007859), which is associated with shoot control (Maier et al., 2011), was also highly expressed in this combination (Table 1). Orthologues of *MYB93* (Prudul26A029785) and *DRMH3* (Prudul26A007496), which participate in regulating root formation but are also expressed in aerial tissues in *Arabidopsis* and grapevine (Fasoli et al., 2012; Gibbs

Table 1
Differentially expressed genes (DEGs) associated with apical dominance and shoot formation.

log ₂ FC Rootpac® 20 - 'Garnem'	log ₂ FC Rootpac® 20 - Rootpac® 40	<i>P. dulcis</i> ID	Gene	GO term	Biological process
1.269	0.679	Prudul26A001569	<i>ABCB15</i>	GO:0055085	transmembrane transport
1.255	1.350	Prudul26A024231	<i>CKX7</i>	GO:0009823	cytokinin catabolic process
2.126	2.071	Prudul26A031352	<i>CLAVATA3</i>	GO:0048507	meristem development
1.706	2.310	Prudul26A007496	<i>DRMH3</i>	GO:0009733	response to auxin
-4.372	-5.185	Prudul26A010631	<i>ESR2</i>	GO:0009733	response to auxin
-2.043	-2.023	Prudul26A017626	<i>GH3.6</i>	GO:0009733	response to auxin
-0.666	-2.747	Prudul26A022681	<i>GSO1</i>	GO:2000280	regulation of root development
-1.909	-2.059	Prudul26A032023	<i>IAA16</i>	GO:0009733	response to auxin
-2.915	-2.680	Prudul26A030184	<i>IAA4</i>	GO:0009733	response to auxin
-0.628	-1.204	Prudul26A031522	<i>LAX3</i>	GO:0060919	auxin influx
2.947	3.244	Prudul26A029785	<i>MYB93</i>	GO:1901332	negative regulation of lateral root development
3.565	3.185	Prudul26A005445	<i>NF-YA10</i>	GO:0006355	regulation of transcription, DNA-dependent
1.258	1.329	Prudul26A007859	<i>PAN</i>	GO:0006355	regulation of transcription, DNA-dependent
-2.262	-2.696	Prudul26A000568	<i>PAR2</i>	GO:0009641	shade avoidance
-1.442	-2.116	Prudul26A009595	<i>PIN6</i>	GO:0055085	transmembrane transport
-2.252	-2.287	Prudul26A005193	<i>RALFL34</i>	GO:0019722	calcium-mediated signaling
-1.645	-1.424	Prudul26A015967	<i>SPL9</i>	GO:0006355	regulation of transcription, DNA-dependent
-1.672	-2.060	Prudul26A024821	<i>SWEET17</i>	GO:0008643	carbohydrate transport

†log₂FC values in cursive represent genes that were not differentially expressed.

et al., 2014; An et al., 2020), were upregulated in ‘Diamar’/Rootpac® 20 (Table 1).

Auxin is synthesized in the apex, but to carry out its function it needs to be transported through specific carriers (Titapiwatanakun and Murphy, 2009; Cho and Cho, 2013; Adamowski and Friml, 2015). Two genes involved in promoting auxin transport, *VAB* (Prudul26A002767) and *ABCB15* (Prudul26A001569) (Kaneda et al., 2011; Naramoto and Kyo-zuka, 2018), and therefore apical dominance, were upregulated in the ‘Diamar’/Rootpac® 20 combination (Table 1).

Rootpac® 20 effect in ‘Diamar’ architecture is characterized by a reduced number of branches and high apical dominance. Here, we saw upregulation of genes related to auxin transport, which might promote apical dominance, and genes likely linked to the inactivation of CK, which promotes branch formation.

3.4. DEGs associated with shoot formation were downregulated in ‘Diamar’/Rootpac® 20

CKs act in opposition to auxins, favoring bud outgrowth and shoot formation (Dun et al., 2012). The *GH3* family is a large group of genes involved in auxin homeostasis, but also in the synthesis of other hormones, such as jasmonic acid (JA) and salicylic acid (SA) (Zhang et al., 2007; Fu et al., 2011). A member of this family, *GH3.6* (Prudul26A017626), was downregulated in the ‘Diamar’/Rootpac® 20 combination (Table 1). *GH3.6* has been described to be CK-dependent and to promote meristem development in roots, being also overexpressed in shoot apex in Arabidopsis (Pierdonati et al., 2019; Tian et al., 2019). Similar expression behaviors were observed for *ESR2* (Prudul26A010631), *RALFL34* (Prudul26A005193), and *GSO1* (Prudul26A022681) (Table 1). As for *GH3.6*, *RALFL34* and *GSO1* have been described participating in root development while being overexpressed in the shoot apex and inflorescences (Schmid et al., 2005; Racolta et al., 2014; Murphy et al., 2016). Therefore, its expression in the shoot apex could be linked to the presence of fewer branches in scions grafted onto Rootpac® 20. *ESR2* is a promoter of shoot formation and cell division in response to CKs (Ikeda et al., 2006).

Auxin carriers not only maintain the auxin flux to favor apical dominance but also can shape plant architecture by redistributing the auxin transport (Sauer et al., 2013). Two transporters expectedly engaged in this mechanism, like *PIN6* (Prudul26A009595) and *LAX3* (Prudul26A031522) (Revalska et al., 2015; Simon et al., 2016) were less expressed in the ‘Diamar’/Rootpac® 20 combination (Table 1), likely provoking an inhibition of branch formation in this combination. *PIN6* localization and expression are mediated through phosphorylation in the plasma membrane and the endoplasmic reticulum influencing auxin

homeostasis and stem elongation (Ditengou et al., 2017). Beside, overexpressing *PIN6* mutants display reduced apical dominance and improved root and shoot development (Cazzonelli et al., 2013).

Apart from its transport, auxin activity is controlled by numerous auxin response proteins, some of which are downregulated in the scions grafted onto Rootpac® 20 (Table 1). *AUX/IAA* proteins repress the expression of auxin response genes in absence of auxin. *IAA16* (Prudul26A032023) has been described as limiting auxin responses and its KO mutants show a reduction in the number of lateral roots in Arabidopsis (Rinaldi et al., 2012). Although its effect on bud outgrowth regulation is unclear, *IAA4* (Prudul26A030184) acts oppositely to auxin in *Populus* (Zhang et al., 2020). *SPL9* (Prudul26A015967) has been observed to act as regulating shoot branching in Arabidopsis, as both repressor and promoter (Jiao et al., 2010; Miura et al., 2010; Lu et al., 2013).

Sugars have been characterized to be a part of bud outgrowth positive regulation (Mason et al., 2014). *SWEET17* (Prudul26A024821) was less expressed in ‘Diamar’/Rootpac® 20 (Table 1). *SWEET17* putatively acts by mobilizing fructose and glucose content (Chardon et al., 2013; Guo et al., 2014). Light availability also affects branching control (Finlayson et al., 2010; Casal, 2012). In Arabidopsis, *PAR1* and *PAR2* play a negative role in the shade avoidance syndrome, acting downstream of *COP1* and being repressed by *phyA* (Bou-Torrent et al., 2008; Zhou et al., 2014). *PAR2* (Prudul26A000568) was downregulated in the ‘Diamar’/Rootpac® 20 combination (Table 1), matching with its repression by *phyA*, which arrests bud outgrowth (Finlayson et al., 2010; Raussenberger et al., 2011).

While auxin activity inhibits branch formation, other processes like CK activity, sugar content, or light perception may favor shoot formation. We observed a downregulation in ‘Diamar’/Rootpac® 20 of genes involved in auxin homeostasis, as with the rest of the mechanisms that promote branch formation.

3.5. DEGs involved in plant growth were affected by rootstock in ‘Diamar’ combinations

GA has been largely known as the growth hormone. Its synthesis and activity are related to active growth and high vigor (Hedden and Thomas, 2012; Binenbaum et al., 2018). Downregulation of genes involved in GA regulation was observed in dwarfing rootstocks in citrus (Liu et al., 2017b). We found various genes associated with GA regulation downregulated in the low vigor ‘Diamar’/Rootpac® 20 combination (Table 2). *YAB1* (Prudul26A023379) is a GA-responsive gene, which is part of regulatory feedback that controls GA levels, being overexpressed when GA levels are high and, thus, repressing its

Table 2
Differentially expressed genes (DEGs) associated with plant growth and vigor.

log ₂ FC Rootpac® 20 - 'Garnem'	log ₂ FC Rootpac® 20 - Rootpac® 40	<i>P. dulcis</i> ID	Gene	GO term	Biological process
-1.989	-2.382	Prudul26A020015	<i>ACL5</i>	GO:0006596	polyamine biosynthetic process
0.842	1.263	Prudul26A009326	<i>ARF16</i>	GO:0009733	response to auxin
-1.303	-1.591	Prudul26A012411	<i>CAX3</i>	GO:0006816	calcium ion transport
1.544	1.341	Prudul26A022494	<i>DAG1</i>	GO:0006355	regulation of transcription, DNA-dependent
-2.597	-2.970	Prudul26A027852	<i>ELP</i>	GO:0009664	plant-type cell wall organization
-0.578	-1.446	Prudul26A006427	<i>EXL5</i>	GO:0009741	response to brassinosteroid
4.663	5.974	Prudul26A026745	<i>EXLB1</i>	GO:0019953	sexual reproduction
-1.033	-1.860	Prudul26A015374	<i>EXT2</i>	GO:0009664	plant-type cell wall organization
-1.746	-2.019	Prudul26A005909	<i>FBL17</i>	GO:0051302	regulation of cell division
3.769	3.085	Prudul26A017080	<i>GA2OX8</i>	GO:0009686	gibberellin biosynthetic process
-3.055	-3.876	Prudul26A015013	<i>GASA1</i>	GO:0009739	response to gibberellin
-2.475	-2.565	Prudul26A023277	<i>GASA6</i>	GO:0009740	gibberellic acid mediated signaling pathway
-1.374	-1.960	Prudul26A011751	<i>GASA9</i>	GO:0009739	response to gibberellin
-3.275	-3.699	Prudul26A010439	<i>GAST1</i>	GO:0009739	response to gibberellin
-0.914	-1.776	Prudul26A009189	<i>NCED5</i>	GO:0009688	abscisic acid biosynthetic process
-0.626	-1.189	Prudul26A025913	<i>NPH3</i>	GO:0009638	phototropism
-1.258	-1.575	Prudul26A012618	<i>RPT2</i>	GO:0009638	phototropism
0.820	1.614	Prudul26A006348	<i>SAUR36</i>	GO:0009733	response to auxin
-0.991	-1.919	Prudul26A025556	<i>SAUR50</i>	GO:0009733	response to auxin
-2.035	-2.794	Prudul26A030325	<i>SAUR50</i>	GO:0009733	response to auxin
-2.986	-3.692	Prudul26A003964	<i>SAUR50</i>	GO:0009733	response to auxin
-1.303	-1.401	Prudul26A023379	<i>YAB1</i>	GO:1902183	regulation of shoot apical meristem development
-1.625	-1.960	Prudul26A020640	<i>YAB5</i>	GO:1902183	regulation of shoot apical meristem development

†log₂FC values in cursive represent genes that were not differentially expressed.

biosynthesis (Dai et al., 2007). Another member of the same family, YAB5 (Prudul26A020640), presented a similar expression profile. GASA6 (Prudul26A023277) is thought to be a positive regulator of GA-dependent processes, which affect growth positively. It is also up-regulated by numerous growth hormones (Qu et al., 2016). ACL5 (Prudul26A020015) is a crucial part of internode elongation and shoot growth, probably acting downstream of GA responses (Hanzawa et al., 1997). GASA1 (Prudul26A015013), GASA9 (Prudul26A011751), or GAST1 (Prudul26A010439) have been described as inhibiting GA response in Arabidopsis (Zhang and Wang, 2008). Therefore, they could be acting here in a feedback regulatory way, being less expressed in combinations with the dwarfing rootstock Rootpac® 20, and expectedly, with lower levels of GA (Table 2).

On the other hand, two genes expected to affect GA biosynthesis were more expressed in the 'Diamar'/Rootpac® 20 combination (Table 2). In Arabidopsis, GA2OX8 (Prudul26A017080) participates in the GA biosynthetic pathway deactivating bioactive GA, while DAG1 inhibits GA biosynthesis genes (Gabriele et al., 2010; Zhou et al., 2012; Liu et al., 2021). A homolog of this gene in citrus, GA2OX1, was also upregulated when grafted onto dwarfing rootstocks (Liu et al., 2017b). Therefore, the low vigor observed in combinations with Rootpac® 20 as rootstock compared to those with Rootpac® 40 or Garnem® may be in part due to reduced GA activity.

Genes related to other hormonal responses were downregulated when grafted onto the dwarfing Rootpac® 20 rootstock (Table 2). NCED5 (Prudul26A009189) participates in maintaining basal abscisic acid (ABA) levels in Arabidopsis, which are necessary to promote plant growth (Frey et al., 2012). CAX3 (Prudul26A005365) participates in Ca²⁺ transport and interacts with auxin response, promoting growth and development in Arabidopsis (Cheng et al., 2005; Cho et al., 2012). The EXORDIUM family is a group of genes that are involved in BR-mediated responses (Coll-Garcia et al., 2004; Schröder et al., 2011). A member of this family, EXL5 (Prudul26A006427), was less expressed in the 'Diamar'/Rootpac® 20 combination, which might indicate lower BR activity in scions grafted onto dwarfing rootstocks (Table 2).

Light response is an important regulator of plant growth (Molas and Kiss, 2009; Casal, 2012; Yadav et al., 2020). Several homologs to the auxin-induced gene SAUR50 (Prudul26A025556, Prudul26A030325, Prudul26A003964) were downregulated when grafted onto the dwarfing rootstock Rootpac® 20 (Table 2). SAUR50 promotes cell expansion and is positively regulated by light (Wang et al., 2020a). A similar

expression profile was presented by other genes associated with light responses (Table 2). NPH3 (Prudul26A025913) and RPT2 (Prudul26A012618), which in Arabidopsis act by linking phototropism and auxin response, modifying polar auxin transport, and promoting growth (Wan et al., 2012; Christie et al., 2018). Oppositely, SAUR36 (Prudul26A006348) and ARF16 (Prudul26A009326), which in Arabidopsis have been described to inhibit cell elongation in response to light or auxin (Hou et al., 2013; Dai et al., 2021), were found being upregulated in 'Diamar'/Rootpac® 20.

At the tissue level, cell proliferation and cell elongation define plant growth. Some effectors of cell proliferation were less expressed in the least vigorous 'Diamar'/Rootpac® 20 combination (Table 2). FBL17 (Prudul26A005909) is a crucial regulator of the cell cycle in Arabidopsis, targeting a negative regulator and hence, promoting cell division (Gusti et al., 2009). Loss-of-function mutants display reduced growth due to decreased cell proliferation, being necessary to keep meristem activity (Noir et al., 2015). ELP (Prudul26A027852) and EXT2 (Prudul26A015374) are homologs of EXT1, whose expression is correlated to tip growth in the roots of Tomato, maybe with a function also in shoot tips (Bucher et al., 2002).

Here, we observed a general downregulation of diverse processes promoting growth in the 'Diamar'/Rootpac® 20 combination. Specially, we have seen that GA regulation is affected by the rootstock.

3.6. DEGs associated with cell wall formation and reorganization were downregulated in combinations with dwarfing rootstock Rootpac® 20

The cell wall defines the ultimate shape of the plant cell, restricting its capacity to elongate or divide (Cosgrove, 2016). Hence, for plants to grow and develop, cells must carry out remodeling of the cell wall. There were multiple genes associated with cell wall reorganization that were downregulated in the 'Diamar'/Rootpac® 20 combination (Table 3). It is indeed plausible that these plants, being less vigorous and thus undergoing fewer cell division and cell elongation cycles, will present reduced cell wall remodeling processes. EXP1 (Prudul26A014459), EXP3 (Prudul26A015151), EXP8 (Prudul26A032368, Prudul26A002026), EXP15 (Prudul26A028987), and EXPB3 (Prudul26A000148) are all members of the expansin family, which acts mediating cell wall loosening, allowing then cell expansion (Cosgrove, 2015; Ramakrishna et al., 2019; Otulak-Kozielec et al., 2020). LRR-extensin proteins like LRX4 (Prudul26A018014) are part of the cell wall formation and deficiencies in

Table 3
Differentially expressed genes (DEGs) associated with cell wall formation and cell wall reorganization.

log ₂ FC Rootpac® 20 - 'Garnem'	log ₂ FC Rootpac® 20 - Rootpac® 40	<i>P. dulcis</i> ID	Gene	GO term	Biological process
−1.346	−2.772	Prudul26A016569	<i>4CLL9</i>	GO:0009809	lignin biosynthetic process
−1.795	−1.876	Prudul26A026119	<i>CSLB4</i>	GO:0030244	cellulose biosynthetic process
−1.147	−1.209	Prudul26A023496	<i>CSLC5</i>	GO:0071555	cell wall organization
−1.481	−1.777	Prudul26A005669	<i>CSLC5</i>	GO:0071555	cell wall organization
−2.225	−2.391	Prudul26A026490	<i>CSLC5</i>	GO:0071555	cell wall organization
−1.510	−1.954	Prudul26A019715	<i>CSLD3</i>	GO:0030244	cellulose biosynthetic process
−2.628	−3.405	Prudul26A014459	<i>EXP1</i>	GO:0009664	plant-type cell wall organization
−3.417	−4.109	Prudul26A028987	<i>EXP15</i>	GO:0009664	plant-type cell wall organization
−1.174	−1.528	Prudul26A015151	<i>EXP3</i>	GO:0009664	plant-type cell wall organization
−4.341	−5.406	Prudul26A032368	<i>EXP8</i>	GO:0009664	plant-type cell wall organization
−2.370	−2.994	Prudul26A002026	<i>EXP8</i>	GO:0009664	plant-type cell wall organization
−2.202	−2.566	Prudul26A000148	<i>EXPB3</i>	GO:0009828	plant-type cell wall loosening
−2.638	−3.705	Prudul26A015935	<i>FLA9</i>	GO:0009834	plant-type secondary cell wall biogenesis
−1.534	−1.898	Prudul26A000195	<i>GRF4</i>	GO:0006355	regulation of transcription, DNA-dependent
−1.963	−2.516	Prudul26A000315	<i>LAC11</i>	GO:0009809	lignin biosynthetic process
−3.545	−3.917	Prudul26A001608	<i>LAC11</i>	GO:0009809	lignin biosynthetic process
−1.718	−2.205	Prudul26A010009	<i>LAC17</i>	GO:0009809	lignin biosynthetic process
−1.858	−2.763	Prudul26A019505	<i>LAC17</i>	GO:0009809	lignin biosynthetic process
−0.818	−1.191	Prudul26A018014	<i>LRX4</i>	GO:0009664	plant-type cell wall organization
−1.248	−1.602	Prudul26A021520	<i>PME3</i>	GO:0042545	cell wall modification
−0.738	−1.412	Prudul26A004552	<i>PME34</i>	GO:0042545	cell wall modification
−1.406	−1.778	Prudul26A029274	<i>PME54</i>	GO:0042545	cell wall modification
−1.629	−2.260	Prudul26A018663	<i>PMR5</i>	GO:0042545	cell wall modification
−2.460	−2.728	Prudul26A012896	<i>TBL19</i>	GO:1990937	xylan acetylation
−2.204	−2.828	Prudul26A009187	<i>TBL19</i>	GO:1990937	xylan acetylation
−2.513	−1.785	Prudul26A011091	<i>TBL19</i>	GO:1990937	xylan acetylation
−2.599	−2.896	Prudul26A009872	<i>XTH5</i>	GO:0010411	xyloglucan metabolic process
−2.889	−2.911	Prudul26A002835	<i>XTH6</i>	GO:0010411	xyloglucan metabolic process
−2.358	−2.763	Prudul26A000404	<i>XTH8</i>	GO:0010411	xyloglucan metabolic process

†log₂FC values in cursive represent genes that were not differentially expressed.

this gene family lead to reduced plant growth (Draeger et al., 2015).

The plant cell wall is formed by numerous components, whose regulation affects cell wall formation and reorganization (Cosgrove, 2016; Meents et al., 2018; Voiniciuc et al., 2018). Various DEGs putatively related to the positive regulation of these processes displayed less expression when ‘Diamar’ was grafted onto Rootpac® 20 (Table 3). More vigor entails tissue growth and a more active cell wall metabolism and reorganization. Lignification is a crucial aspect of the secondary cell wall formation, with laccases like *LAC11* (Prudul26A000315, Prudul26A0016089, and *LAC17* (Prudul26A010009, Prudul26A019505) playing an important role in assuring proper cell structure, controlling lignin deposition (Ranocha et al., 2002; Berthet et al., 2011; Zhao et al., 2013; Liu et al., 2018a). *4CLL9* (Prudul26A016569) is a regulator of lignin biosynthesis in rice, both promoting and repressing it (Liu et al., 2017a). Cell wall hemicellulose is formed by several molecules, including xyloglucans, which in case of cell wall reorganization are hydrolyzed or remodeled (Park and Cosgrove, 2015). *XTH5* (Prudul26A009872), *XTH6* (Prudul26A002835), and *XTH8* (Prudul26A000404) are involved in loosening the cell wall, allowing cell elongation in Arabidopsis (Liu et al., 2007; Muñoz and Calderini, 2015; Takahashi et al., 2021). *CSLC5* (Prudul26A026490, Prudul26A005669) is part of the xyloglucan biosynthetic pathway, while *TBL19* (Prudul26A012896, Prudul26A009187, Prudul26A011091) controls xylan acetylation (Gao et al., 2017; Kim et al., 2020).

Cellulose and pectins are major cell wall components and their synthesis and organization are crucial aspects of cell wall formation (Meents et al., 2018; Saffer, 2018). FLA proteins, like *FLA9* (Prudul26A015935), are associated with wood formation, affecting secondary cell wall formation and structure (Wang et al., 2015; He et al., 2019). They participate in the organization of cell wall polysaccharides like cellulose and pectins, with mutants presenting reduced cellulose content (Liu et al., 2020). *CSLD3* (Prudul26A019715) plays a role in the cellulose biosynthetic pathway (Park et al., 2011; Yang et al., 2020). Although its specific role is yet to be characterized, *CSLB4* (Prudul26A026119) seems to be also required for cellulose biosynthesis (Youngs et al., 2007). *GRF4* (Prudul26A000195) is expected to

positively regulate cellulose biosynthesis and biomass accumulation, controlling *MYB61* transcription. A member of its family in citrus has been characterized as being more expressed in vigor-inducing rootstocks (Liu et al., 2017b; Gao et al., 2020). *PMR5* (Prudul26A018663) is a member of the TBL family, likely participating in pectin acetylation (Chiniquy et al., 2019). Pectin methylesterases like *PME3* (Prudul26A021520) and *PM34* (Prudul26A004552, Prudul26A029274) affect cell wall composition and cell expansion in Arabidopsis (Kohorn et al., 2014).

When grafted onto Rootpac® 20, ‘Diamar’ displayed a broad downregulation of mechanisms involved in cell wall formation and reorganization compared to vigor-conferring rootstock combinations. Lower expression in this combination may be associated with a less active metabolism, likely due to a less active cell division, which translates into a less cell wall modifications.

3.7. Nitrogen metabolism was less active in the ‘Diamar’/Rootpac® 20 combination

Nitrogen assimilation is vital for plant growth and development as it is an indispensable nutrient for the mechanisms involved in tree vigor (Krouk et al., 2011). The rootstock effect in nitrogen assimilation has been described in grapevine, where changes in nitrogen availability affect the expression profile of genes in dwarfing rootstocks (Cochetel et al., 2017). *NIR1* (Prudul26A012711) and *NIA1* (Prudul26A000078) perform two crucial successive steps in nitrate assimilation, converting NO into assimilable molecules for plant metabolism (Solomonson and Barber, 1990; Tanaka et al., 1994). Deficiencies in these genes lead to severely impaired growth in Arabidopsis (Costa-Broseta et al., 2020). In Arabidopsis, *TIP2;3* (Prudul26A020819) mediates NH₃ transport and is upregulated under conditions of high nitrogen availability (Loqué et al., 2005). These three genes were downregulated in the ‘Diamar’/Rootpac® 20 combination, evidencing that nitrogen metabolism is less active in scions grafted onto dwarfing rootstocks (Table 4). Various homologs to the *NRT1.1* (Prudul26A015004, Prudul26A008539, and Prudul26A010496) transporter were also less expressed when grafted

Table 4
Differentially expressed genes (DEGs) associated with nitrogen assimilation and flowering meristem development.

log ₂ FC Rootpac® 20 - 'Garnem'	log ₂ FC Rootpac® 20 - Rootpac® 40	<i>P. dulcis</i> ID	Gene	GO term	Biological process
-1.025	-1.616	Prudul26A005648	<i>CIB1</i>	GO:0009908	flower development
2.863	3.562	Prudul26A019427	<i>DAM5</i>	GO:0009910	negative regulation of flower development
2.081	2.128	Prudul26A006108	<i>FD</i>	GO:0009909	regulation of flower development
1.615	2.472	Prudul26A024273	<i>NF-YA3</i>	GO:0006355	regulation of transcription, DNA-dependent
-2.401	-1.542	Prudul26A000078	<i>NIA1</i>	GO:0042128	nitrate assimilation
-3.929	-2.589	Prudul26A012711	<i>NIR</i>	GO:0042128	nitrate assimilation
-1.422	-1.460	Prudul26A015004	<i>NRT1</i>	GO:0010167	response to nitrate
-2.414	-3.720	Prudul26A008539	<i>NTL1</i>	GO:0055085	transmembrane transport
-1.550	-1.799	Prudul26A010496	<i>NTL1</i>	GO:0055085	transmembrane transport
0.819	2.080	Prudul26A030680	<i>SIP1</i>	GO:0005975	carbohydrate metabolic process
-2.104	-2.610	Prudul26A021958	<i>TFL1</i>	GO:0009910	negative regulation of flower development
-5.937	-9.422	Prudul26A020819	<i>TIP2;3</i>	GO:0055085	transmembrane transport

†log₂FC values in cursive represent genes that were not differentially expressed.

onto Rootpac® 20 (Table 4). NRT1.1 carriers participate in the regulation of architecture processes like root branching, slowing down their development in response to auxin, which they seem able to transport (Krouk et al., 2010; Wang et al., 2020b). This function would not match the observed phenotype, since individuals grafted onto Rootpac® 40 or Garnem® displayed reduced apical dominance and numerous branches in comparison to those grafted onto Rootpac® 20, a different regulatory function in the nitrogen metabolism cannot be excluded for these homologs.

Nitrogen availability is crucial for tree growth and development. Here we detected a downregulation of genes involved in nitrogen assimilation and transport in the reduced vigor 'Diamar'/Rootpac® 20 combination.

3.8. Characterization of DEGs associated with meristem differentiation in 'Diamar' combinations

Flowering has been previously linked to the regulation of tree architecture, though the relation between them is not fully characterized (Seleznova et al., 2008; Foster et al., 2014). We observed mixed results, with genes promoting and repressing flowering being more expressed in both combinations with the dwarfing rootstock Rootpac® 20 and the vigor-inducing rootstocks Rootpac® 40 and Garnem®. Various flowering inductors were less expressed when grafted onto Rootpac® 20 (Table 4). *CIB1* (Prudul26A005648) activates *FT* transcription in Arabidopsis, thus regulating flowering positively (Liu et al., 2018b). Nevertheless, the flowering repressor *TFL1* (Prudul26A021958) was downregulated in 'Diamar'/Rootpac® 20 (Table 4). *TFL1* acts antagonistically to *FT*, repressing flowering and increasing vegetative growth (Moraes et al., 2019). The effect of *TFL1* in growth promotion may concur with the reduced vigor observed in the 'Diamar'/Rootpac® 20 combination compared with 'Diamar'/Rootpac® 40. A gene encoding a homolog of *DAM5* (Prudul26A019427) was upregulated in the 'Diamar'/Rootpac® 20 combination (Biswajit et al., 2011Table 4). *DAM5* and *DAM6* participate in flowering by negatively regulating bud dormancy release in *Prunus* (Biswajit et al., 2011). Several flowering inductors were also upregulated in the 'Diamar'/Rootpac® 20 combination (Table 4). *FD* (Prudul26A006108) performs a pivotal step in flowering development, being required for *FT* activity, which regulates directly forming a complex (Wigge et al., 2005; Collani et al., 2019). Upstream of this step, *NF-YA3* (Prudul26A024273) interacts with the flowering regulator *CO*, positively affecting floral organ development in Arabidopsis (Fornari et al., 2013; Su et al., 2018). Lastly, *SIP1* (Prudul26A030680) has been described in rice to promote early flowering in response to light signaling (Kim et al., 2013; Kang et al., 2015; Jiang et al., 2018).

Given these results, it is unclear how genes associated with flowering interact with the regulatory pathways involved in the tree architecture. Instead of an overall interaction between these two biological processes, individual genes may carry out specific functions that affect both

pathways.

4. Conclusions

Tree architecture is dependent on numerous processes such as light perception, gravity sensing, sugar availability, or nutrient supply that take part in the tree's physiological and hormonal regulation. Rootstock interaction with the scion may transform how cultivars respond to the same environmental cues. Previous studies had described how the rootstock effect can alter scion architecture traits like the number of branches or axis height in tree species, including Almond. After carrying out a transcriptome analysis in nine cultivar/rootstock combinations, we report our results and considerations of the biological processes that are affected by scion/rootstock interaction possibly affecting tree architecture. As a prospect, the evaluation of these hormones and metabolites concentrations would help to support our hypothesis based on phenotypic and molecular observations of the rootstock effect on tree architecture. Our results show that while the expression profile of cultivars with strong scion phenotypes is not significantly altered by the rootstock, that of cultivars whose phenotype is affected by the rootstock presents a strong modification. Regulation of genes associated with hormones involved in apical dominance and branch formation, like auxin and CKs, are in this case influenced by the rootstock. Moreover, mechanisms associated with vigor control, such as GA response or nitrogen assimilation, were shown to also be affected by the rootstock, being limited when grafted onto dwarfing rootstocks. Rootstock interaction can also modify the expression of genes involved in cell wall formation and reorganization, being less active in combinations with dwarfing rootstocks. In conclusion, described effects on scion architecture correlate with significant differences in the transcriptome of those combinations, affecting several hormonal responses and molecular mechanisms.

Funding sources

This research was financed by grants RTI- 2018-507 094210-R-100 and FPI-INIA CPD2016-0056 funded by MCIN/AEI/ 10.13039/ 501100011033.

CRedit authorship contribution statement

Álvaro Montesinos: Investigation, Writing – original draft, Methodology, Formal analysis, Writing – review & editing. María José Rubio-Cabetas: Investigation, Writing – original draft, Writing – review & editing. Jérôme Grimplet: Investigation, Writing – original draft, Formal analysis, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal

relationships which may be considered as potential competing interests:

This research was financed by grants RTI- 2018-507 094210-R-100 and FPI-INIA CPD2016-0056 funded by MCIN/AEI/ 10.13039/501100011033

Data availability

The datasets generated and/or analyzed during the current study are available in the European Nucleotide Archive and are accessible through the accession number PRJEB47633.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.scienta.2023.112628](https://doi.org/10.1016/j.scienta.2023.112628).

References

- Adamowski, M., Friml, J., 2015. PIN-dependent auxin transport: action, regulation, and evolution. *Plant Cell* 27, 20–32. <https://doi.org/10.1105/tpc.114.134874>. Online.
- Alabadí, D., Gallego-Bartolomé, J., Orlando, L., et al., 2008. Gibberellins modulate light signaling pathways to prevent Arabidopsis seedling de-etiolation in darkness. *Plant J.* 53, 324–335. <https://doi.org/10.1111/j.1365-313X.2007.03346.x>.
- Albacete, A., Martínez-Andújar, C., Martínez-Pérez, A., et al., 2015. Unravelling rootstock×scion interactions to improve food security. *J. Exp. Bot.* 66, 2211–2226. <https://doi.org/10.1093/jxb/erv027>.
- Alioto, T., Alexiou, K.G., Bardil, A., et al., 2020. Transposons played a major role in the diversification between the closely related almond and peach genomes: results from the almond genome sequence. *Plant J.* 101, 455–472. <https://doi.org/10.1111/tj.14538>.
- Aloni, B., Cohen, R., Karni, L., et al., 2010. Hormonal signaling in rootstock-scion interactions. *Sci. Hortic.* 127, 119–126. <https://doi.org/10.1016/j.scienta.2010.09.003> (Amsterdam).
- An, H., Zhang, J., Xu, F., et al., 2020. Transcriptomic profiling and discovery of key genes involved in adventitious root formation from green cuttings of highbush blueberry (*Vaccinium corymbosum* L.). *BMC Plant Biol.* 20, 1–14. <https://doi.org/10.1186/s12870-020-02398-0>.
- Balducci, F., Capriotti, L., Mazzoni, L., et al., 2019. The rootstock effects on vigor, production and fruit quality in sweet cherry (*Prunus avium* L.). *J. Berry Res.* 9, 249–265. <https://doi.org/10.3233/JBR-180345>.
- Barbier, F.F., Dun, E.A., Kerr, S.C., et al., 2019. An update on the signals controlling shoot branching. *Trends Plant Sci.* 24, 220–236. <https://doi.org/10.1016/j.tplants.2018.12.001>.
- Barbier, F.F., Lunn, J.E., Beveridge, C.A., 2015. Ready, steady, go! A sugar hit starts the race to shoot branching. *Curr. Opin. Plant Biol.* 25, 39–45. <https://doi.org/10.1016/j.cpb.2015.04.004>.
- Bennett, R., Liang, Y., Seale, M., et al., 2016. Strigolactone regulates shoot development through a core signalling pathway. *Biol. Open*, 021402. <https://doi.org/10.1242/bio.021402>.
- Berthet, S., Demont-Caulet, N., Pollet, B., et al., 2011. Disruption of LACCASE4 and 17 results in tissue-specific alterations to lignification of Arabidopsis thaliana stems. *Plant Cell* 23, 1124–1137. <https://doi.org/10.1105/tpc.110.082792>.
- Binenbaum, J., Weinstein, R., Shani, E., 2018. Gibberellin localization and transport in plants. *Trends Plant Sci.* 23, 410–421. <https://doi.org/10.1016/j.tplants.2018.02.005>.
- Biswajit, D., Ahmed, N., Pushkar, S., 2011. Prunus diversity-early and present development: a review. *Int. J. Biodivers. Conserv.* 3 (14), 721–734. <https://doi.org/10.5897/IJBCX11.003>.
- Blankenbush, D., Gordon, A., Von Kuster, G., et al., 2010. Manipulation of FASTQ data with galaxy. *Bioinformatics* 26, 1783–1785. <https://doi.org/10.1093/bioinformatics/btq281>.
- Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30, 2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>.
- Bou-Torrent, J., Roig-Villanova, I., Galstyan, A., Martínez-García, J.F., 2008. PAR1 and PAR2 integrate shade and hormone transcriptional networks. *Plant Signal. Behav.* 3, 453–454. <https://doi.org/10.4161/psb.3.7.5599>.
- Bucher, M., Brunner, S., Zimmermann, P., et al., 2002. The expression of an extensin-like protein correlates with cellular tip growth in tomato. *Plant Physiol.* 128, 911–923. <https://doi.org/10.1104/pp.010998>.
- Busov, V.B., Brunner, A.M., Straus, S.H., 2008. Genes for control of plant stature and form. *New Phytol.* 177, 589–607. <https://doi.org/10.1111/j.1469-8137.2007.02324.x>.
- Casal, J.J., 2012. Shade avoidance. *Arab. Book* 10, e0157. <https://doi.org/10.1199/tab.0157>.
- Cazzonelli, C.I., Vanstraelen, M., Simon, S., et al., 2013. Role of the arabidopsis PIN6 auxin transporter in auxin homeostasis and auxin-mediated development. *PLoS One* 8. <https://doi.org/10.1371/journal.pone.0070069>.
- Chang, S., Puryear, J., Cairney, J., 1993. A simple and efficient method for isolating RNA from pine trees. *Plant Mol. Biol. Rep.* 11, 113–116. <https://doi.org/10.1007/BF02670468>.
- Chardon, F., Bedu, M., Calenge, F., et al., 2013. Leaf fructose content is controlled by the vacuolar transporter SWEET17 in Arabidopsis. *Curr. Biol.* 23, 697–702. <https://doi.org/10.1016/j.cub.2013.03.021>.
- Cheng, N.H., Pittman, J.K., Shigaki, T., et al., 2005. Functional association of Arabidopsis CAX1 and CAX3 is required for normal growth and ion homeostasis. *Plant Physiol.* 138, 2048–2060. <https://doi.org/10.1104/pp.105.061218>.
- Chiniquy, D., Underwood, W., Corwin, J., et al., 2019. PMR5, an acetylation protein at the intersection of pectin biosynthesis and defense against fungal pathogens. *Plant J.* 100, 1022–1035. <https://doi.org/10.1111/tj.14497>.
- Cho, D., Villiers, F., Kroniewicz, L., et al., 2012. Vacuolar CAX1 and CAX3 influence auxin transport in guard cells via regulation of apoplastic pH. *Plant Physiol.* 160, 1293–1302. <https://doi.org/10.1104/pp.112.201442>.
- Cho, M., Cho, H.T., 2013. The function of ABCB transporters in auxin transport. *Plant. Signal. Behav.* 8, e22990. <https://doi.org/10.4161/psb.22990>.
- Christie, J.M., Suetsugu, N., Sullivan, S., Wada, M., 2018. Shining light on the function of nph3/rpt2-like proteins in phototropin signaling. *Plant Physiol.* 176, 1015–1024. <https://doi.org/10.1104/pp.17.00835>.
- Cochetel, N., Escudé, F., Cookson, S.J., et al., 2017. Root transcriptomic responses of grafted grapevines to heterogeneous nitrogen availability depend on rootstock genotype. *J. Exp. Bot.* 68, 4339–4355. <https://doi.org/10.1093/jxb/erx224>.
- Coenye, T., 2021. Do results obtained with RNA-sequencing require independent verification? *Biofilm* 3, 100043. <https://doi.org/10.1016/j.bioflm.2021.100043>.
- Coll-García, D., Mazuch, J., Altmann, T., Müssig, C., 2004. EXORDIUM regulates brassinosteroid-responsive genes. *FEBS Lett.* 563, 82–86. [https://doi.org/10.1016/S0014-5793\(04\)00255-8](https://doi.org/10.1016/S0014-5793(04)00255-8).
- Collani, S., Neumann, M., Yant, L., Schmid, M., 2019. FT modulates genome-wide DNA-binding of the bZIP transcription factor FD. *Plant Physiol.* 180, 367–380. <https://doi.org/10.1104/pp.18.01505>.
- Cosgrove, D.J., 2016. Plant cell wall extensibility: connecting plant cell growth with cell wall structure, mechanics, and the action of wall-modifying enzymes. *J. Exp. Bot.* 67, 463–476. <https://doi.org/10.1093/jxb/erv511>.
- Cosgrove, D.J., 2015. Plant expansins: diversity and interactions with plant cell walls. *Curr. Opin. Plant Biol.* 25, 162–172. <https://doi.org/10.1016/j.cpb.2015.05.014>.
- Costa-Broseta, A., Castillo, M., León, J., 2020. Nitrite reductase 1 is a target of nitric oxide-mediated post-translational modifications and controls nitrogen flux and growth in arabidopsis. *Int. J. Mol. Sci.* 21, 1–13. <https://doi.org/10.3390/ijms21197270>.
- Dai, M., Zhao, Y., Ma, Q., et al., 2007. The rice YABBY1 gene is involved in the feedback regulation of gibberellin metabolism. *Plant Physiol.* 144, 121–133. <https://doi.org/10.1104/pp.107.096586>.
- Dai, X., Lu, Q., Wang, J., et al., 2021. MiR160 and its target genes ARF10, ARF16 and ARF17 modulate hypocotyl elongation in a light, BRZ, or PAC-dependent manner in Arabidopsis: miR160 promotes hypocotyl elongation. *Plant Sci.* 303, 110686. <https://doi.org/10.1016/j.plantsci.2020.110686>.
- Depuydt, S., Hardtke, C.S., 2011. Hormone signalling crosstalk in plant growth regulation. *Curr. Biol.* 21, R365–R373. <https://doi.org/10.1016/j.cub.2011.03.013>.
- Dierck, R., De Keyser, E., De Riek, J., et al., 2016a. Change in auxin and cytokinin levels coincides with altered expression of branching genes during axillary bud outgrowth in chrysanthemum. *PLoS One* 11, 1–30. <https://doi.org/10.1371/journal.pone.0161732>.
- Dierck, R., Dhooche, E., Van Huylenbroeck, J., et al., 2016b. Response to strigolactone treatment in chrysanthemum axillary buds is influenced by auxin transport inhibition and sucrose availability. *Acta Physiol. Plant.* 38 <https://doi.org/10.1007/s11738-016-2292-6>.
- Ditengou, F.A., Gomes, D., Nziengui, H., et al., 2017. Characterization of auxin transporter PIN6 plasma membrane targeting reveals a function for PIN6 in plant bolting. *New Phytol.* 217 (4), 1610–1624. <https://doi.org/10.1111/nph.14923>.
- Draeger, C., Ndinyanka Fabrice, T., Gineau, E., et al., 2015. Arabidopsis leucine-rich repeat extensin (LRX) proteins modify cell wall composition and influence plant growth. *BMC Plant Biol.* 15, 1–11. <https://doi.org/10.1186/s12870-015-0548-8>.
- Dun, E.A., Saint, Germain A de, Rameau, C., Beveridge, C.A., 2012. Antagonistic action of strigolactone and cytokinin in bud outgrowth control. *Plant Physiol.* 158, 487–498. <https://doi.org/10.1104/pp.111.186783>.
- Everaert, C., Luypaert, M., Maag, J.L.V., et al., 2017. Benchmarking of RNA-sequencing analysis workflows using whole- transcriptome RT-qPCR expression data. *Sci. Rep.* 1–11. <https://doi.org/10.1038/s41598-017-01617-3>.
- Fasoli, M., Dal Santo, S., Zenoni, S., et al., 2012. The grapevine expression atlas reveals a deep transcriptome shift driving the entire plant into a maturation program. *Plant Cell* 24, 3489–3505. <https://doi.org/10.1105/tpc.112.100230>.
- Felipe, A.J., 2009. Felinem, “Garnem”, and “Monegro” almond x peach hybrid rootstocks. *HortScience* 44, 196–197. <https://doi.org/10.21273/HORTSCI.44.1.196>.
- Filo, J., Wu, A., Eliason, E., et al., 2015. Gibberellin driven growth in elf3 mutants requires PIF4 and PIF5. *Plant. Signal. Behav.* 10, 1–9. <https://doi.org/10.4161/15592324.2014.992707>.
- Finlayson, S.A., Krishnareddy, S.R., Kebrom, T.H., Casal, J.J., 2010. Phytochrome regulation of branching in arabidopsis. *Plant Physiol.* 152, 1914–1927. <https://doi.org/10.1104/pp.109.148833>.
- Font i Forcada, C., Reig, G., Mestre, L., Mignard, P., Betrán, J., Moreno, M.A., 2020. Scion x rootstock response on production, mineral composition and fruit quality under heavy-calcareous soil and hot climate. *Agronomy* 10, 1159. <https://doi.org/10.3390/agronomy10081159>.

- Fornari, M., Calvenzani, V., Masiero, S., et al., 2013. The Arabidopsis NF-YA3 and NF-YA8 genes are functionally redundant and are required in early embryogenesis. *PLoS One* 8. <https://doi.org/10.1371/journal.pone.0082043>.
- Foster, T.M., Celson, J.M., Chagne, D., et al., 2015. Two quantitative trait loci, Dw1 and Dw2, are primarily responsible for rootstock-induced dwarfing in apple. *Hortic. Res.* 2 (9) <https://doi.org/10.1038/hortres.2015.1>.
- Foster, T.M., Watson, A.E., van Hooijdonk, B.M., Schaffer, R.J., 2014. Key flowering genes including FT-like genes are upregulated in the vasculature of apple dwarfing rootstocks. *Tree Genet. Genomes* 10, 189–202. <https://doi.org/10.1007/s11295-013-0675-z>.
- Frey, A., Effroy, D., Lefebvre, V., et al., 2012. Epoxycarotenoid cleavage by NCED5 fine-tunes ABA accumulation and affects seed dormancy and drought tolerance with other NCED family members. *Plant J.* 70, 501–512. <https://doi.org/10.1111/j.1365-3113.2011.04887.x>.
- Fridman, Y., Savaldi-Goldstein, S., 2013. Brassinosteroids in growth control: how, when and where. *Plant Sci.* 209, 24–31. <https://doi.org/10.1016/j.plantsci.2013.04.002>.
- Fu, J., Yu, H., Li, X., et al., 2011. Rice GH3 gene family: regulators of growth and development. *Plant. Signal. Behav.* 6, 570–574. <https://doi.org/10.4161/psb.6.4.14947>.
- Gabriele, S., Rizza, A., Martone, J., et al., 2010. The Dof protein DAG1 mediates PIL5 activity on seed germination by negatively regulating GA biosynthetic gene AtGA3ox1. *Plant J.* 61, 312–323. <https://doi.org/10.1111/j.1365-3113.2009.04055.x>.
- Gallego-Bartolomé, J., Alabadi, D., Blázquez, M.A., 2011. DELLA-induced early transcriptional changes during etiolated development in Arabidopsis thaliana. *PLoS One* 6. <https://doi.org/10.1371/journal.pone.0023918>.
- Gao, Y., He, C., Zhang, D., et al., 2017. Two trichome birefringence-like proteins mediate xylan acetylation, which is essential for leaf blight resistance in rice. *Plant Physiol.* 173, 470–481. <https://doi.org/10.1104/pp.16.01618>.
- Gao, Y., Xu, Z., Zhang, L., et al., 2020. MYB61 is regulated by GRF4 and promotes nitrogen utilization and biomass production in rice. *Nat. Commun.* 11, 1–12. <https://doi.org/10.1038/s41467-020-19019-x>.
- Gibbs, D.J., Voß, U., Harding, S.A., et al., 2014. AtMYB93 is a novel negative regulator of lateral root development in Arabidopsis. *New Phytol.* 203, 1194–1207. <https://doi.org/10.1111/nph.12879>.
- Griffiths, J., Murase, K., Rieu, I., et al., 2006. Genetic characterization and functional analysis of the GID1 gibberellin receptors in Arabidopsis. *Plant Cell* 18, 3399–3414. <https://doi.org/10.1105/tpc.106.047415>.
- Guo, W.J., Nagy, R., Chen, H.Y., et al., 2014. SWEET17, a facilitative transporter, mediates fructose transport across the tonoplast of Arabidopsis roots and leaves. *Plant Physiol.* 164, 777–789. <https://doi.org/10.1104/pp.113.232751>.
- Gusti, A., Baumberg, N., Nowack, M., et al., 2009. The Arabidopsis thaliana F-box protein FBL17 is essential for progression through the second mitosis during pollen development. *PLoS One* 4. <https://doi.org/10.1371/journal.pone.0004780>.
- Hanzawa, Y., Takahashi, T., Komeda, Y., 1997. ACL5: an Arabidopsis gene required for internodal elongation after flowering. *Plant J.* 12, 863–874. <https://doi.org/10.1046/j.1365-3113.1997.12040863.x>.
- He, J., Zhao, H., Cheng, Z., et al., 2019. Evolution analysis of the fasciclin-like arabinogalactan proteins in plants shows variable fasciclin-AGP domain constitutions. *Int. J. Mol. Sci.* 20. <https://doi.org/10.3390/ijms20081945>.
- Hedden, P., Thomas, S.G., 2012. Gibberellin biosynthesis and its regulation. *Biochem. J.* 444, 11–25. <https://doi.org/10.1042/BJ20120245>.
- Holalu, S.V., Finlayson, S.A., 2017. The ratio of red light to far red light alters Arabidopsis axillary bud growth and abscisic acid signalling before stem auxin changes. *J. Exp. Bot.* 68, 943–952. <https://doi.org/10.1093/jxb/erw479>.
- Hollender, C.A., Dardick, C., 2015. Molecular basis of angiosperm tree architecture. *New Phytol.* 541–556. <https://doi.org/10.1111/nph.13204>.
- Hollender, C.A., Hadiarto, T., Srinivasan, C., et al., 2016. A brachytic dwarfism trait (dw) in peach trees is caused by a nonsense mutation within the gibberellin acid receptor PpGID1c. *New Phytol.* 210, 227–239. <https://doi.org/10.1111/nph.13772>.
- Hou, K., Wu, W., Gan, S.S., 2013. SAUR36, a SMALL AUXIN UP RNA gene, is involved in the promotion of leaf senescence in Arabidopsis. *Plant Physiol.* 161, 1002–1009. <https://doi.org/10.1104/pp.112.212787>.
- Huang, X., Ding, J., Effgen, S., et al., 2013. Multiple loci and genetic interactions involving flowering time genes regulate stem branching among natural variants of Arabidopsis. *New Phytol.* 199, 843–857. <https://doi.org/10.1111/nph.12306>.
- Ikeda, Y., Banno, H., Niu, Q.W., et al., 2006. The ENHANCER OF SHOOT REGENERATION 2 gene in Arabidopsis regulates CUP-SHAPED COTYLEDON 1 at the transcriptional level and controls cotyledon development. *Plant Cell Physiol.* 47, 1443–1456. <https://doi.org/10.1093/pcp/pcl023>.
- Jiang, P., Wang, S., Zheng, H., et al., 2018. SIP1 participates in regulation of flowering time in rice by recruiting OsTrx1 to Ehd1. *New Phytol.* 219, 422–435. <https://doi.org/10.1111/nph.15122>.
- Jiao, Y., Wang, Y., Xue, D., et al., 2010. Regulation of OsSPL14 by OsmiR156 defines ideal plant architecture in rice. *Nat. Genet.* <https://doi.org/10.1038/ng.591>.
- Kaneda, M., Schuetz, M., Lin, B.S.P., et al., 2011. ABC transporters coordinately expressed during lignification of Arabidopsis stems include a set of ABCBs associated with auxin transport. *J. Exp. Bot.* 62, 2063–2077. <https://doi.org/10.1093/jxb/erq416>.
- Kang, M.J., Jin, H.S., Noh, Y.S., Noh, B., 2015. Repression of flowering under a noninductive photoperiod by the HDA9-AGL19-FT module in Arabidopsis. *New Phytol.* 206, 281–294. <https://doi.org/10.1111/nph.13161>.
- Kim, D., Langmead, B., Salzberg, S.L., 2015. HISAT : a fast spliced aligner with low memory requirements. *Nat. Methods.* <https://doi.org/10.1038/nmeth.3317>.
- Kim, S.J., Chandrasekar, B., Rea, A.C., et al., 2020. The synthesis of xyloglucan, an abundant plant cell wall polysaccharide, requires CSLC function. *Proc. Natl. Acad. Sci. U. S. A.* 117, 20316–20324. <https://doi.org/10.1073/PNAS.2007245117>.
- Kim, W., Latrasse, D., Servet, C., Zhou, D.X., 2013. Arabidopsis histone deacetylase HDA9 regulates flowering time through repression of AGL19. *Biochem. Biophys. Res. Commun.* 432, 394–398. <https://doi.org/10.1016/j.bbrc.2012.11.102>.
- Klepikova, A.V., Kasianov, A.S., Gerasimov, E.S., et al., 2016. A high resolution map of the Arabidopsis thaliana developmental transcriptome based on RNA-seq profiling. *Plant J.* 88, 1058–1070. <https://doi.org/10.1111/tjp.13312>.
- Kohorn, B.D., Kohorn, S.L., Saba, N.J., Martinez, V.M., 2014. Requirement for pectin methyl esterase and preference for fragmented over native pectins for wall-associated kinase-activated, EDS1/PAD4-dependent stress response in Arabidopsis. *J. Biol. Chem.* 289, 18978–18986. <https://doi.org/10.1074/jbc.M114.567545>.
- Köllmer, I., Novák, O., Strnad, M., et al., 2014. Overexpression of the cytosolic cytokinin oxidase/dehydrogenase (CKX7) from Arabidopsis causes specific changes in root growth and xylem differentiation. *Plant J.* 78, 359–371. <https://doi.org/10.1111/tjp.12477>.
- Krouk, G., Lacombe, B., Bielach, A., et al., 2010. Nitrate-regulated auxin transport by NRT1.1 defines a mechanism for nutrient sensing in plants. *Dev. Cell* 18, 927–937. <https://doi.org/10.1016/j.devcel.2010.05.008>.
- Krouk, G., Ruffel, S., Gutiérrez, R.A., et al., 2011. A framework integrating plant growth with hormones and nutrients. *Trends Plant Sci.* 16, 178–182. <https://doi.org/10.1016/j.tplants.2011.02.004>.
- Langfelder, P., Horvath, S., 2008. WGCNA: an R package for weighted correlation network analysis. *BMC Bioinform.* 9. <https://doi.org/10.1186/1471-2105-9-559>.
- Liao, Y., Smyth, G.K., Shi, W., 2014. FeatureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* 30, 923–930. <https://doi.org/10.1093/bioinformatics/btt656>.
- Liu, B., Zhao, S., Li, P., et al., 2021. Plant buffering against the high-light stress-induced accumulation of CsGA2ox8 transcripts via alternative splicing to finely tune gibberellin levels and maintain hypocotyl elongation. *Hortic. Res.* 8. <https://doi.org/10.1038/s41438-020-00430-w>.
- Liu, E., MacMillan, C.P., Shafee, T., et al., 2020. Fasciclin-like arabinogalactan-protein 16 (FLA16) is required for stem development in Arabidopsis. *Front. Plant Sci.* 11, 1–16. <https://doi.org/10.3389/fpls.2020.615392>.
- Liu, H., Guo, Z., Gu, F., et al., 2017a. 4-Coumarate-CoA ligase-like gene OsAAE3 negatively mediates the rice blast resistance, floret development and lignin biosynthesis. *Front. Plant Sci.* 7, 1–13. <https://doi.org/10.3389/fpls.2016.02041>.
- Liu, Q., Luo, L., Zheng, L., 2018a. Lignins: biosynthesis and biological functions in plants. *Int. J. Mol. Sci.* 19. <https://doi.org/10.3390/ijms19020335>.
- Liu, X.Y., Li, J., Liu, M.M., et al., 2017b. Transcriptome profiling to understand the effect of citrus rootstocks on the growth of “Shatangju” mandarin. *PLoS One* 12, 1–22. <https://doi.org/10.1371/journal.pone.0169897>.
- Liu, Y., Li, X., Ma, D., et al., 2018b. CIB 1 and CO interact to mediate CRY 2-dependent regulation of flowering. *EMBO Rep.* 19, 1–10. <https://doi.org/10.15252/embr.201845762>.
- Liu, Y.B., Lu, S.M., Zhang, J.F., et al., 2007. A xyloglucan endotransglucosylase/hydrolase involves in growth of primary root and alters the deposition of cellulose in Arabidopsis. *Planta* 226, 1547–1560. <https://doi.org/10.1007/s00425-007-0591-2>.
- Lo, S.F., Yang, S.Y., Chen, K.T., et al., 2008. A novel class of gibberellin 2-oxidases control semidwarfism, tillering, and root development in rice. *Plant Cell* 20, 2603–2618. <https://doi.org/10.1105/tpc.108.060913>.
- Loqué, D., Ludewig, U., Yuan, L., Von Wirén, N., 2005. Tonoplast intrinsic proteins ATIP2:1 and ATIP2:3 facilitate NH₃ transport into the vacuole. *Plant Physiol.* 137, 671–680. <https://doi.org/10.1104/pp.104.051268>.
- Lordan, J., Zazurka, L., Maldonado, M., et al., 2019. Horticultural performance of ‘Marinada’ and ‘Vairo’ almond cultivars grown on a genetically diverse set of rootstocks. *Sci. Hortic.* 256, 108558. <https://doi.org/10.1016/j.scienta.2019.108558> (Amsterdam).
- Lu, Z., Yu, H., Xiong, G., et al., 2013. Genome-wide binding analysis of the transcription activator IDEAL PLANT ARCHITECTURE1 reveals a complex network regulating rice plant architecture. *Plant Cell* 25, 3743–3759. <https://doi.org/10.1105/tpc.113.113639>.
- Ma, Y., Xue, H., Zhang, L., et al., 2016. Involvement of auxin and brassinosteroid in dwarfism of autotetraploid apple (Malus × domestica). *Sci. Rep.* 6, 1–14. <https://doi.org/10.1038/srep26719>.
- Maier, A.T., Stehling-Sun, S., Offenburger, S.L., Lohmann, J.U., 2011. The bZIP transcription factor PERIANTHIA: a multifunctional hub for meristem control. *Front. Plant Sci.* 2, 1–17. <https://doi.org/10.3389/fpls.2011.00079>.
- Martínez-Ballesta, M.C., Alcaraz-López, C., Muries, B., et al., 2010. Physiological aspects of rootstock-scion interactions. *Sci. Hortic.* 127, 112–118. <https://doi.org/10.1016/j.scienta.2010.08.002> (Amsterdam).
- Mason, M.G., Ross, J.J., Babst, B.A., et al., 2014. Sugar demand, not auxin, is the initial regulator of apical dominance. *Proc. Natl. Acad. Sci. U. S. A.* 111, 6092–6097. <https://doi.org/10.1073/pnas.1322045111>.
- Meents, M.J., Watanabe, Y., Samuels, A.L., 2018. The cell biology of secondary cell wall biosynthesis. *Ann. Bot.* 121, 1107–1125. <https://doi.org/10.1093/aob/mcy005>.
- Meisel, L., Fonseca, B., González, S., et al., 2005. A rapid and efficient method for purifying high quality total RNA from peaches (Prunus persica) for functional genomics analyses. *Biol. Res.* 38, 83–88. <https://doi.org/10.4067/S0716-9762005000100010>.
- Miura, K., Ikeda, M., Matsubara, A., et al., 2010. OsSPL14 promotes panicle branching and higher grain productivity in rice. *Nat. Genet.* 42, 545–549. <https://doi.org/10.1038/ng.592>.
- Molas, M.L., Kiss, J.Z., 2009. Phototropism and gravitropism in plants. *Adv. Bot. Res.* 49, 1–34. [https://doi.org/10.1016/S0065-2296\(08\)00601-0](https://doi.org/10.1016/S0065-2296(08)00601-0).

- Montesinos, Á., Thorp, G., Grimplet, J., Rubio-Cabetas, M., 2021. Phenotyping almond orchards for architectural traits influenced by rootstock choice. *Horticulturae* 7, 159. <https://doi.org/10.3390/horticulturae7070159>.
- Moraes, T.S., Dornelas, M.C., Martinelli, A.P., 2019. FT/TFL1: calibrating plant architecture. *Front. Plant Sci.* 10 <https://doi.org/10.3389/fpls.2019.00097>.
- Muñoz, M., Calderini, D.F., 2015. Volume, water content, epidermal cell area, and XTH5 expression in growing grains of wheat across ploidy levels. *Field Crop Res.* 173, 30–40. <https://doi.org/10.1016/j.fcr.2014.12.010>.
- Murphy, E., Vu, L.D., Van Den Broeck, L., et al., 2016. RALFL34 regulates formative cell divisions in Arabidopsis pericycle during lateral root initiation. *J. Exp. Bot.* 67, 4863–4875. <https://doi.org/10.1093/jxb/erw281>.
- Naramoto, S., Kyoizuka, J., 2018. ARF GTPase machinery at the plasma membrane regulates auxin transport-mediated plant growth. *Plant Biotechnol.* 35, 155–159. <https://doi.org/10.5511/plantbiotechnology.18.0312a>.
- Narandžić, T., Ljubojević, M., 2022. Breeding size-controlling cherry rootstocks for changing environmental conditions. *Hortic. Environ. Biotechnol.* 63, 719–733. <https://doi.org/10.1007/s13580-022-00432-8>.
- Noir, S., Marrocco, K., Masoud, K., et al., 2015. The control of arabidopsis thaliana growth by cell proliferation and endoreplication requires the F-box protein FBL17. *Plant Cell* 27, 1461–1476. <https://doi.org/10.1105/tpc.114.135301>.
- Ogawa, M., Hanada, A., Yamauchi, Y., et al., 2003. Gibberellin biosynthesis and response during Arabidopsis seed germination. *Plant Cell* 15, 1591–1604. <https://doi.org/10.1105/tpc.011650>.
- Otulak-Kozielec, K., Kozielec, E., Lockhart, B.E.L., Bujarski, J.J., 2020. The expression of potato Expansin A3 (StEXPA3) and Extensin4 (StEXT4) genes with distribution of StEXPA3 and HRGPs-extensin changes as an effect of cell wall rebuilding in two types of PVYNTN-Solanum tuberosum interactions. *Viruses* 12 (1), 66. <https://doi.org/10.3390/v12010066>.
- Park, J., Lee, Y., Martinoia, E., Geisler, M., 2017. Plant hormone transporters: what we know and what we would like to know. *BMC Biol.* 15, 1–15. <https://doi.org/10.1186/s12915-017-0443-x>.
- Park, S., Szumlanski, A.L., Gu, F., et al., 2011. A role for CSLD3 during cell-wall synthesis in apical plasma membranes of tip-growing root-hair cells. *Nat. Cell Biol.* 13, 973–980. <https://doi.org/10.1038/ncb2294>.
- Park, Y.B., Cosgrove, D.J., 2015. Xyloglucan and its interactions with other components of the growing cell wall. *Plant Cell Physiol.* 56, 180–194. <https://doi.org/10.1093/pcp/pcu204>.
- Pierdonati, E., Unterholzner, S.J., Salvi, E., et al., 2019. Cytokinin-dependent control of GH3 group II family genes in the arabidopsis root. *Plants* 8, 1–9. <https://doi.org/10.3390/plants8040094>.
- Pin, P.A., Nilsson, O., 2012. The multifaceted roles of FLOWERING LOCUS T in plant development. *Plant Cell Environ.* 35, 1742–1755. <https://doi.org/10.1111/j.1365-3040.2012.02558.x>.
- Qu, J., Kang, S.G., Hah, C., Jang, J.C., 2016. Molecular and cellular characterization of GA-stimulated transcripts GAS4A and GAS4B in Arabidopsis thaliana. *Plant Sci.* 246, 1–10. <https://doi.org/10.1016/j.plantsci.2016.01.009>.
- Racolta, A., Bryan, A.C., Tax, F.E., 2014. The receptor-like kinases GSO1 and GSO2 together regulate root growth in arabidopsis through control of cell division and cell fate specification. *Dev. Dyn.* 243, 257–278. <https://doi.org/10.1002/dvdy.24066>.
- Ramakrishna, P., Duarte, P.R., Rance, G.A., et al., 2019. EXPANSIN A1-mediated radial swelling of pericycle cells positions anticlinal cell divisions during lateral root initiation. *Proc. Natl. Acad. Sci. U. S. A.* 116, 8597–8602. <https://doi.org/10.1073/pnas.1820882116>.
- Ranocha, P., Chabannes, M., Chamayou, S., et al., 2002. Laccase down-regulation causes alterations in phenolic metabolism and cell wall structure in poplar. *Plant Physiol.* 129, 145–155. <https://doi.org/10.1104/pp.010988>.
- Rausenberger, J., Tscheuschler, A., Nordmeier, W., et al., 2011. Photoconversion and nuclear trafficking cycles determine phytochrome A's response profile to far-red light. *Cell* 146, 813–825. <https://doi.org/10.1016/j.cell.2011.07.023>.
- Reddy, S.K., Finlayson, S.A., 2014. Phytochrome B promotes branching in Arabidopsis by suppressing auxin signaling. *Plant Physiol.* 164, 1542–1550. <https://doi.org/10.1104/pp.113.234021>.
- Reig, G., Iglesias, I., Zazurca, L., Torguet, L., Martinez, G., Miarnau, X., 2022. Physiological and agronomical responses of 'Vairo' almond and 'Big Top' nectarine cultivars grafted onto different Prunus rootstocks and grown under semiarid Mediterranean conditions. *Agronomy* 12, 821. <https://doi.org/10.3390/agronomy12040821>.
- Revalsa, M., Vassileva, V., Zechirov, G., Iantcheva, A., 2015. Is the auxin influx carrier LAX3 essential for plant growth and development in the model plants *Medicago truncatula*, *Lotus japonicus* and *Arabidopsis thaliana*? *Biotechnol. Biotechnol. Equip.* 29, 786–797. <https://doi.org/10.1080/13102818.2015.1031698>.
- Rinaldi, M.A., Liu, J., Enders, T.A., et al., 2012. A gain-of-function mutation in IAA16 confers reduced responses to auxin and abscisic acid and impedes plant growth and fertility. *Plant Mol. Biol.* 79, 359–373. <https://doi.org/10.1007/s11103-012-9917-y>.
- Robinson, M.D., McCarthy, D.J., Smyth, G.K., 2009. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 26, 139–140. <https://doi.org/10.1093/bioinformatics/btp616>.
- Rubio-Cabetas, M.J., Felipe, A.J., Reighard, G.L., 2017. Socias. In: i Company, R, Gradziel, T.M. (Eds.), Rootstock development. CABI, Wallingford, UK, pp. 209–227. <https://doi.org/10.1079/9781780643540.0209>.
- Sablowski, R., 2016. Coordination of plant cell growth and division: collective control or mutual agreement? *Curr. Opin. Plant Biol.* 34, 54–60. <https://doi.org/10.1016/j.pbi.2016.09.004>.
- Saffer, A.M., 2018. Expanding roles for pectins in plant development. *J. Integr. Plant Biol.* 60, 910–923. <https://doi.org/10.1111/jipb.12662>.
- Salzman, R.A., Fujita, T., Zhu-Salzman, K., et al., 1999. An improved RNA isolation method for plant tissues containing high levels of phenolic compounds or carbohydrates. *Plant Mol. Biol. Report.* 17, 11–17. <https://doi.org/10.1023/A:1007520314478>.
- Sauer, M., Robert, S., Kleine-Vehn, J., 2013. Auxin: simply complicated. *J. Exp. Bot.* 64, 2565–2577. <https://doi.org/10.1093/jxb/ert139>.
- Scalisi, A., Lo Bianco, R., Caruso, T., et al., 2018. Preliminary evaluation of six Prunus rootstocks for peach in Italy. *Acta Hort.* 1228, 273–278. <https://doi.org/10.17660/ActaHortic.2018.1228.41>.
- Schmid, M., Davison, T.S., Henz, S.R., et al., 2005. A gene expression map of Arabidopsis thaliana development. *Nat. Genet.* 37, 501–506. <https://doi.org/10.1038/ng1543>.
- Schröder, F., Liss, J., Müssig, C., 2011. Exordium-like promotes growth during low carbon availability in arabidopsis. *Plant Physiol.* 156, 1620–1630. <https://doi.org/10.1104/pp.111.177204>.
- Seleznyova, A.N., Tustin, D.S., Thorp, T.G., 2008. Apple dwarfing rootstocks and interstocks affect the type of growth units produced during the annual growth cycle: precocious transition to flowering affects the composition and vigour of annual shoots. *Ann. Bot.* 101, 679–687. <https://doi.org/10.1093/aob/mcn007>.
- Shinohara, N., Taylor, C., Leyser, O., 2013. Strigolactone can promote or inhibit shoot branching by triggering rapid depletion of the auxin efflux protein PIN1 from the plasma membrane. *PLoS Biol.* 11 <https://doi.org/10.1371/journal.pbio.1001474>.
- Simon, S., Sküpa, P., Viane, T., et al., 2016. PIN6 auxin transporter at endoplasmic reticulum and plasma membrane mediates auxin homeostasis and organogenesis in Arabidopsis. *New Phytol.* 211, 65–74. <https://doi.org/10.1111/nph.14019>.
- Solomonson, L.P., Barber, M.J., 1990. Assimilatory nitrate reductase: functional properties and regulation. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 41, 225–253. <https://doi.org/10.1146/annurev.pp.41.060190.001301>.
- Sorin, C., Declercq, M., Christ, A., et al., 2014. A miR169 isoform regulates specific NF-YA targets and root architecture in Arabidopsis. *New Phytol.* 202, 1197–1211. <https://doi.org/10.1111/nph.12735>.
- Srikanth, A., Schmid, M., 2011. Regulation of flowering time: all roads lead to Rome. *Cell. Mol. Life Sci.* 68, 2013–2037. <https://doi.org/10.1007/s00018-011-0673-y>.
- Stokes, M.E., Chattopadhyay, A., Wilkins, O., et al., 2013. Interplay between sucrose and folate modulates auxin signaling in arabidopsis. *Plant Physiol.* 162, 1552–1565. <https://doi.org/10.1104/pp.113.215095>.
- Su, H., Cao, Y., Ku, L., et al., 2018. Dual functions of ZmNF-YA3 in photoperiod-dependent flowering and abiotic stress responses in maize. *J. Exp. Bot.* 69, 5177–5189. <https://doi.org/10.1093/jxb/ery299>.
- Sun, T., 2010. Gibberellin-GID1-DELLA: a pivotal regulatory module for plant growth and development. *Plant Physiol.* 154, 567–570. <https://doi.org/10.1104/pp.110.161554>.
- Takahashi, D., Johnson, K.L., Hao, P., et al., 2021. Cell wall modification by the xyloglucan endotransglucosylase/hydrolase XTH19 influences freezing tolerance after cold and sub-zero acclimation. *Plant Cell Environ.* 44, 915–930. <https://doi.org/10.1111/pce.13953>.
- Tanaka, S., Ida, S., Irfune, K., Oeda, K., Morikawa, H., 1994. Nucleotide sequence of a gene for nitrite reductase from arabidopsis thaliana. *Mitochondrial DNA* 5, 57–61. <https://doi.org/10.3109/10425179409039705>.
- Tian, C., Wang, Y., Yu, H., et al., 2019. A gene expression map of shoot domains reveals regulatory mechanisms. *Nat. Commun.* 10, 1–12. <https://doi.org/10.1038/s41467-018-08083-z>.
- Titapiwatanakun, B., Murphy, A.S., 2009. Post-transcriptional regulation of auxin transport proteins: cellular trafficking, protein phosphorylation, protein maturation, ubiquitination, and membrane composition. *J. Exp. Bot.* 60, 1093–1107. <https://doi.org/10.1093/jxb/ern240>.
- Tworokski, T., Fazio, G., 2015. Effects of size-controlling apple rootstocks on growth, abscisic acid, and hydraulic conductivity of scion of different vigour. *Int. J. Fruit Sci.* 15, 369–381. <https://doi.org/10.1080/15538362.2015.1009973>.
- Tworokski, T., Miller, S., 2007. Rootstock effect on growth of apple scions with different growth habits. *Sci. Hortic.* 111, 335–343. <https://doi.org/10.1016/j.scienta.2006.10.034> (Amsterdam).
- Vaahter, L., Schulz, J., Hamann, T., 2019. Cell wall integrity maintenance during plant development and interaction with the environment. *Nat. Plants* 5, 924–932. <https://doi.org/10.1038/s41477-019-0502-0>.
- Van Hooijdonk, B.M., Woolley, D.J., Warrington, I.J., Tustin, D.S., 2010. Initial alteration of scion architecture by dwarfing apple rootstocks may involve shoot-root-shoot signalling by auxin, gibberellin, and cytokinin. *J. Hortic. Sci. Biotechnol.* 85, 59–65. <https://doi.org/10.1080/14620316.2010.11512631>.
- Voiniciuc, C., Pauly, M., Usadel, B., 2018. Monitoring polysaccharide dynamics in the plant cell wall. *Plant Physiol.* 176, 2590–2600. <https://doi.org/10.1104/pp.17.01776>.
- Waldie, T., Leyser, O., 2018. Cytokinin targets auxin transport to promote shoot branching. *Plant Physiol.* 177, 803–818. <https://doi.org/10.1104/pp.17.01691>.
- Wan, Y., Jasik, J., Wang, L., et al., 2012. The signal transducer NPH3 integrates the phototropin1 photosensor with PIN2-based polar auxin transport in Arabidopsis root phototropism. *Plant Cell* 24, 551–565. <https://doi.org/10.1105/tpc.111.094284>.
- Wang, B., Smith, S.M., Li, J., 2018a. Genetic regulation of shoot architecture. *Annu. Rev. Plant Biol.* 69, 437–468. <https://doi.org/10.1146/annurev-arplant-042817-040422>.
- Wang, H., Jiang, C., Wang, C., et al., 2015. Antisense expression of the fasciclin-like arabinogalactan protein FLA6 gene in Populus inhibits expression of its homologous genes and alters stem biomechanics and cell wall composition in transgenic trees. *J. Exp. Bot.* 66, 1291–1302. <https://doi.org/10.1093/jxb/eru479>.
- Wang, J., Sun, N., Zhang, F., et al., 2020a. SAUR17 and SAUR50 differentially regulate PP2C-D1 during apical hook development and cotyledon opening in arabidopsis. *Plant Cell* 32, 3792–3811. <https://doi.org/10.1105/tpc.20.00283>.

- Wang, W., Hu, B., Li, A., Chu, C., 2020b. NRT1.1s in plants: functions beyond nitrate transport. *J. Exp. Bot.* 71, 4373–4379. <https://doi.org/10.1093/jxb/erz554>.
- Wang, Y.Y., Cheng, Y.H., Chen, K.E., Tsay, Y.F., 2018b. Nitrate transport, signaling, and use efficiency. *Annu. Rev. Plant Biol.* 69, 85–122. <https://doi.org/10.1146/annurev-arplant-042817-040056>.
- Warschefsky, E.J., Klein, L.L., Frank, M.H., et al., 2016. Rootstocks: diversity, domestication, and impacts on shoot phenotypes. *Trends Plant Sci.* 21, 418–437. <https://doi.org/10.1016/j.tplants.2015.11.008>.
- Wei, Z., Li, J., 2016. Brassinosteroids regulate root growth, development, and symbiosis. *Mol. Plant* 9, 86–100. <https://doi.org/10.1016/j.molp.2015.12.003>.
- White, C.N., Rivin, C.J., 2000. Gibberellins and seed development in maize. II. Gibberellin synthesis inhibition enhances abscisic acid signaling in cultured embryos. *Plant Physiol.* 122, 1089–1097. <https://doi.org/10.1104/pp.122.4.1089>.
- Wigge, P.A., Kim, M.C., Jaeger, K.E., et al., 2005. Integration of spatial and temporal information during floral induction in Arabidopsis. *Science* 309 (80), 1056–1059. <https://doi.org/10.1126/science.1114358>.
- Yadav, A., Singh, D., Lingwan, M., et al., 2020. Light signaling and UV-B-mediated plant growth regulation. *J. Integr. Plant Biol.* 62, 1270–1292. <https://doi.org/10.1111/jipb.12932>.
- Yahmed, J.B., Ghrab, M., Moreno, M.A., et al., 2016. Performance of ‘Subirana’ flat peach cultivar budded on different Prunus rootstocks in a warm production area in North Africa. *Sci. Hortic.* 206, 24–32. <https://doi.org/10.1016/j.scienta.2016.04.031>.
- Yamaguchi, S., 2008. Gibberellin metabolism and its regulation. *Annu. Rev. Plant Biol.* 59, 225–251. <https://doi.org/10.1146/annurev-arplant.59.032607.092804>.
- Yang, J., Bak, G., Burgin, T., et al., 2020. Biochemical and genetic analysis identify CSLD3 as a beta-1,4-glucan synthase that functions during plant cell wall synthesis. *Plant Cell* 32, 1749–1767. <https://doi.org/10.1105/tpc.19.00637>.
- Yeh, S.Y., Chen, H.W., Ng, C.Y., et al., 2015. Down-regulation of cytokinin oxidase 2 expression increases tiller number and improves rice yield. *Rice* 8, 1–13. <https://doi.org/10.1186/s12284-015-0070-5>.
- Youngs, H.L., Hamann, T., Osborne, E., Somerville, C., n.d. Chapter 3 the cellulose synthase superfamily. 35–48 2007. In: Brown, R.M., Saxena, I.M. (Eds.), *Cellulose: Molecular and Structural Biology*. Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-5380-1_3.
- Zeng, Y., Yang, T., 2002. RNA isolation from highly viscous samples rich in polyphenols and polysaccharides. *Plant Mol. Biol. Report.* 20, 5223210 <https://doi.org/10.1007/BF02772130>.
- Zhai, L., Wang, X., Tang, D., et al., 2021. Molecular and physiological characterization of the effects of auxin-enriched rootstock on grafting. *Hortic. Res.* 8 <https://doi.org/10.1038/s41438-021-00509-y>.
- Zhang, M., Hu, X., Zhu, M., et al., 2017. Transcription factors NF-YA2 and NF-YA10 regulate leaf growth via auxin signaling in Arabidopsis. *Sci. Rep.* 7, 1–9. <https://doi.org/10.1038/s41598-017-01475-z>.
- Zhang, S., Wang, X., 2008. Expression pattern of GASA, downstream genes of DELLA, in Arabidopsis. *Chin. Sci. Bull.* 53, 3839–3846. <https://doi.org/10.1007/s11434-008-0525-9>.
- Zhang, Y., Yang, X., Cao, P., et al., 2020. The bZIP53-IAA4 module inhibits adventitious root development in Populus. *J. Exp. Bot.* 71, 3485–3498. <https://doi.org/10.1093/jxb/eraa096>.
- Zhang, Z., Li, Q., Li, Z., et al., 2007. Dual regulation role of GH3.5 in salicylic acid and auxin signaling during arabidopsis-Pseudomonas syringae interaction. *Plant Physiol.* 145, 450–464. <https://doi.org/10.1104/pp.107.106021>.
- Zhao, Q., Nakashima, J., Chen, F., et al., 2013. LACCASE is necessary and nonredundant with PEROXIDASE for lignin polymerization during vascular development in Arabidopsis. *Plant Cell* 25, 3976–3987. <https://doi.org/10.1105/tpc.113.117770>.
- Zhou, B., Lin, J., Peng, W., et al., 2012. Dwarfism in Brassica napus L. induced by the over-expression of a gibberellin 2-oxidase gene from Arabidopsis thaliana. *Mol. Breed.* 29, 115–127. <https://doi.org/10.1007/s11032-010-9530-1>.
- Zhou, P., Song, M., Yang, Q., et al., 2014. Both PHYTOCHROME RAPIDLY REGULATED1 (PAR1) and PAR2 promote seedling photomorphogenesis in multiple light signaling pathways. *Plant Physiol.* 164, 841–852. <https://doi.org/10.1104/pp.113.227231>.