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Phosphate Starvation Triggers Transcriptional Changes in the Biosynthesis and Signaling Pathways of Phytohormones in *Marchantia polymorpha* †

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Abstract: Plant hormones are master regulators of developmental and genetic mechanisms to deal with diverse environmental cues. Upon phosphate (Pi) limitation, vascular plants modify phytohormone metabolism to coordinate diverse mechanisms to overcome such stress. However, the transcriptional program underlying hormonal signaling in response to Pi scarcity in early branches of land plant phylogeny, remains unclear. Therefore, we explored the transcriptional dynamics of key genes involved in auxin, cytokinin, ethylene, jasmonate, gibberellin and abscisic acid metabolism in the early divergent land plant *Marchantia polymorpha*, upon Pi starvation. Our RNAseq approach revealed major changes in genes associated with auxin and ethylene biosynthesis. Genes involved in cytokinin synthesis are repressed. Interestingly, genes involved in auxin and ethylene signaling such as Mp*ARF1* and Mp*ARF2* are upregulated. In contrast, Mp*ARRb* is down-regulated. Moreover, genes involved in the synthesis of jasmonates were highly upregulated, but those related to signaling did not change in expression. Our data suggest that auxin and ethylene act as positive regulators of rhizoid development under Pi-limited conditions, whereas cytokinin may act as a negative regulator. The transcriptional behaviour of some hormone-related genes in Marchantia is similar to those described in controlling root hair development in Arabidopsis, Maize and Rice, upon Pi scarcity.

Keywords: Marchantia polymorpha; Pi starvation; Phytohormones and land plant evolution

1. Introduction

During the process of land colonization by plants (470 mya), one of the most challenging conditions was poor nutrient availability on primitive earth crust [1,2]. In this scenario, physico-

chemical properties of phosphate (Pi) reduce the accessible amount for plant uptake [3,4], which allowed the selection of novel morphological and molecular strategies to satisfy its requirements and colonize novel ecosystems. Those biochemical, genetic and morphological adaptations include the symbiosis with mycorrhizal fungi, development of novel plant structures and the rewiring of gene networks to cope with low Pi availability. However, there is a bias to angiosperms in the characterization of PSR along plant phylogeny and little is known in early divergent plants. Those mechanisms have been deeply explored in the flowering plant *Arabidopsis thaliana*, showing that phenotypic modifications in root system architecture (RSA) such as primary root growth inhibition, formation of lateral roots and root hairs elongation are triggered by the external Pi concentrations, defined as the local response (LR) to Pi starvation [5,6]. In contrast, at the molecular level the transcription factor *PHOSPHATE STARVATION RESPONSE 1 (AtPHR1)* activates the expression of multiple target genes involved in Pi uptake, recycling and scavenging, the so called systemic response (SR) [7,8]. The AtPHR1 activity is negatively regulated by the proteins encoding *SIG1-PHO81-XPR1* (*SPX*) domain, by a protein-protein interaction that depends on internal inositol polyphosphate (IPP) concentration [9,10].

Among the mechanisms developed to cope with low Pi, the roles of plant phytohormones have been explored at multiple levels, uncovering highly relevant changes in auxin, ethylene, jasmonate, gibberellin and cytokinin metabolism and signaling [11,12]. Auxin impacts as a positive regulator of morphological changes in RSA, in particular promoting lateral root development and primary root growth inhibition [11]. Under limited Pi conditions *AUXIN RESPONSE FACTOR 19 (AtARF19)* and *TRANSPORT INHIBITOR RESPONSE1 (AtTIR1)* are transcriptionally induced suggesting that TIR1, together with the SCF complex, promotes the ubiquitination of AUXIN/INDOLE-3-ACETIC ACID (AtAUX/IAA) for its degradation, in order to facilitate the transcription of AtARF19 targets [13]. In another hand, the expression of *AtPHR1* is driven by both AUXIN RESPONSE FACTOR 7/19 (AtARF7 and AtARF19) throughout three auxin response elements (AREs) present in the promoter region of *AtPHR1* [14]. Hence, auxin plays important roles in both local and systemic responses of *A. thaliana* to Pi starvation [11,14]. Also, ethylene biosynthetic genes such as *AMINOCYCLOPROPANE-1-CARBOXILATE SYNTHASE 2/4/6* (*AtACS2*/4/6) were found upregulated in Pi-starved Arabidopsis plants [15]. Further, pharmacological and genetic experiments showed that addition of ethylene precursor mimics the RSA phenotype displayed by low Pi [11]. Another study, using ethylene signaling mutants, showed the role of this hormone in root hair development triggered by Pi starvation [16]. Other experiments revealed that ETHYLENE INSENSITIVE 3 (AtEIN3), a transcription factor involved in ethylene signaling, promotes the transcription of *AtPHR1* [17]. In contrast, cytokinin negatively impacts the expression of PSR genes, since exogenous application of kinetin was enough to repress the expression of IPS, however the changes in RSA induced by Pi scarcity were maintained [18]. In addition, mutations on *CYTOKININ RESPONSE 1 (AtCRE1)* and *ARABIDOPSIS HISTIDINE KINASE 4 (AtAHK4)* genes, which code for membrane receptors, impair the transcriptional repression of PSR [19]. In the case of jasmonate, it has been reported that its concentration increases in plants grown under low Pi conditions, which potentiates the tolerance to herbivory attacks [20]. Some genes involved in the biosynthesis and signaling of JA such as *JASMONATE ZIM DOMAIN 10 (AtJAZ10)*, *VEGETATIVE STORAGE PROTEIN 2 (AtVSP2)* and *LIPOXYGENASE 2 (AtLOX2)* are transcriptionally upregulated under low Pi conditions [20]. Moreover, genetic evidence suggests that several JA-responsive genes are also under the control of AtPHR1, some of which participate in the anthocyanin accumulation phenotype observed in plants grown in low Pi [20]. On the other hand, it has been found that the active form of Gibberellin decreases under Pi starvation, the low levels of this hormone allows DELLA transcription factors to participate in the regulation of anthocyanin accumulation and RSA modifications [21]. In contrast, the exogenous addition of gibberellins suppresses the transcription of diverse PSR genes, while mutants in genes involved in GA biosynthesis also impair the expression of genes induced by low Pi, when compared to the Wt [21].

In *M. polymorpha* the phenotypic alterations under Pi starvation include changes in thallus pigmentation by auronodin accumulation, reduction of thallus weight, decreased internal Pi

accumulation and changes in rhizoid development [22,23]. Underlying such morpho-physiological responses are transcriptional changes such as the upregulation of Mp*PHR1,* which codes for the homolog of the major transcriptional regulator of PSR, as well as the upregulation of Mp*MYB14* gene, a master regulator of aronodin biosynthesis, and phosphate transporters encoding genes such as Mp*PHTs* and Mp*PTBs,* among others [23]. However, the transcriptional changes triggered by Pi starvation on genes involved in the biosynthesis and signaling of the diverse hormonal pathways remain poorly understood in the liverworth *M. polymorpha*. Here, we explore the transcriptional changes of such putative orthologous genes, in order to explore the probable role of hormonal fluctuations in the response to Pi starvation in Marchantia. These analyses also allow us to speculate on the conservation or divergence in the response along land plant evolution. The resulting transcriptional patterns of genes involved in auxin, ethylene, cytokinin, gibberellin, abscisic acid and jasmonate pathways revealed that the most relevant changes occur in genes related to auxin, ethylene and cytokinin metabolism. Pi scarcity modulates auxin and ethylene biosynthesis, apparently to promote the synthesis of both hormones in these conditions. We speculate that downstream the transcriptional activation of Mp*ARF1/2* and Mp*ERF* points to promote the expression of theris targets in response to Pi limitation. By contrast the biosynthesis of cytokinins are impaired at transcriptional level and correlates with the down regulation of MpARRb in the same conditions.

2. Methods

(1) Survey of hormone-related homologs in *M. polymorpha*

To determine the transcriptional behavior of genes related to hormone biosynthesis and signaling in *M. polymorpha,* we first search for those genes annotated in the genome version 3.1 (Bowman eta la., 2017) using the Pfam, KEGG or OrthoKEGG identifiers. Then, using the *A. thaliana* sequences from tair.V10 (available on: https://phytozome.jgi.doe.gov/pz/portal.html) were used as a query, we searched by sequence homology using the blast software. As an additional step we compared the conserved domains identified on the Marchantia proteins with the online tool conserved domains available at: https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). Those sequences that did not show conserved domains with their respective *A. thaliana* orthologs were discarded for posterior analyses.

(2) RNA-seq conditions and differential gene expression analysis

We used the RNA-seq data for *M. polymorpha* in low and high Pi conditions previously reported [23]. Briefly, plants were grown in high Pi conditions for 10 days and then transferred to high (500 **µ**M) and low (10 **µ**M) Pi availability on liquid media. Total RNA was sampled at 12, 24 and 150 h post transference (HPT). The differential gene expression for each time was determined with the online server of DNAsubway available on the cyverse platform. We use the MpIDs to search into the table of differential expressed genes induced or repressed in response to low Pi availability. The criteria to define a gene as differentially expressed were FDR < 0.001 and 0.5 > log2FC < -0.5.

(3) Determination of P1BS enrichment

We searched for the consensus sequence P1BS along the promoter regions of each gene, then we performed an enrichment analysis for P1BS. The results were reported previously [23].

3. Results

(1) Pi limitation positive regulates expression of auxin related genes in *M. polymorpha*

The role of auxin on Pi-deprived plants has been extensively studied in angiosperms, mainly the relationship of this hormone with the changes in Arabidopsis SA and the expression of *AtPHR1* under low Pi conditions in *A. thaliana* [24,25]. Perturbations in auxin metabolism, perception and transduction have been associated with primary root growth adjust, increased development and emergence of lateral roots and induction, and elongation of root hairs, all diverse phenotypes altered in response to Pi availability [11]. These are examples of morphological adaptations which lead plants to improve the nutrient assimilation capacity in the richest soil strata [26]. Here, we explore the transcriptional landscape of genes related to auxin biosynthesis, transport and signaling in the rootless plant *M. polymorpha*, under low Pi conditions. We hypothesized that changes observed in the

rhizoids development that occur under low Pi conditions (reference de papers, incluye el tuyo) are part of Marchantia strategies to enhance nutrient uptake, which are underpinned by auxin concentration and signaling. Therefore, we searched for putative homologs related to auxin metabolism and found that genes involved in auxin biosynthesis are miss regulated under Pi starvation. Mp*TAA1* is down-regulated at early times (12 and 24 HPT) and turned up-regulated after 150 HPT. On the other hand the transcripts of Mp*YUC6* are upregulated in the three time points sampled, interestingly we identified two P1BS motives present in the 1 kb promoter region analyzed of this gene. It points to the hypothesis that *MpPHR1* could be involved directly in the positive regulation of auxin biosynthesis under low Pi conditions. In case of auxin transporters two loci were differentially expressed, *MpPIN2* is down regulated three times and *MpPIN3* is down regulated at early times and then shows a strong induction after 150 HPT. In this case, the *MpPIN2* possess two P1BS motives enriched and suggest a role as negative regulator for MpPHR1. Moreover, the MpTIR locus display changes on its expression decreased at 12 HPT, induced at 24 HPT and repressed at 150 HPT. While the MpAUX/IAA1 is induced at early times and downregulated after 150 HPT. Downstream we found the induction of MpARF1/2 at early times and downregulated at 150 HPT which suggest the activation of its direct targets in order to modify the rhizoid development under low Pi. In another hand, taken in consideration the transcriptional induction of AtPHR1 by the AtARF7/19 under low Pi conditions we hypothesized that probably the MpARF1/2 promotes the expression of MpPHR1 under low Pi conditions, similar than occurs in *A. thaliana*.

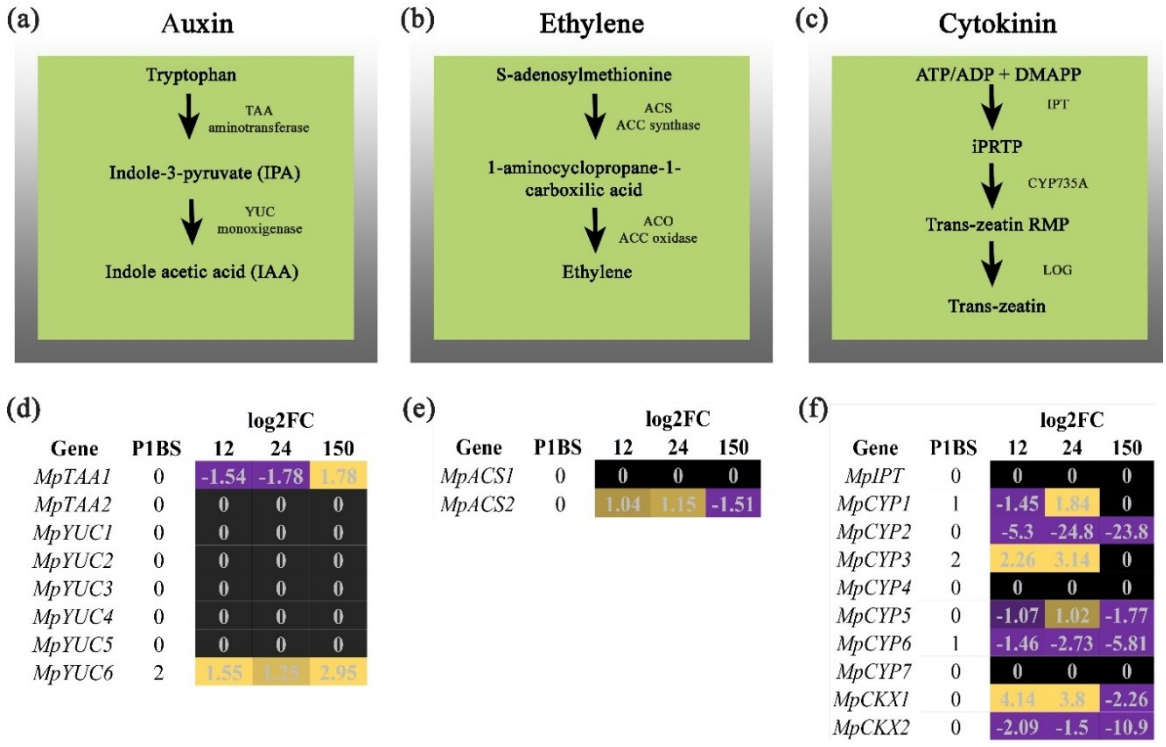
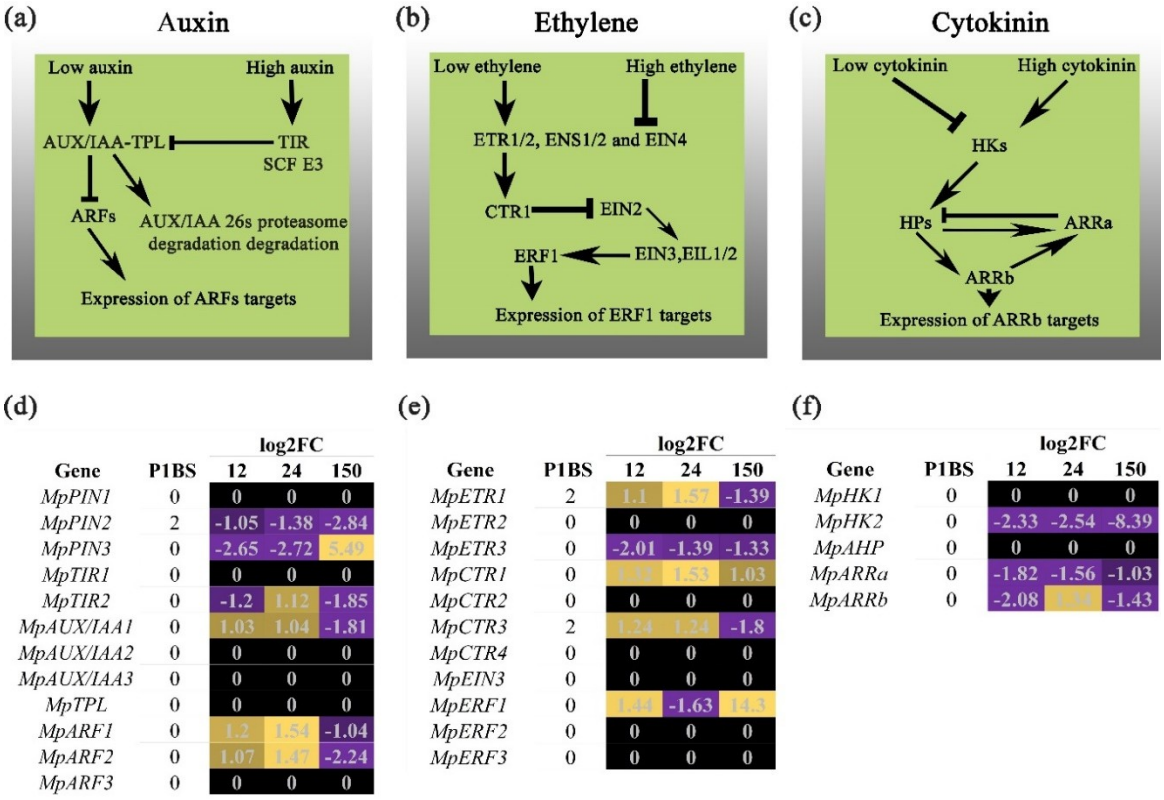


Figure 1. Transcriptional changes of hormonal biosynthetic pathways. Summary of biosynthetic steps of auxin, ethylene and cytokinin (**a**, **b** and **c** panels, respectively). Heatmaps where purple color means downregulated values and yellow shows upregulated genes for auxin, ethylene and cytokinin in panels (**d–f**), respectively. Also, we show genes with P1BS present the 1 Kb promoter region of the respective genes in the heatmap.



(d)

Gene	P1BS	log2FC 12	24	150
MpPIN1	0	0	0	0
MpPIN2	2	-1.05	-1.38	-2.84
MpPIN3	0	-2.65	-2.72	5.49
MpTIR1	0	0	0	0
MpTIR2	0	-1.2	1.12	-1.85
MpAUX/IAA1	0	1.03	1.04	-1.81
MpAUX/IAA2	0	0	0	0
MpAUX/IAA3	0	0	0	0
MpTPL	0	0	0	0
MpARF1	0	1.2	1.54	-1.04
MpARF2	0	1.07	1.47	-2.24
MpARF3	0	0	0	0

(e)

Gene	P1BS	log2FC 12	24	150
MpETR1	2	1.1	1.57	-1.39
MpETR2	0	0	0	0
MpETR3	0	-2.01	-1.39	-1.33
MpCTR1	0	1.32	1.53	1.03
MpCTR2	0	0	0	0
MpCTR3	2	1.24	1.24	-1.8
MpCTR4	0	0	0	0
MpEIN3	0	0	0	0
MpERF1	0	1.44	-1.63	14.3
MpERF2	0	0	0	0
MpERF3	0	0	0	0

(f)

Gene	P1BS	log2FC 12	24	150
MpHK1	0	0	0	0
MpHK2	0	-2.33	-2.54	-8.39
MpAHP	0	0	0	0
MpARRa	0	-1.82	-1.56	-1.03
MpARRb	0	-2.08	1.32	-1.43

Figure 2. Expression of hormonal signaling pathways. Summary of the main genes related to auxin, ethylene and cytokinin signaling (**a**, **b** and **c** panels, respectively). Heatmaps show in purple color the downregulated genes and the upregulated ones in yellow for auxin, ethylene and cytokinin in (**d**–**f**), respectively. We also show those genes that contain the P1BS motif in their promoter regions.

(2) Low Pi activated expression of ethylene signaling genes

It has been reported that Pi starvation impacts ethylene metabolism in *A. thaliana*, changes on its biosynthesis and signaling were linked to the regulation of root hair elongation, also *AtPHR1* transcriptional levels are modulated by ethylene [16,17]. Our results in the search of homologs related to ethylene metabolism unveil that putative genes coding for ACC oxidase (ACO) are absent in the *M. polymorpha* genome while two loci that code for ACC synthase were identified. We found that Mp*ACS2* is up regulated at early times and turned repressed after 150 HPT under low Pi conditions. In the case of ethylene biosynthesis our results were not conclusive, further experiments are necessary to determine the genes involved in the complete enzymatic pathway for ethylene production in Marchantia. Downstream, the putative Marchantia homologs involved in ethylene signaling show transcriptional changes in the expression of four members of ethylene receptors. Mp*CTR1* is induced in the three times sampled, while the *MpETR3* is down regulated in all time points sampled. In addition, the *MpETR1* and *MpCTR3* were upregulated at early times and repressed after 150 HPT. The search for the P1BS reveals that *MpETR1* and *MpCTR3* have two motives on its respective promoter regions, suggesting that MpPHR1 drives the transcriptional changes of these genes in response to low Pi conditions. Finally, the putative homolog for *MpERF1,* is upregulated at 12 HPT, downregulated at 24 FPT and upregulated again at 150 HPT. The transcriptional activation of Mp*ERF1* points to the hypothesis that ethylene biosynthesis and/or perception is promoted by Pi starvation, but how this occurs, and what are the phenotypic consequences of such crosstalk requires additional experiments.

(3) Cytokinin metabolism negatively regulates Pi starvation responses

Pioneering experiments to elucidate the role of cytokinins in the responses of Arabidopsis to cope with low Pi availability revealed that under this nutritional stress the plant changes its metabolism to decrease the concentration of active cytokinin [18]. The addition of exogenous

cytokinin represses the transcription of several genes involved in diverse responses triggered by Pi starvation, supporting the notion that this hormone acts as a negative regulator of such responses [19]. We searched for putative homologs of genes related to CK biosynthesis and signaling, and found one locus coding for IPT, seven loci coding for CYP735 and two coding for cytokinin oxidases. Also, two histidine kinase receptor genes were found, a single gene coding AHP was identified and two genes coding for ARR transcription factors. The transcriptional dynamics for these genes show changes induced by Pi starvation in five members of the CYP735 family. Mp*CYP1* is downregulated at 12 HPT and upregulated after 24 HPT, while the Mp*CYP2/6* are down regulated in the three time points sampled. The transcript levels of Mp*CYP3* increase early times, whilo those of Mp*CYP5* are down regulated at 12 HPT, upregulated at 24 HPT and down regulated again at 150 HPT. One and two P1BS motifs were located in the promoter regions of t Mp*CYP1/6* e and Mp*CYP3*, respectively. Moreover, we found that the transcript levels of Mp*HK2* and Mp*ARRb* are down regulated at three time points sampled while those of Mp*ARRa* are downregulated at 12 HPT, up regulated at 24 HPT and downregulated after 150 HPT. Altogether our data show that although some of the Marchantia homologos involved in cytokinin biosynthesis were upregulated, the major transcriptional regulators such as Mp*ARRa/b* are repressed, suggesting that the negative regulatory effect of CKs in Pi starvation responses could be conserved in *M. polymorpha*.

(4) Other hormonal-related genes regulated by Pi scarcity

Several other plant hormones explored in the context of Pi starvation response are gibberellins (GA), abscisic acid (ABA) and jasmonates [12]. In *A. thaliana* the role of GA and ABA were described as negative regulators of PSR, while the jasmonates are implicated in the induction of ROS and local modification of RSA [12,28]. Among the biosynthetic genes we found several of them deregulated, including the Mp*GA20ox* which expression is upregulated in the three times sampled. The Mp*DELLA2* gene is upregulated at 12 HPT and downregulated after 24 and 150 HPT, similar to what was described in *A. thaliana*. In the case of ABAm we observed that genes involved in the biosynthesis are mainly down regulated under low Pi conditions. Interestingly, Mp*ABI4* is up-regulated at 12 HPT and turn down-regulated after 24 and 150 HPT. In contrast, the genes involved in the biosynthesis of jasmonates are strongly induced in Pi-deprived plants, but those involved in the perception and transcriptional response to this hormone, which include the MYB TFs, do not show differential expression. Only, the Mp*JAZ* gene, which codes for a transcriptional repressor, was induced in the three time points of our transcriptomic approach.

4. Discussion

The signaling cascades and transcriptional programs that rely upstream the morphological and physiological adaptations to deal with low Pi availability have been widely studied in the flowering plant *A. thaliana* [24,25]. In this field, the potential role of hormones as master coordinators of the Pi starvation response is well known, metabolic changes in hormones regulate the modificaations in RSA and affect the expression of genes that are responsive to this nutritional stress [11,27]. However, how this rescue mechanism evolved is poorly understood. In order to gain insight in how the early divergent land plants respond to this nutrient restriction the morphophysiological and transcriptional characterization was performed in the evolutionary model *M. polymorpha* [23]. The phenotypical changes correlate with the transcriptional dynamic underpinning the accumulation of auronidins, changes in thallus size, disminution of internal Pi concentration and developmental modifications of rhizoids [23]. The local and systemic responses were dissected at transcriptional level, but genes related to metabolism of hormones remain unexplored. Here, we search by sequence homology and conserved domains the putative homologs related to hormone metabolism and discuss their transcriptional behavior under low Pi.

Our observation related to auxin biosynthesis and transport points to Pi limitation modulate the biosynthesis and transport, apparently MpPHR1 promotes the induction of *MpYUC6* through two P1BS motives enriched present on its promoter region. The presumably induction of this monooxygenase enzyme, via MpPHR1, links auxin synthesis directly to the PSR. These changes in biosynthetic genes are associated with the induction and elongation of rhizoids, example if this is the

effect of exogenous application of auxin on root hairs which phenocopies the effect of low Pi on trychoblasts [29,30]. In agreement with this, Marchantia auxin insensitive mutants did not show rhizoid alterations in media supplemented with this hormone [29,30]. Also, we found transcriptional changes in the auxin transporters, it is noteworthy the repression of *MpPIN2,* which promoter region posses two P1BS cis regulatory elements. This is in agreement with a previously established hypothesis which suggests that MpPHR1 displays dual role inducing and repressing subsets of genes via its binding to P1BS in a selective manner and depending on the fluctuations of environmental factors, similar to what occurs in *A. thaliana* [23,31]. Another notable finding is the induction of *MpARF1/2* at early times (12 and 24 HPT), suggesting that Pi starvation: (1) increases auxin levels, (2) induces the expression of targets involved in rhizoid development. These observations led us to suggest that MpARFs could be involved in the transcriptional regulation of Mp*PHR1*, as reported for *A. thaliana* upon Pi starvation [13]. The notion of a link between auxin signaling and rhizoid development under low Pi conditions is supported by the fact that loss of function mutants *mparf1-1/2* are impaired in the developmental responses of rhizoids after exogenous application of auxin [29,30]. Hence, the alteration of auxin metabolism under Pi limitation probably allows the rhizoid response via the induction of MpARF targets, including Mp*PHR1*.

In the case of ethylene role in the response of *A. thaliana* to Pi scarcity, changes in the metabolism of this hormone were linked to the elongation of root hairs in Pi starved plants [16]. The inhibition of ethylene synthesis or its promotion, by the addition of silver nitrate or ACC on media respectively, impact the development of these structures in the roots [11]. In this study we were not able to identify a complete biosynthetic pathway in *M. polymorpha*, a putative homolog to AtACO was not found according to our search criteria. This suggests two probable scenarios: (1) the ACC oxidase enzyme in Marchantia may possess non-canonical domains which are not conserved in angiosperms and (2) there is no ACO coding gene in the *M. polymorpha*.genome. Transcriptional changes in other genes, related to ethylene biosynthesis and signaling, suggest that the plant may respond to a crosstalk between Pi starvation and this hormone, However, further experiments are necessary to decipher and understand such molecular interactions. The activation of Mp*ERF1* suggests that some players of the ethylene signaling pathway respond to low Pi availability, perhaps to modulate the elongation of rhizoids similar to what occurs with *A. thaliana* root hairs [16].

In the case of the cytokinins, the antagonic role of this hormone into the low Pi response has been reported [27], Based on our results, we hypothesized that such antagonism is partially conserved in *M. polymorpha*. This is in agreement with the downregulation of key genes such as *MpCYP2/6* and some signaling genes. Moreover, we observed that the *MpCKX1* is up regulated at 12 and 24 HPT suggesting that, in low Pi, cytokinins are inactivated. Previous reports show that the over expression of this enzyme decreases the active cytokinin amount [32]. Interestingly, the characterization of the loss of function mutants for *MpARRb* and the overexpression of *MpARRa* are involved in the regulation of rhizoid development [32]. We found that under low Pi conditions both *MpARRa/b* are down regulated, this correlates with rhizoid modifications observed in response to Pi starvation [23].

Altogether, our results point to a major participation of hormone metabolism and signaling in rhizoid developmental changes in response to low Pi. Our transcriptional analyses suggest that under Pi scarcity auxin and ethylene levels may be increasing, acting as positive regulators of rhizoid development, while the cytokinin levels, which act as negative regulators of root hair development in other species, may be decreasing.

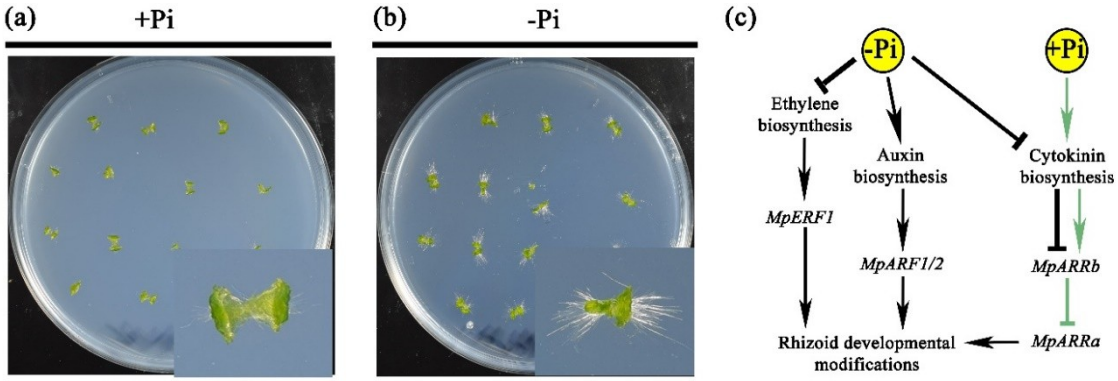


Figure 3. Changes of rhizoid development under low Pi and the putative model of regulation at hormonal level. The phenotype of plants 7 days after sown in high and low Pi availability conditions (panels **a** and **b**, respectively). The putative model of rhizoid development under Pi deprivation shows the induction of auxin biosynthesis and represses the synthesis of ethylene and cytokinin. The *MpERF1* and *MpARF1/2* are induced in low Pi conditions and promote the elongation of rhizoids. While the *MpARRb* is down regulated allowing the activity of *MpARRa* to promote the rhizoid modifications.

5. Conclusions

- Low Pi availability modifies the expression of diverse genes involved in the biosynthesis of auxin, ethylene, cytokinin, gibberellins, abscisic acid and jasmonates.
- Auxin and ethylene probably act as positive regulators of changes observed in rhizoids under Pi scarcity.
- Cytokinin probably acts as a negative regulator of the PSR and rhizoid developmental modifications.
- The induction of Mp*ARF1/2* and *MpERF1* transcription may result in the modification of hormonal levels, promoting the expression of several targets that allow rhizoid elongation.
- The downregulation of Mp*ARRa/b* could participate in the phenotypical changes observed in rhizoids.

Author Contributions: F.R.-R., M.A.A.-V., L.H.-E. and A.C.-R. conceived the project. F.R.-R., L.H.-E. and A.C.-R. designed the experiments. F.R.-R., Z.H.U.D.-S. and M.D.-A. performed the experiments. F.R.-R., Z.H.U.D.-S., M.D.-A., K.I., M.A.A.-V. and A.C.-R. analyzed the data. K.I., A.C.-H., J.L.B., M.A.A.-V., L.H.-E. and A.C.-R. contributed key bioinformatic resources, biological materials, and reagents. F.R.-R. and A.C.-R. wrote the manuscript with inputs from all co-authors. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

Pi	Phosphate
RSA	Root system architecture
SR	Systemic response
IPP	Inositol poly-phosphate
PSR	Phosphate starvation response
Wt	Wild type
μM	Micro molar
HPT	Hours post transference

FDR	False discovery rate
P1BS	PHR1 binding site
Kb	Kilo base

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