# Characterization of almond scion/rootstock communication in cultivar and rootstock tissues through a RNA-Seq approach

**Jérôme Grimplet** ( jgrimplet@cita-aragon.es )
Centro de Investigación y Tecnología Agroalimentaria de Aragón
**Álvaro Montesinos**
Centro de Investigación y Tecnología Agroalimentaria de Aragón
**María José Rubio-Cabetas**
Centro de Investigación y Tecnología Agroalimentaria de Aragón

**Research Article**

**Keywords:** Prunus dulcis, transcriptome, tree architecture, root development

**Posted Date:** February 11th, 2022

**DOI:** https://doi.org/10.21203/rs.3.rs-1320607/v1

# Abstract

**Background:** Rootstock genotype determines multiple aspects of the scion development, including the scion three-dimensional structure, or tree architecture. Thus, rootstock choice is an important factor in the establishment of new almond (*Prunus amygdalus* (L.) Batsch, syn *P. dulcis* (Mill.)) planting systems, which demand cultivars whose vigor and shape adapt to these new requirements. However, if the rootstock genotype is able to alter scion development, it is likely that the scion genotype affects the rootstock performance.

**Results:** We carried out a transcriptomic analysis of the scion/rootstock interaction in young trees, focusing on the scion effect in the rootstock molecular response. Two commercial almond cultivars were grafted onto two hybrid rootstocks, resulting in four combinations, whose gene expression in both scion and rootstock tissue was analyzed via RNA-Seq. We observed that, in fact, the scion genotype has an impact on the rootstock expression profile, affecting the expression of genes associated with hormonal regulation, root development and light signaling.

**Conclusions:** Scion/rootstock communication has a pivotal role in the development of both scion and rootstock, accentuating the importance of a correct choice when establishing new almond orchards.


# Background

In modern orchards, rootstocks are used both to select specific root system traits and to confer traits of agronomic interest to trees and fruits [1,2]. These effects on scion development have been described in numerous trees species; ranging from tree vigor to yield or fruit quality [1,3–6]. Recently, molecular approaches have been carried out in woody plant species to describe how these effects happen at the molecular level [7,8]. In a recent study where almond commercial cultivars were grafted onto hybrid rootstocks, a differential expression of genes associated to hormones involved in the regulation of apical dominance, branch formation and vigor control was observed, while genes related to cell wall reorganization and formation were also affected.

The analysis of the scion effect on the rootstock has been limited to the graft formation, analyzing the processes that happens in the moment of that vascular union, leading to vascular regeneration and the establishment of the graft junction [9,10]. However, little is known about how the scion can modulate the phenotypes displayed by the rootstock, from nutrient assimilation to pathogen resistance or root development [11]. These traits might be affected differently depending which scion cultivar is grafted onto them.

Rootstock development is controlled by various phytohormones, which have roles in regulating cell elongation, cell division and cell differentiation [12,13]. As it happens with the aerial part of the plant, auxin has an important role in regulating diverse processes in roots, like root patterning, cell division and cell elongation [14–17]. Strigolactones (SLs) act in consonance with auxin, controlling lateral root formation and root-hair elongation, while mediating root responses to environment changes [18–20]. Cytokinins (CKs) promote root cell differentiation and cell division in various root tissues and inhibits lateral root formation in opposition to auxin [17,21–23]. Gibberellic acid (GA) is involved in maintaining root cell proliferation and cell elongation in the meristem while arresting lateral root formation [24–26]. Brassinosteroids (BRs) play a crucial role in controlling the root meristem activity, also participating in the regulation of lateral root initiation or root cell elongation [27,28]. Ethylene (ET) modulates the meristem maintenance, promoting cell division; whilst opposing auxin in lateral root formation [29,30].

Light signaling can also control plant development through different mechanisms. In plants, the circadian clock regulates several developmental processes in response to light changes, from seed germination, to hypocotyl elongation, root growth or flowering [31,32]. Carbohydrate metabolism and nutrient assimilation are also linked to the regulation of the circadian clock [33]. The shade avoidance response also regulates plant growth which is based on the ratio between red light and far

red light (R:FR), captured by phytochrome photoreceptors phyA and phyB. Changes in this ratio provoke a redistribution in the auxin flux, changing the direction and activity of the plant growth [34–38].

In this study, we have analyzed both the rootstock influence on the scion and the scion influence on the rootstock at the transcriptional response level. We grafted two commercial almond cultivars with opposite architecture and vigor characteristics onto two almond × peach (*Prunus amygdalus* (L.) Batsch, syn *P. dulcis* (Mill.). × *P. persica* (L.) Batsch) hybrid rootstocks for a total of four combinations. Our goal was to identify which biological processes and molecular responses were affected above and below the graft site.

## Results And Discussion

# ‘Isabelona’ and ‘Lauranne’ vigor was influenced by the rootstock

Tree architecture data was collected for the four combinations, ‘Isabelona’/Garnem®, ‘Isabelona’/‘GN-8’, ‘Lauranne’/Garnem® and ‘Lauranne’/‘GN-8’ (Fig. 1). Since trees were too young to have developed any branches, only trunk length (Length) and the diameter of both the scion (d_Scion) and the rootstock (d_Rootstock) was measured. Due to the intrinsic difficulties of its measurement, no data was collected of the root architecture.

In a previous study with thirty different scion/rootstock combinations [39], we reported that ‘Isabelona’ displayed reduced vigor paired with strong apical dominance, which resulted in a phenotype with reduced branching and long trunks. On the contrary, ‘Lauranne’ presented high vigor and weak apical dominance, resulting in numerous branching and a shortening of the trunk. Here, combinations with ‘Lauranne’ as scion presented higher Length values, and hence, longer trunks (Table 1, Fig. 1). In this case, trees are in their first year of growth, so there are no branches yet that compete with the main axis growth. As a result, ‘Lauranne’ more vigor leads to higher Length values. Regarding the rootstocks, Garnem® effect as a vigorous rootstock was present on both cultivars, presenting higher Length values than when grafted onto the dwarfing rootstock ‘GN-8’ (Table 1, Fig. 1).

**Table 1**

**Analysis of architectural traits related to vigor in one-year-old scion/rootstock combinations.**

| Cultivar | Rootstock | Length (mm) | d_Scion (mm) | d_Rootstock (mm) |
|---|---|---|---|---|
| **‘Isabelona’** | **‘GN-8’** | 210 a | 2.63 a | 4.25 a |
| | **Garnem®** | 260 b | 3.25 ab | 4.36 a |
| **‘Lauranne’** | **‘GN-8’** | 310 c | 2.97 ab | 4.50 a |
| | **Garnem®** | 400 d | 3.32 b | 4.56 a |

Assessed with Tukey’s test. Values within columns followed by the same letter were not significantly different ($p < 0.05$).

Trunk diameter (d_Scion) is typically used as a vigor measure, normally presented as TCSA (Trunk Cross Sectional Area). As it happened with Length values, ‘Lauranne’ presented higher d_Scion values than ‘Isabelona’. Besides, cultivars grafted onto Garnem® had also higher d_Scion values than when grafted onto ‘GN-8’ (Table 1). However, we did not observe a significant difference in the rootstock diameters (d_Rootstock), though mean values were slightly lower with ‘Isabelona’ (Table 1).

The observed phenotype differences seem to depend mostly on the vigor that each combination displays. Though is likely that the biological processes that will shape the specific tree architecture of each combination are already developed and their phenotypic effects are not yet visible in these one-year-old plants.

# Rootstock only influenced gene expression in combinations with ‘Isabelona’

We reported in a previous experiment that combinations with ‘Lauranne’ and ‘Isabelona’ did show little phenotypic differences when grafted onto different rootstocks, which was correlated with a lack of differentially expressed genes (DEGs) [39]. However, in the present experiment, ‘Lauranne’ and ‘Isabelona’ were selected because of their consistent scion phenotype, expecting that they could influence rootstock transcriptome. In addition, we analyzed the gene expression in the scion in order to determine if the rootstock influences gene expression at an early development stage (Supplementary Data 1).

A PCA (Principal Component Analysis) was carried out using expression for each gene as variables for the four combinations, with the first (PC1; 33.2% of variability explained) and third (PC3; 11.8%) component selected to represent the data (Fig. 2). As we observed previously, combinations with ‘Lauranne’ as scion were not differentiated according to rootstock, grouping together (Fig. 2). However, we did observe that gene expression in combinations with ‘Isabelona’ is influenced by the rootstock. These individuals could be separated in two groups in the PCA, depending on whether they were grafted onto Garnem® or ‘GN-8’.

Looking at the global picture of gene expression by functional categories, we performed a Gene Ontology (GO) enrichment analysis but due to the low number of genes we did not obtain any significant categories. However, we found a molecular response similar to what we observed in previous analysis of almond scion-rootstock combinations. When grafted onto the vigor-conferring rootstock Garnem®, ‘Isabelona’ displayed several DEGs overexpressed involved in auxin regulation, mostly in a repressive manner. Besides, DEGs promoting CKs or GA activity or repressing abscisic acid (ABA) response were also overexpressed in these combinations (Supplementary Data 2). Therefore, Garnem® influence hormonal regulation here in a similar manner to what we observed before, with auxin responses being downregulated, hence reducing apical dominance [40,41]. Moreover, as it happened previously, we found overexpression of DEGs involved in processes associated with active growth, like cell proliferation and cell expansion, or promoting nitrogen and sugar assimilation (Supplementary Data 2).

Genes related to ET regulation were overexpressed when ‘Isabelona’ was grafted onto the dwarfing rootstock ‘GN-8’ (Supplementary Data 2). Contrary to what happened when grafted onto Garnem®, DEGs related to low nitrogen or sugar content were upregulated (Supplementary Data 2). However, some genes involved in cell wall reorganization were overexpressed (Supplementary Data 2), while in a previous experiment, these genes were only upregulated in combinations with vigor-conferring rootstocks.

In general, although the effects in the phenotype are not yet visible, we observed a similar expression profile to what has been previously described, with auxin responses downregulated in combinations with a vigor-inducing rootstock, while branching and growth are upregulated in combinations with Garnem®.

# Scion/rootstock interaction in almond affected rootstock molecular profile

The cultivar effect of commercial almond cultivars ‘Lauranne’ and ‘Isabelona’ on the rootstock development was analyzed in a vigorous rootstock like Garnem®, and a dwarfing rootstock such as ‘GN-8’ (Supplementary Data 3). We carried out a PCA using the expression of each gene as variables for the four different scion/rootstock combinations. The first two components explained 50.1% of the variability, while none of the other variables explained more than a 10%. PC1 and PC2 explained 32.6% and 17.6% of the variability respectively. In the PCA, there was a clear separation between the four different combinations (Fig. 3). Combinations with Garnem® as rootstock are in the lower-left corner while combinations with ‘GN-8’

are in the upper-right corner. Therefore, there is a clear effect of the rootstock and it can be observed in the gene expression, with individuals clearly segregating depending on which scion, 'Lauranne' or 'Isabelona', is grafted onto them (Fig. 3).

A total of 168 DEGs were overexpressed in combinations with 'Isabelona' as scion respective to those with 'Lauranne', of which 100 appeared in the combination with Garnem® and 52 in combination with 'GN-8', while only 16 DEGs were in both combinations (Fig. 4a). A similar display was observed with DEGs that were underexpressed when 'Isabelona' was the scion. A total of 71 DEGs appeared only in Garnem®, while 74 DEGs were found in 'GN-8'. A total of 34 DEGs were present in both rootstocks (Fig. 4b).

Therefore, while both Garnem® and 'GN-8' expression profiles are influenced by the scion that is grafted onto them, responses seem to be specific for each rootstock; at least regarding which specific genes are involved. In any case, that does not mean that the regulatory pathways affected by the scion influence are not similar.

# DEGs associated with hormonal regulation were influenced by the cultivar in rootstock tissue

We have seen that changes in hormonal response prompted by a different rootstock affect the almond scion architecture, modifying the number of branches or the growth of the main axis. Therefore, it is likely that the grafted scion also has an effect on the rootstocks, triggering different mechanisms that could affect the rootstock properties. This reciprocal effect has been already described in other species regarding different traits (regulation of rootstock responses to low Pi and phloem sap metabolites) [42,43]. Here, we reported that hormonal response is affected by the scion, presumably leading to changes in the root architecture. Although samples were collected from the rootstock trunk, we expect that the variation of the dynamics of hormone flux found there affect the rest of the root system.

In contrast to its function in shoots, auxin has been described to promote the formation of lateral roots [14–17]. Various DEGs involved positively in auxin response were downregulated when 'Isabelona' was the scion in Garnem® (Table 2). *BUD2* (Prudul26A013026) is an auxin inducible member of the SAMDC family, playing a part in mechanisms promoted by auxin, like apical dominance and root branching [44,45]. *IAR3* (Prudul26A016337) releases IAA from its conjugate form, regulating the levels of free auxin [46,47]. *ZIFL1* (Prudul26A023995) positively regulates polar auxin transport, favoring processes like lateral root development (Remy et al., 2013). On the other hand, *GH3.6* (Prudul26A017626), a negative regulator of auxin levels [48,49], appeared overexpressed in combinations with 'Isabelona' as scion (Table 2). Here, the fact that auxin processes are downregulated in combinations with 'Isabelona' as scion suggests that rootstocks with this cultivar may display hormonal conditions required to develop less lateral roots. Whereas, rootstocks with 'Lauranne' as scion could develop an increased number of lateral roots, which would correlate to higher substrate availability and therefore affect their vigor and aerial branching phenotype [39].

**Table 2**

**Differentially expressed genes (DEGs) associated with hormonal regulation.**

| logFC 'Isabelona'/Garnem - 'Lauranne'/Garnem | logFC 'Isabelona'/'GN8' - 'Lauranne'/'GN-8' | *P. dulcis* ID | Gene | GO term | Biological process |
|---|---|---|---|---|---|
| 0.920 | **1.003** | Prudul26A011001 | *ACO* | GO:0009693 | ethylene biosynthetic process |
| -0.416 | **-1.243** | Prudul26A007830 | *ACO* | GO:0009693 | ethylene biosynthetic process |
| **1.404** | 0.024 | Prudul26A030744 | *BAS1* | GO:0055114 | oxidation-reduction process |
| -0.731 | **-1.197** | Prudul26A013026 | *BUD2* | GO:0006557 | S-adenosylmethioninamine biosynthetic process |
| **1.228** | 0.717 | Prudul26A008430 | *bZIP58* | GO:0006355 | regulation of transcription, DNA-templated |
| **1.755** | 0.337 | Prudul26A017801 | *CKX5* | GO:0009823 | cytokinin catabolic process |
| 0.740 | **1.106** | Prudul26A028543 | *CVIF2* | GO:0043086 | negative regulation of catalytic activity |
| 0.808 | **1.014** | Prudul26A016230 | *CVIF2* | GO:0043086 | negative regulation of catalytic activity |
| 0.221 | **-1.434** | Prudul26A017398 | *CYP94C1* | GO:0009611 | response to wounding |
| 0.991 | **1.295** | Prudul26A002650 | *ERF12* | GO:0009873 | ethylene-activated signaling pathway |
| 0.423 | **1.232** | Prudul26A022504 | *ERF12* | GO:0009873 | ethylene-activated signaling pathway |
| **-2.000** | **-2.949** | Prudul26A000689 | *GA2OX8* | GO:0009686 | gibberellin biosynthetic process |
| 0.669 | **1.358** | Prudul26A017626 | *GH3.6* | GO:0010252 | auxin homeostasis |
| **-5.950** | -0.941 | Prudul26A016337 | *IAR3* | GO:0009850 | auxin metabolic process |
| **1.007** | 0.135 | Prudul26A016134 | *LOL1* | GO:0034052 | positive regulation of plant-type hypersensitive response |
| **1.821** | -0.772 | Prudul26A022418 | *MAX1* | GO:0016117 | carotenoid biosynthetic process |
| **-1.005** | **-1.005** | Prudul26A005107 | *RCA* | GO:0050790 | regulation of catalytic activity |
| **1.102** | 0.681 | Prudul26A028381 | *SPL8* | GO:0030154 | cell differentiation |
| **-1.640** | -0.515 | Prudul26A006492 | *SWEET2* | GO:0008643 | carbohydrate transport |
| **-1.176** | -0.730 | Prudul26A023995 | *ZIFL1* | GO:0010540 | basipetal auxin transport |

Only genes with a logFC superior or infertior to 1 (highlighted in bold) were considered as differentially expressed.

GA acts mostly in opposition to the auxin response, inhibiting lateral root formation while promoting cell elongation and proliferation in the central root [25,26]. Three genes related positively to GA activity were found to be upregulated in

rootstock tissues in combinations with 'Isabelona' as the scion (Table 2). *LOL1* (Prudul26A016134) and *bZIP58* (Prudul26A008430) modulate GA levels, favoring its activity and acting in numerous pathways regulated by this hormone [50]. *SPL8* (Prudul26A028381) can act both in a positive or negative manner, although has been described to negatively affect root elongation in Arabidopsis [51]. On the other hand, *GA2OX8* (Prudul26A000689) is downregulated in combinations with 'Isabelona' (Table 2). GA2OX8 catalyzes the deactivation of active GA, hence reducing its levels and activity [52,53]. In general, genes related to increased GA levels are upregulated in rootstocks when 'Isabelona' is the scion. This could lead to the elongation of the central root, in a similar manner of what we observed in the scion, while inferior expression of GA responses in combinations with 'Lauranne' would favor the development of numerous lateral roots.

ET response was also affected by the scion. 1-Aminocyclopropane-1-Carboxylic Acid Oxidase (*ACO)* carries out a crucial step in ET biosynthesis, controlling ET production [54,55]. Homologues of this gene (Prudul26A011001, Prudul26A007830) were found both upregulated and downregulated in 'Isabelona'/'GN-8' combinations (Table 2). *ERF12* has been described to participate in floral transition and seed dormancy in response to ethylene, being activated by its presence [56,57]. Here, two homologues (Prudul26A002650, Prudul26A022504) where upregulated when 'Isabelona' was grafted onto 'GN-8' (Table 2). ET acts by opposing auxin effect in lateral root formation [29,30], which matches the reduced auxin response that has been reported in combinations with 'Isabelona'. BRs have an opposite function to ET, favoring the initiation of lateral roots [28,58]. BAS1 (Prudul26A030744), an enzyme that catalyzes BR inactivation [59], is overexpressed in Garnem® when 'Isabelona' is the scion (Table 2).

Scion also influenced the expression of genes involved in other hormonal responses. *CKX5* (Prudul26A017801) was overexpressed in the 'Isabelona'/Garnem® combination (Table 2). As a CK dehydrogenase, CKX5 participates in degrading CKs [60]. *MAX1* (Prudul26A022418), which is part of the SL biosynthetic pathway [61,62], is also upregulated when Garnem® had 'Isabelona' as scion (Table 2). Jasmonic acid (JA) is typically activated in stress responses [63]. *CYP94C1* (Prudul26A017398) carries out the oxidative inactivation of this hormone [64]. This gene was less expressed in the 'Isabelona'/'GN-8' combination too, suggesting a negative regulation of growth in this combination (Table 2). Finally, a couple of genes related to sugar availability were affected by the scion. Two *CVIF2* homologues (Prudul26A028543, Prudul26A016230) were overexpressed in the 'Isabelona'/'GN-8' combination (Table 2). *CVIF2* might regulate sucrose cleaving, therefore negatively affecting plant sugar levels [65]. *RCA* (Prudul26A005107), which was downregulated with 'Isabelona' as scion (Table 2), promotes RuBisCO activity and therefore sugar production [66]. Moreover, the sugar transporter *SWEET2* (Prudul26A006492), which is especially active in roots [67], was also less expressed when 'Isabelona' was the scion. Therefore, 'Isabelona' seems to negatively influence sugar production in roots, which might lead to a reduction in the formation of roots.

In conclusion, the presence of a different scion affects the hormonal response in the rootstock. In this case, we observed that rootstocks with 'Isabelona' as scion present a hormonal framework that should inhibit the formation of lateral roots, while those with 'Lauranne' as scion are prompted to develop more lateral roots.

# Root development and root cell wall reorganization are negatively influenced by 'Isabelona'

Root architecture is regulated by numerous genes that mediate the formation of the primary root and others, like lateral roots or adventitious roots [68]. Though samples were collected from below the grafting site in 'GN-8' and Garnem® rootstocks, we would expect that changes in the expression profile would condition the behavior of other parts of the rootstock.

Two inhibitors of lateral root formation were overexpressed in combinations with 'Isabelona' (Table 3). *AGL79* (Prudul26A020939) acts as a repressor of lateral root development [69]. While not affecting lateral root initiation, *LRP1*

(Prudul26A023724) does affect its progression. Its overexpression in Arabidopsis reduced the number of lateral roots [70]. *IAA4* (Prudul26A024452) is also overexpressed in the 'Isabelona'/Garnem® combination (Table 3). *IAA4* acts in opposition to auxin response, inhibiting the formation of adventitious roots [71]. Therefore, there is an upregulation of processes that lead to reduce lateral root formation when 'Isabelona' is the scion. Moreover, two homologues of *FIP37* (Prudul26A025382, Prudul26A011653) were highly overexpressed in the 'Isabelona'/Garnem® combination (Table 3). *FIP37* effect in meristem development has been mostly described in shoots, but it acts preventing meristem proliferation and therefore bud outgrowth [72]. A similar function is carried out by *TSO1* [73,74]. Here, we found a homologue of this gene, *TCX2* (Prudul26A017201), which is downregulated when 'Isabelona' was the scion (Table 3).

**Table 3**

**Differentially expressed genes (DEGs) associated with root development and root cell wall reorganization.**

| logFC 'Isabelona'/Garnem - 'Lauranne'/Garnem | logFC 'Isabelona'/'GN8' - 'Lauranne'/'GN-8' | *P. dulcis* ID | Gene | GO term | Biological process |
|---|---|---|---|---|---|
| **-1.023** | 0.073 | Prudul26A020211 | *4CLL6* | GO:0006744 | ubiquinone biosynthetic process |
| 0.030 | **-1.627** | Prudul26A014215 | *4CLL9* | GO:0000272 | polysaccharide catabolic process |
| **1.094** | 0.291 | Prudul26A020939 | *AGL79* | GO:0006355 | regulation of transcription, DNA-templated |
| -0.986 | **-1.062** | Prudul26A005381 | *ERF3* | GO:0072659 | protein localization in plasma membrane |
| **-1.265** | 1.319 | Prudul26A009806 | *EXPL1* | GO:0019953 | sexual reproduction |
| **3.089** | 0.646 | Prudul26A025382 | *FIP37* | GO:0010073 | meristem maintenance |
| **3.072** | 0.623 | Prudul26A011653 | *FIP37* | GO:0010073 | meristem maintenance |
| **1.048** | **1.468** | Prudul26A000195 | *GRF4* | GO:0006355 | regulation of transcription, DNA-templated |
| -0.466 | **-1.032** | Prudul26A031613 | *GUX3* | GO:0045492 | xylan biosynthetic process |
| **1.180** | 0.462 | Prudul26A024452 | *IAA4* | GO:0009733 | response to auxin |
| **1.174** | 0.195 | Prudul26A009950 | *KING1* | GO:0042128 | nitrate assimilation |
| 0.401 | **1.024** | Prudul26A023724 | *LRP1* | GO:0048364 | root development |
| **1.334** | **1.832** | Prudul26A008528 | *MYB103* | GO:0006355 | regulation of transcription, DNA-templated |
| **-1.300** | -0.538 | Prudul26A012897 | *MYB20* | GO:1901141 | regulation of lignin biosynthetic process |
| **-1.074** | -0.532 | Prudul26A014014 | *ROL1* | GO:0071555 | cell wall organization |
| -0.031 | **-1.022** | Prudul26A008007 | *SKP2A* | GO:0010311 | lateral root formation |
| **-1.694** | **-2.203** | Prudul26A014041 | *SNAK2* | GO:0006952 | defense response |
| -0.794 | **-1.850** | Prudul26A015706 | *SNAK2* | GO:0006952 | defense response |

| | | | | | |
|---|---|---|---|---|---|
| **-1.034** | **-3.009** | Prudul26A007951 | *TBL19* | GO:0045492 | xylan biosynthetic process |
| -0.544 | **-1.001** | Prudul26A014994 | *TBL29* | GO:0045492 | xylan biosynthetic process |
| **-1.405** | 0.011 | Prudul26A017201 | *TCX2* | GO:0006355 | regulation of transcription, DNA-templated |

Only genes with a logFC superior or infertior to 1 (highlighted in bold) were considered as differentially expressed.

'Lauranne' has been proved to be a more vigorous scion than 'Isabelona'. Here, we also observed several genes involved in cell proliferation being downregulated in the rootstock in combinations with 'Isabelona' (Table 3). *ERF3* (Prudul26A005381) promotes cell division and cell elongation of the root meristem [75]. *SKP2A* (Prudul26A008007) is a regulator of cell proliferation, promoting cell division in lateral root primordium, whose degradation is stimulated by auxin [76,77]. Two homologues of *SNAK2* (Prudul26A014041, Prudul26A015706) were found. *SNAK1* has been described to promote cell division in response to external stimuli [78,79]. *SnRK1* is involved in repressing growth in response to low energy supplies [81]. Here, a member of its family, *KING1* (Prudul26A009950), was upregulated in the 'Isabelona'/Garnem® combination (Table 3).

The regulation of several components that are part of the cell wall, like lignins, xyloglucans or pectins, is essential in the control of cell wall formation and cell wall reorganization [81–83]. Numerous genes associated to their synthesis or transport were downregulated in combinations with 'Isabelona' compared to those with 'Lauranne' as the scion (Table 3). Members of the 4CL family like *4CLL6* (Prudul26A020211) and *4CLL9* (Prudul26A014215) are part of the phenylpropanoid metabolism pathway, participating in lignin biosynthesis [84]. The MYB transcription factor, *MYB20* (Prudul26A012897), promotes the lignin biosynthesis pathway [85]. However, another MYB TF linked to lignin biosynthesis, *MYB103* (Prudul26A008528), was overexpressed in combinations with 'Isabelona' as scion [86]. *GUX3* (Prudul26A031613) is involved in xylan modification while *TBL19* (Prudul26A007951) and *TBL29* (Prudul26A014994) participate in xylan acetylation [87–89]. These modifications are crucial to ensure xylan integrity and cell wall strength. Knockout mutants of *ROL1* (Prudul26A014014) produce aberrant pectin structure which leads to reduced elongation growth, highlighting a role for *ROL1* in cell wall reorganization [90,91]. Nevertheless, some genes associated also to cell wall formation were found to be upregulated when 'Isabelona' was the scion (Table 3). *GRF4* (Prudul26A000195) promotes cellulose biosynthesis in a response involving *MYB61* transcription factor [92]. *EXPL1* (Prudul26A009806) is associated to cell wall remodeling in response to auxin and lateral root initiation [93]. Contradictorily, *EXPL1* was overexpressed in 'GN-8', while being downregulated in the 'Isabelona'/Garnem® combination (Table 3). This could mean a differential response for this gene depending on which rootstock is affected by the scion, maybe linked to the fact that 'GN-8' is a prominently less vigorous rootstock than Garnem®.

In general, processes related to root formation or active tissue growth like cell wall reorganization were downregulated when 'Isabelona' was the scion, expecting that these combinations should present a root system with fewer lateral roots. This response is in line with the hormonal status reported previously, that favored root formation in rootstocks with 'Lauranne' as scion, and not in those with 'Isabelona'.

# DEGs associated with light responses are affected by cultivar in rootstock tissue

Light regulates numerous processes related to plant development, and several pathways are involved in growth control [94,95]. Light availability mediates the formation of lateral branches, through several responses like shade avoidance

[34,35]. In the root, we observed an upregulation of genes involved in responses related to reduced light in combinations that had 'Isabelona' as scion, with *ABR* (Prudul26A020068) being overexpressed and several homologues of *phyE* (Prudul26A014761, Prudul26A002019) and *UVR8* (Prudul26A018495, Prudul26A003343, Prudul26A011979) downregulated (Table 4). *ABR* is involved in ABA responses and it is induced by light deprivation [96]. *phyE* regulates responses to low R/FR, in consonance with *phyB* [97]. The photoreceptor *UVR8* mediates the signal produced by UV-B that inhibits shade avoidance responses [98]. Auxin and light responses are tightly integrated, affecting tree architecture [99]. Two inhibitors of auxin response affected by light were overexpressed in combinations with 'Isabelona' (Table 4). *NPH3* (Prudul26A013341) participates in an auxin feedback response, modifying auxin transport in response to phototropism [100]. *RVE7* (Prudul26A019438) is a member of the same family of *RVE1*, which modulates plant growth through repression of auxin levels [101]. 'Lauranne', which shows numerous branching, is expected not to be affected as acutely by light availability than 'Isabelona', which displays reduced branching. Here, this effect is more prevalent in Garnem®, while 'GN-8' is less affected by the scion light perception. This could be caused by the higher vigor presented by Garnem®, which is more influenceable by changes that favor growth.

**Table 4**

**Differentially expressed genes (DEGs) associated with light responses and circadian clock regulation**

| logFC 'Isabelona'/Garnem - 'Lauranne'/Garnem | logFC 'Isabelona'/'GN8' - 'Lauranne'/'GN-8' | *P. dulcis* ID | Gene | GO term | Biological process |
|---|---|---|---|---|---|
| **1.899** | **1.208** | Prudul26A020068 | *ABR* | GO:0009733 | response to auxin |
| **1.596** | -0.580 | Prudul26A024462 | *COL6* | GO:0006355 | regulation of transcription, DNA-templated |
| **-1.751** | **-1.054** | Prudul26A016707 | *GI* | GO:0042752 | regulation of circadian rhythm |
| **1.061** | 0.101 | Prudul26A014609 | *JMJD5* | GO:0042752 | regulation of circadian rhythm |
| **-1.542** | 0.905 | Prudul26A026608 | *MDL1* | GO:0055114 | oxidation-reduction process |
| **1.368** | **1.015** | Prudul26A013341 | *NPH3* | GO:0009638 | phototropism |
| **-1.339** | 0.051 | Prudul26A014761 | *phyE* | GO:0009585 | red, far-red light phototransduction |
| **-1.364** | 0.042 | Prudul26A002019 | *phyE* | GO:0009585 | red, far-red light phototransduction |
| **-1.453** | -0.886 | Prudul26A027917 | *PRR7* | GO:0007623 | circadian rhythm |
| **1.009** | 0.772 | Prudul26A019438 | *RVE7* | GO:0007623 | circadian rhythm |
| **-1.078** | -0.643 | Prudul26A018495 | *UVR8* | GO:0009649 | entrainment of circadian clock |
| **-1.078** | **-1.143** | Prudul26A003343 | *UVR8* | GO:0009649 | entrainment of circadian clock |
| **-1.798** | **-1.042** | Prudul26A011979 | *UVR8* | GO:0009649 | entrainment of circadian clock |

Only genes with a logFC superior or infertior to 1 (highlighted in bold) were considered as differentially expressed.

The circadian clock, which is controlled by light, among other environmental responses, regulates numerous processes in plant development, including root growth [31–33]. We detected a mixed pattern of expression profiles of genes involved in circadian clock regulation. *COL6* (Prudul26A024462) and *JMJD5* (Prudul26A014609) were overexpressed in the 'Isabelona'/Garnem® combination (Table 4). CO-like genes are light responsive genes under circadian clock control and affecting circadian rhythms [102,103]. *JMJD5* is integrated in various responses regulated by circadian period, including flowering regulation [104]. On the other hand, the circadian clock regulator *GI* (Prudul26A016707) was downregulated in combinations with 'Isabelona' (Table 4). This gene participates in regulating daily *CO* expression and in activating *FT* expression, being controlled by light [105–107]. While we do not observe any clear trend in the influence of the scion in the circadian clock regulation, it seems clear that these processes can be affected by the interaction between scion and rootstock.

## Conclusions

Interaction between scion and rootstock in almond trees occur in both directions, influencing both the scion and the rootstock development. Here, we identified multiple biological processes which were differentially affected according to the grafted almond cultivar. Among the differentially expressed genes , we observed genes involved in hormonal regulation, root development, cell wall reorganization, light perception and circadian clock regulation (Figure 5). This influence seems to have a feedback effect in the development of the scion. We report that cultivars displaying more vigor like 'Lauranne' influence positively root development, including lateral root formation. This would favor the capture of nutrients by the radicular system and, in consequence, would promote scion growth, resulting in the vigorous phenotype that 'Lauranne' presents when compared to 'Isabelona'. Therefore, choosing the correct scion/rootstock combination is essential to the success of the orchard. In intensive systems, the rootstock effect in tree vigor depends not only on its genotype, but also their complementarity as the scion is determinant in root development, and hence, tree growth.

## Methods

# Plant material and growth conditions

For the experiment, two almond commercial cultivars, 'Isabelona' and 'Lauranne' were grafted onto two hybrid rootstocks, Garnem®, a commercial rootstock, and 'GN-8', a new selection, obtaining four different combinations. Both rootstocks are almond × peach *(P. amygdalus* (L.) Batsch, syn *P. dulcis* (Mill.). × *P. persica* (L.) Batsch) hybrid rootstocks. The two cultivars were selected because the weak influence that the rootstock displays in their apical dominance and branch formation phenotype [39]. Grafted plants were supplied by the Agromillora Iberia S.L. nursery in 2020 (Barcelona, Spain). Plants were kept in a nursery shortly until sample collection at the Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA), where conventional orchard practices were applied.

# Phenotypic data collection

Phenotypic data was collected for ten replicates of each of the four combinations, before sample collection. Three parameters related to vigor were measured: scion axe length (Length), scion trunk diameter (d_Scion) and rootstock trunk diameter (d_Rootstock). Length was determined from the graft union. d_Scion and d_Rootstock were quantified using a caliper, measuring from 20 mm above and 20 mm below of the graft union respectively.

# RNA-Seq analysis

Samples from the four combinations mentioned were collected from 50 mm below and above the graft union of three different individuals per combination during summer 2020. RNA extraction was performed from these samples using the CTAB method described previously [108] with some modifications [109–111]. 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 2×50bp. FASTQ files were converted with FASTQ Groomer (Galaxy Version 1.1.1) [112]. Adapter sequences were removed by processing the reads sequences of the 27 individual datasets with Trimmomatic (Galaxy Version 0.38.0) [113]. RNA-Seq data alignment was carried out by TopHat (Galaxy Version 2.1.1), with a maximum intron length of 1,000 bp, [114] on the *P. dulcis* 'Texas' Genome v2.0 [115]. 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 1.6.4+galaxy2) was used to measure gene expression [116] using the gene annotation *P. dulcis* 'Texas' Genome v2.0 containing 27044 genes (https://www.rosaceae.org/analysis/295). Differential analysis of count data was performed by edgeR (Galaxy Version 3.24.1) with default settings [117]. All procedures were carried out using the Galaxy platform.

# Statistical analysis

All statistical analyses were carried out in the R platform (https://cran.r-project.org/). Significant differences in phenotypic data were evaluated using an ANOVA test to find. These were assessed with a Tukey's test ($p < 0.05$) using the agricolae R package (https://CRAN.R-project.org/package=agricolae). PCA was carried out using R stats package with default parameters on the gene expression values for the all the genes in the four combinations.

## Abbreviations

**ABA:** Abscisic acid

**BR:** Brassinosteroid

**CK:** Cytokinin

**DEG:** Differentially expressed gene

**ET:** Ethylene

**GA:** Gibberellic acid

**PCA:** Principal component analysis

**R:FR:** Red:far red ratio

**SL:** Strigolactone

## Declarations

# Ethics approval and consent to participate

Experimental research has been carried out in compliance with relevant national and international guidelines, and legislation regarding plant research.

# Consent for publication

Not applicable.

# Availability of data and materials

The datasets generated and/or analyzed during the current study are available in the European Nucleotide Archive (https://www.ebi.ac.uk/ena/browser/home) and are accessible through the accession number PRJEB50411.

# Competing interests

The authors declare that they have no competing interests.

# Funding

This research was funded by the Spanish Research State Agency (AEI), grant numbers RTI- 2018-507 094210-R-100 and FPI-INIA CPD2016-0056.

# Authors' contributions

AM, MJRC and JG designed the experiment. AM measured the phenotypical data, collected the plant tissues and performed the RNA extraction. AM and JG analyzed and interpreted the data obtained from the RNA-Seq. AM and JG drafted the manuscript. MJRC substantively revised the manuscript. All authors read and approved the final manuscript.

# Acknowledgements

Not applicable.

## References

1. Warschefsky EJ, Klein LL, Frank MH, Chitwood DH, Londo JP, von Wettberg EJB, et al. Rootstocks: Diversity, Domestication, and Impacts on Shoot Phenotypes. Trends Plant Sci. 2016;21(5):418–37.
2. Rubio-Cabetas MJ, Felipe AJ, Reighard GL.(2017). Rootstock Development. In: Socias i Company R., Gradziel TM, editors. Almonds: botany, production and uses. CABI: Wallingford, UK. p. 193–220.
3. Albacete A, Martínez-Andújar C, Martínez-Pérez A, Thompson AJ, Dodd IC, Pérez-Alfocea F. Unravelling rootstock×scion interactions to improve food security. J Exp Bot. 2015;66(8):2211–26.
4. Aloni B, Cohen R, Karni L, Aktas H, Edelstein M. Hormonal signaling in rootstock-scion interactions. Sci Hortic (Amsterdam). 2010;127(2):119–26.
5. Foster TM, Celton JM, Chagne D, Tustin DS, Gardiner SE.Two quantitative trait loci, Dw1 and Dw2, are primarily responsible for rootstock-induced dwarfing in apple. Hortic Res. 2015;2:9.
6. Martínez-Ballesta MC, Alcaraz-López C, Muries B, Mota-Cadenas C, Carvajal M. Physiological aspects of rootstock-scion interactions.Sci Hortic (Amsterdam). 2010;127(2):112–8.
7. López-Hinojosa M, de María N, Guevara MA, Vélez MD, Cabezas JA, Díaz LM, et al..Rootstock effects on scion gene expression in maritime pine. Sci. Rep. 2021;11:1–16.

8. Ou C, Jiang S, Wang F, Tang C, Hao N. An RNA-Seq analysis of the pear (Pyrus communis L.) transcriptome, with a focus on genes associated with dwarf. Plant Gene. 2015;4:69–77.
9. Melnyk CW, Gabel A, Hardcastle TJ, Robinson S, Miyashima S, Grosse I, et al. Transcriptome dynamics at Arabidopsis graft junctions reveal an intertissue recognition mechanism that activates vascular regeneration. Proc Natl Acad Sci. 2018;115(10):201718263.
10. Wulf KE, Reid JB, Foo E. Auxin transport and stem vascular reconnection - has our thinking become canalized? Ann Bot. 2019;123(3):429–39.
11. Li G, Ma J, Tan M, Mao J, An N, Sha G, et al.Transcriptome analysis reveals the effects of sugar metabolism and auxin and cytokinin signaling pathways on root growth and development of grafted apple. BMC Genomics. 2016;17(1):1–17.
12. Motte H, Vanneste S, Beeckman T. Molecular and environmental regulation of root development. Annu. Rev. Plant Biol. 2019;70:465–488.
13. Takatsuka H, Umeda M. Hormonal control of cell division and elongation along differentiation trajectories in roots. J Exp Bot. 2014;65(10):2633–43.
14. Ding Z, Friml J. Auxin regulates distal stem cell differentiation in Arabidopsis roots. Proc Natl Acad Sci U S A. 2010;107(26):12046–51.
15. Overvoorde P, Fukaki H, Beeckman T. Auxin control of root development. Cold Spring Harb Perspect Biol. 2010;2(6).
16. Petersson S V., Johansson AI, Kowalczyk M, Makoveychuk A, Wang JY, Moritz T, et al. An auxin gradient and maximum in the arabidopsis root apex shown by high-resolution cell-specific analysis of IAA distribution and synthesis. Plant Cell. 2009;21(6):1659–68.
17. Saini S, Sharma I, Kaur N, Pati PK. Auxin: A master regulator in plant root development. Plant Cell Rep. 2013;32(6):741–57.
18. Jiang L, Matthys C, Marquez-Garcia B, De Cuyper C, Smet L, De Keyser A, et al.Strigolactones spatially influence lateral root development through the cytokinin signaling network. J Exp Bot. 2016;67(1):379–89.
19. Koltai H. Strigolactones are regulators of root development. New Phytol. 2011;190(3):545–9.
20. Sun H, Tao J, Liu S, Huang S, Chen S, Xie X, et al. Strigolactones are involved in phosphate- and nitrate-deficiency-induced root development and auxin transport in rice. J Exp Bot. 2014;65(22):6735–46.
21. Jing H, Strader LC. Interplay of auxin and cytokinin in lateral root development. Int J Mol Sci. 2019;20(3).
22. Liu J, Moore S, Chen C, Lindsey K. Crosstalk Complexities between Auxin, Cytokinin, and Ethylene in Arabidopsis Root Development: From Experiments to Systems Modeling, and Back Again. Mol Plant. 2017;10(12):1480–96.
23. Márquez G, Alarcón MV, Salguero J. Cytokinin Inhibits Lateral Root Development at the Earliest Stages of Lateral Root Primordium Initiation in Maize Primary Root. J Plant Growth Regul. 2019;38(1):83–92.
24. Gou J, Strauss SH, Tsai CJ, Fang K, Chen Y, Jiang X, et al.Gibberellins regulate lateral root formation in Populus through interactions with auxin and other hormones. Plant Cell. 2010;22(3):623–39.
25. Ubeda-Tomás S, Swarup R, Coates J, Swarup K, Laplaze L, Beemster GTS, et al. Root growth in Arabidopsis requires gibberellin/DELLA signalling in the endodermis. Nat Cell Biol. 2008;10(5):625–8.
26. Yaxley JR, Ross JJ, Sherriff LJ, Reid JB. Gibberellin biosynthesis mutations and root development in pea. Plant Physiol.2001;125(2):627–33.
27. Li T, Lei W, He R, Tang X, Han J, Zou L, et al.Brassinosteroids regulate root meristem development by mediating BIN2-UPB1 module in Arabidopsis. PLoS Genet. 2020;16(7):1–27.
28. Wei Z, Li J. Brassinosteroids Regulate Root Growth, Development, and Symbiosis. Mol Plant. 2016;9(1):86–100.
29. Lewis DR, Negi S, Sukumar P, Muday GK. Ethylene inhibits lateral root development, increases IAA transport and expression of PIN3 and PIN7 auxin efflux carriers. Development. 2011;138(16):3485–95.

30. Qin H, He L, Huang R. The coordination of ethylene and other hormones in primary root development. Front Plant Sci. 2019;10.
31. Farré EM. The regulation of plant growth by the circadian clock. Plant Biol. 2012;14(3):401–10.
32. Inoue K, Araki T, Endo M. Circadian clock during plant development. J Plant Res. 2018;131(1):59–66.
33. Sanchez SE, Kay SA. The plant circadian clock: From a simple timekeeper to a complex developmental manager. Cold Spring Harb Perspect Biol. 2016;8(12).
34. Casal JJ. Shade Avoidance. Arab B. 2012;10:e0157.
35. Finlayson SA, Krishnareddy SR, Kebrom TH, Casal JJ. Phytochrome regulation of branching in arabidopsis. Plant Physiol. 2010;152(4):1914–27.
36. Holalu S V., Finlayson SA. 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. 2017;68(5):943–52.
37. Rausenberger J, Tscheuschler A, Nordmeier W, Wüst F, Timmer J, Schäfer E, et al. Photoconversion and nuclear trafficking cycles determine phytochrome A's response profile to far-red light. Cell. 2011;146(5):813–25.
38. Reddy SK, Finlayson SA. Phytochrome B promotes branching in Arabidopsis by suppressing auxin signaling. Plant Physiol. 2014;164(3):1542–50.
39. Montesinos Á, Thorp G, Grimplet J, Rubio-Cabetas M. Phenotyping Almond Orchards for Architectural Traits Influenced by Rootstock Choice. Horticulturae. 2021;7(7):159.
40. Barbier FF, Dun EA, Kerr SC, Chabikwa TG, Beveridge CA. An Update on the Signals Controlling Shoot Branching. Trends Plant Sci. 2019;24(3):220–36.
41. Hill JL, Hollender CA. Branching out: new insights into the genetic regulation of shoot architecture in trees. Curr Opin Plant Biol. 2019;47:73–80.
42. Gautier AT, Merlin I, Doumas P, Cochetel N, Mollier A, Vivin P, et al. Identifying roles of the scion and the rootstock in regulating plant development and functioning under different phosphorus supplies in grapevine. Environ Exp Bot. 2021;185.
43. Tietel Z, Srivastava S, Fait A, Tel-zur N, Carmi N, Id ER. Impact_of_scionrootstock_reciprocal_effects_on_met template proposal. 2020;1–17.
44. Cui X, Ge C, Wang R, Wang H, Chen W, Fu Z, et al. The BUD2 mutation affects plant architecture through altering cytokinin and auxin responses in Arabidopsis.Cell Res. 2010;20(5):576–86.
45. Ge C, Cui X, Wang Y, Hu Y, Fu Z, Zhang D, et al.BUD2, encoding an S-adenosylmethionine decarboxylase, is required for Arabidopsis growth and development. Cell Res. 2006;16(5):446–56.
46. Davies RT, Goetz DH, Lasswell J, Anderson MN, Bartel B. IAR3 encodes an auxin conjugate hydrolase from Arabidopsis. Plant Cell. 1999;11(3):365–76.
47. Widemann E, Miesch L, Lugan R, Holder E, Heinrich C, Aubert Y, et al. The amidohydrolases IAR3 and ILL6 contribute to jasmonoyl-isoleucine hormone turnover and generate 12-hydroxyjasmonic acid upon wounding in arabidopsis leaves.J Biol Chem. 2013;288(44):31701–14.
48. Pierdonati E, Unterholzner SJ, Salvi E, Svolacchia N, Bertolotti G, Dello Ioio R, et al.Cytokinin-dependent control of GH3 group II family genes in the arabidopsis root. Plants. 2019;8(4):1–9.
49. Zhang Z, Li Q, Li Z, Staswick PE, Wang M, Zhu Y, et al. Dual regulation role of GH3.5 in salicylic acid and auxin signaling during arabidopsis-Pseudomonas syringae interaction. Plant Physiol.2007;145(2):450–64.
50. Wu J, Zhu C, Pang J, Zhang X, Yang C, Xia G, et al.OsLOL1, a C2C2-type zinc finger protein, interacts with OsbZIP58 to promote seed germination through the modulation of gibberellin biosynthesis in Oryza sativa. Plant J. 2014;80(6):1118–30.

51. Zhang Y, Schwarz S, Saedler H, Huijser P. SPL8, a local regulator in a subset of gibberellin-mediated developmental processes in Arabidopsis. Plant Mol Biol. 2007;63(3):429–39.
52. Liu B, Zhao S, Li P, Yin Y, Niu Q, Yan J, et al.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. 2021;8(1).
53. Zhou B, Lin J, Peng W, Peng D, Zhuo Y, Zhu D, et al.Dwarfism in Brassica napus L. induced by the over-expression of a gibberellin 2-oxidase gene from Arabidopsis thaliana. Mol Breed. 2012;29(1):115–27.
54. Houben M, Van de Poel B. 1-aminocyclopropane-1-carboxylic acid oxidase (ACO): The enzyme that makes the plant hormone ethylene. Front Plant Sci. 2019;10:1–15.
55. Ruduś I, Sasiak M, Kępczyński J. Regulation of ethylene biosynthesis at the level of 1-aminocyclopropane-1-carboxylate oxidase (ACO) gene. Acta Physiol Plant. 2013;35(2):295–307.
56. Chandler JW, Werr W. A phylogenetically conserved APETALA2/ETHYLENE RESPONSE FACTOR, ERF12, regulates Arabidopsis floral development. Plant Mol Biol. 2020;102(1–2):39–54.
57. Li J, Chen F, Li Y, Li P, Wang Y, Mi G, et al. ZmRAP2.7, an AP2 transcription factor, is involved in maize brace roots development. Front Plant Sci. 2019;10:1–11.
58. Li X, Chen T, Li Y, Wang Z, Cao H, Chen F, et al.ETR1/RDO3 regulates seed dormancy by relieving the inhibitory effect of the ERF12-TPL complex on DELAY OF GERMINATION1 expression. Plant Cell. 2019;31(4):832–47.
59. Neff MM, Nguyen SM, Malancharuvil EJ, Fujioka S, Noguchi T, Seto H, et al. Bas1: A gene regulating brassinosteroid levels and light responsiveness in Arabidopsis. Proc Natl Acad Sci U S A. 1999;96(26):15316–23.
60. Ha S, Vankova R, Yamaguchi-Shinozaki K, Shinozaki K, Tran LSP. Cytokinins: Metabolism and function in plant adaptation to environmental stresses. Trends Plant Sci. 2012;17(3):172–9.
61. Booker J, Sieberer T, Wright W, Williamson L, Willett B, Stirnberg P, et al. MAX1 encodes a cytochrome P450 family member that acts downstream of MAX3/4 to produce a carotenoid-derived branch-inhibiting hormone. Dev Cell. 2005;8(3):443–9.
62. Challis RJ, Hepworth J, Mouchel C, Waites R, Leyser O. A role for More Axillary Growth1 (MAX1) in evolutionary diversity in strigolactone signaling upstream of MAX2. Plant Physiol. 2013;161(4):1885–902.
63. Ruan J, Zhou Y, Zhou M, Yan J, Khurshid M, Weng W, et al. Jasmonic acid signaling pathway in plants. Int J Mol Sci. 2019;20(10).
64. Bruckhoff V, Haroth S, Feussner K, König S, Brodhun F, Feussner I. Functional characterization of CYP94-genes and identification of a novel jasmonate catabolite in flowers. PLoS One. 2016;11(7):1–26.
65. Yang W, Chen S, Cheng Y, Zhang N, Ma Y, Wang W, et al.Cell wall/vacuolar inhibitor of fructosidase 1 regulates ABA response and salt tolerance in Arabidopsis. Plant Signal Behav. 2020;15(4).
66. Portis AR, Li C, Wang D, Salvucci ME.Regulation of Rubisco activase and its interaction with Rubisco. J Exp Bot. 2008;59(7):1597–604.
67. Chen HY, Huh JH, Yu YC, Ho LH, Chen LQ, Tholl D, et al. The Arabidopsis vacuolar sugar transporter SWEET2 limits carbon sequestration from roots and restricts Pythium infection. Plant J. 2015;83(6):1046–58.
68. Eshel A, Beeckman T. Plant roots: the hidden half. CRC press; 2013.
69. Gao J, Zhang T, Xu B, Jia L, Xiao B, Liu H, et al.CRISPR / Cas9-Mediated Mutagenesis of Carotenoid Cleavage Dioxygenase 8 ( CCD8 ) in Tobacco Affects Shoot and Root Architecture. 2018;8.
70. Singh S, Yadav S, Singh A, Mahima M, Singh A, Gautam V, et al. Auxin signaling modulates LATERAL ROOT PRIMORDIUM1 (LRP1) expression during lateral root development in Arabidopsis. Plant J. 2020;101(1):87–100.
71. Zhang Y, Peng D, Song Y, Jin C, Ji J, Wang G, et al.Enhancement of methyl salicylate accumulation promotes early flowering in transgenic tobacco plants by overexpressing a carboxymethyl transferase (SAMT) gene from Lycium chinense. Mol Breed. 2020;40(6).

72. Shen L, Liang Z, Gu X, Chen Y, Teo ZWN, Hou X, et al.N6-Methyladenosine RNA Modification Regulates Shoot Stem Cell Fate in Arabidopsis. Dev Cell. 2016;38(2):186–200.
73. Song JY, Leung T, Ehler LK, Wang C, Liu Z. Regulation of meristem organization and cell division by TSO1, an Arabidopsis gene with cysteine-rich repeats. Development. 2000;127(10):2207–17.
74. Wang W, Sijacic P, Xu P, Lian H, Liu Z. Arabidopsis TSO1 and MYB3R1 form a regulatory module to coordinate cell proliferation with differentiation in shoot and root. Proc Natl Acad Sci U S A. 2018;115(13):E3045–54.
75. Zhao Y, Cheng S, Song Y, Huang Y, Zhou S, Liu X, et al.The interaction between rice ERF3 and WOX11 promotes crown root development by regulating gene expression involved in cytokinin signaling.Plant Cell. 2015;27(9):2469–83.
76. Jurado S, Abraham Z, Manzano C, López-Torrejón G, Pacios LF, del Pozo JC.The arabidopsis cell cycle F-Box protein SKP2A binds to auxin.Plant Cell. 2010;22(12):3891–904.
77. Jurado S, Díaz-Triviño S, Abraham Z, Manzano C, Gutierrez C, Pozo C Del.SKP2A, an F-box protein that regulates cell division, is degraded via the ubiquitin pathway.Plant J. 2008;53(5):828–41.
78. Nahirñak V, Almasia NI, Fernandez PV, Hopp HE, Estevez JM, Carrari F, et al.Potato Snakin-1 gene silencing affects cell division, primary metabolism, and cell wall composition.Plant Physiol. 2012;158(1):252–63.
79. Nahirñak V, Rivarola M, Almasia NI, Barón MPB, Hopp HE, Vile D, et al.Snakin-1 affects reactive oxygen species and ascorbic acid levels and hormone balance in potato. PLoS One. 2019;14(3):1–18.
80. Baena-González E, Hanson J. Shaping plant development through the SnRK1–TOR metabolic regulators. Curr Opin Plant Biol. 2017;35:152–7.
81. Cosgrove DJ. Plant cell wall extensibility: Connecting plant cell growth with cell wall structure, mechanics, and the action of wall-modifying enzymes. J Exp Bot. 2016;67(2):463–76.
82. Meents MJ, Watanabe Y, Samuels AL. The cell biology of secondary cell wall biosynthesis. Ann Bot. 2018;121(6):1107–25.
83. Voiniciuc C, Pauly M, Usadel B. Monitoring polysaccharide dynamics in the plant cell wall. Plant Physiol. 2018;176(4):2590–600.
84. Liu H, Guo Z, Gu F, Ke S, Sun D, Dong S, et al. 4-Coumarate-CoA ligase-like gene OsAAE3 negatively mediates the rice blast resistance, floret development and lignin biosynthesis. Front Plant Sci. 2017;7:1–13.
85. Geng P, Zhang S, Liu J, Zhao C, Wu J, Cao Y, et al. MYB20, MYB42, MYB43, and MYB85 regulate phenylalanine and lignin biosynthesis during secondary cell wall formation1. Plant Physiol. 2020;182(3):1272–83.
86. Ohman D, Demedts B, Kumar M, Gerber L, Gorzsas A, Goeminne G, et al. MYB103 is required for FERULATE-5-HYDROXYLASE expression and syringyl lignin biosynthesis in Arabidopsis stems. Plant J. 2013;73(1):63–76.
87. Gao Y, He C, Zhang D, Liu X, Xu Z, Tian Y, et al.Two trichome birefringence-like proteins mediate xylan acetylation, which is essential for leaf blight resistance in rice. Plant Physiol. 2017;173(1):470–81.
88. Grantham NJ, Wurman-Rodrich J, Terrett OM, Lyczakowski JJ, Stott K, Iuga D, et al. An even pattern of xylan substitution is critical for interaction with cellulose in plant cell walls.Nat Plants. 2017;3(11):859–65.
89. Mortimer JC, Faria-Blanc N, Yu X, Tryfona T, Sorieul M, Ng YZ, et al.An unusual xylan in Arabidopsis primary cell walls is synthesised by GUX3, IRX9L, IRX10L and IRX14. Plant J. 2015;83(3):413–26.
90. Ringli C, Bigler L, Kuhn BM, Leiber RM, Diet A, Santelia D, et al. The modified flavonol glycosylation profile in the Arabidopsis rol1 mutants results in alterations in plant growth and cell shape formation. Plant Cell. 2008;20(6):1470–81.
91. Schumacher I, Ndinyanka Fabrice T, Abdou MT, Kuhn BM, Voxeur A, Herger A, et al. Defects in Cell Wall Differentiation of the Arabidopsis Mutant rol1-2 Is Dependent on Cyclin-Dependent Kinase CDK8. Cells. 2021;10(3):1–16.
92. Gao Y, Xu Z, Zhang L, Li S, Wang S, Yang H, et al. MYB61 is regulated by GRF4 and promotes nitrogen utilization and biomass production in rice. Nat Commun. 2020;11(1):1–12.

93. Ramakrishna P, Duarte PR, Rance GA, Schubert M, Vordermaier V, Vu LD, et al.EXPANSIN A1-mediated radial swelling of pericycle cells positions anticlinal cell divisions during lateral root initiation. Proc Natl Acad Sci U S A. 2019;116(17):8597–602.
94. Molas ML, Kiss JZ. Chapter 1 Phototropism and Gravitropism in Plants. Adv Bot Res. 2009;49(C):1–34.
95. Yadav A, Singh D, Lingwan M, Yadukrishnan P, Masakapalli SK, Datta S. Light signaling and UV-B-mediated plant growth regulation. J Integr Plant Biol. 2020;62(9):1270–92.
96. Su M, Huang G, Zhang Q, Wang X, Li C, Tao Y, et al.The LEA protein, ABR, is regulated by ABI5 and involved in dark-induced leaf senescence in Arabidopsis thaliana. Plant Sci. 2016;247:93–103.
97. Devlin PF, Patel SR, Whitelam GC. Phytochrome E influences internode elongation and flowering time in arabidopsis. Plant Cell. 1998;10(9):1479–87.
98. Sharma A, Sharma B, Hayes S, Kerner K, Hoecker U, Jenkins GI, et al. UVR8 disrupts stabilisation of PIF5 by COP1 to inhibit plant stem elongation in sunlight. Nat Commun. 2019;10(1):1–10.
99. Keuskamp DH, Pollmann S, Voesenek LACJ, Peeters AJM, Pierik R. Auxin transport through PIN-FORMED 3 (PIN3) controls shade avoidance and fitness during competition. Proc Natl Acad Sci U S A. 2010;107(52):22740–4.
100. Wan Y, Jasik J, Wang L, Hao H, Volkmann D, Menzel D, et al. The signal transducer NPH3 integrates the phototropin1 photosensor with PIN2-based polar auxin transport in Arabidopsis root phototropism. Plant Cell. 2012;24(2):551–65.
101. Rawat R, Schwartz J, Jones MA, Sairanen I, Cheng Y, Andersson CR, et al. The circadian clock and auxin pathways. For Genet. 2009;106(39):1–6.
102. Chia TYP, Müller A, Jung C, Mutasa-Göttgens ES. Sugar beet contains a large CONSTANS-LIKE gene family including a CO homologue that is independent of the early-bolting (B) gene locus. J Exp Bot. 2008;59(10):2735–48.
103. Ledger S, Strayer C, Ashton F, Kay SA, Putterill J. Analysis of the function of two circadian-regulated CONSTANS-LIKE genes. Plant J. 2001;26(1):15–22.
104. Jones MA, Morohashi K, Grotewold E, Harmer SL.Arabidopsis JMJD5/JMJ30 acts independently of LUX ARRHYTHMO within the plant circadian clock to enable temperature compensation. Front Plant Sci. 2019;10:1–12.
105. Sawa M, Nusinow DA, Kay SA, Imaizumi T. FKF1 and GIGANTEA complex formation is required for day-length measurement in Arabidopsis. Science (80- ). 2007;318(5848):261–5.
106. Sawa M, Kay SA. GIGANTEA directly activates Flowering Locus T in Arabidopsis thaliana. Proc Natl Acad Sci U S A. 2011;108(28):11698–703.
107. Song YH, Estrada DA, Johnson RS, Kim SK, Lee SY, MacCoss MJ, et al. Distinct roles of FKF1, GIGANTEA, and ZEITLUPE proteins in the regulation of constans stability in Arabidopsis photoperiodic flowering.Proc Natl Acad Sci U S A. 2014;111(49):17672–7.
108. Meisel L, Fonseca B, González S, Baeza-Yates R, Cambiazo V, Campos R, et al.A rapid and efficient method for purifying high quality total RNA from peaches (Prunus persica) for functional genomics analyses. Biol Res. 2005;38(1):83–8.
109. Salzman RA, Fujita T, Zhu-Salzman K, Hasegawa PM, Bressan RA. An Improved RNA Isolation Method for Plant Tissues Containing High Levels of Phenolic Compounds or Carbohydrates. Plant Mol Biol Report. 1999;17(1):11–7.
110. Zeng Y, Yang T. RNA isolation from highly viscous samples rich in polyphenols and polysaccharides. Plant Mol Biol Report. 2002;20(4):5223210.
111. Chang S, Puryear J, and Cairney J. A simple and efficient method for isolating RNA from pine trees. Plant Mol Biol Rep. 1993;11:113–116.
112. Blankenberg D, Gordon A, Von Kuster G, Coraor N, Taylor J, Nekrutenko A, et al. Manipulation of FASTQ data with galaxy. Bioinformatics. 2010;26(14):1783–5.
113. Bolger AM, Lohse M, Usadel B. Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics. 2014;30(15):2114–20.

114. Kim D, Cho YH, Ryu H, Kim Y, Kim TH, Hwang I. BLH1 and KNAT3 modulate ABA responses during germination and early seedling development in Arabidopsis. Plant J. 2013;75(5):755–66.
115. Alioto T, Alexiou KG, Bardil A, Barteri F, Castanera R, Cruz F, et al. Transposons played a major role in the diversification between the closely related almond and peach genomes: results from the almond genome sequence. Plant J. 2020;101(2):455–72.
116. Liao Y, Smyth GK, Shi W. FeatureCounts: An efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics. 2014;30(7):923–30.
117. Robinson MD, McCarthy DJ, Smyth GK. edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics. 2009;26(1):139–40.

# Figures

### Figure 1

**Scion/rootstock combinations showed differences in vigor response.** From left to right: 'Isabelona'/'GN-8', 'Isabelona'/Garnem®, 'Lauranne'/'GN-8' and 'Lauranne'/Garnem®.

### Figure 2

**Principal component analysis (PCA) of the global expression profile data from cultivar samples of the four scion/rootstock combinations.** ISA/GN: 'Isabelona'/Garnem®; ISA/G8: 'Isabelona'/'GN-8'; LAU/GN: 'Lauranne'/Garnem®; LAU/G8: 'Lauranne'/'GN-8'.

### Figure 3

**Principal component analysis (PCA) of the global expression profile data from rootstock samples of the four scion/rootstock combinations.** ISA/GN: 'Isabelona'/Garnem®; ISA/G8: 'Isabelona'/'GN-8'; LAU/GN: 'Lauranne'/Garnem®; LAU/G8: 'Lauranne'/'GN-8'.

### Figure 4

**Venn diagrams of differentially expressed genes (DEGs) for the four scion/roostock combinations. a.** DEGs more expressed in combinations with 'Isabelona' as scion than with 'Lauranne'. **b.** DEGs less expressed in combinations with 'Isabelona' as scion than with 'Lauranne'.

### Figure 5

**Schematic representation of the scion effect in the rootstock hormonal regulation.** + indicates upregulation, while – indicates downregulation. Arrows only in downward direction indicate apical origin. GA: Gibberelic acid; BRs: Brassinosteroids; ET: Ethylene.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryData1.xlsx
- SupplementaryData2.xlsx
- SupplementaryData3.xlsx