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1 TransDetect identifies a new regulatory module controlling phosphate  
2 accumulation

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25 **Abstract**

26

27 Identifying transcription factors (TFs) cooperation controlling target gene expression is still an arduous  
28 challenge. The accuracy of current methods at genome scale significantly drops with the increase in  
29 number of genes, which limits their applicability to more complex genomes, like animals and plants. Here,  
30 we developed an algorithm, TransDetect, able to predict TFs combinations controlling the expression  
31 level of a given gene. TransDetect was used to identify novel TFs modules regulating the expression of  
32 Arabidopsis phosphate transporter *PHO1;H3* comprising MYB15, MYB84, bHLH35 and ICE1. These  
33 TFs were confirmed to interact between themselves and with the *PHO1;H3* promoter. Phenotypic and  
34 genetic analyses of TF mutants enable the organization of these four TFs and *PHO1;H3* in a new gene  
35 regulatory network controlling phosphate accumulation in zinc-dependent manner. This demonstrates the  
36 reliability of TransDetect to extract directionality in non-dynamic transcriptomes and to provide blueprint  
37 to identify gene regulatory network involved in a given biological process.

38

39 **Key words:** Computational biology, transcription factor, mineral nutrition, phosphate, zinc

40 **Short title:** TransDetect reveals key transcription factors involved in Pi homeostasis

41 **Summary:** TransDetect uses non-dynamic transcriptomes to defining regulatory pathways controlling  
42 phosphate accumulation in zinc-deficient plants.

43

## 44 **Introduction**

45 Transcription factors are recognized as important orchestrators of living organisms response to  
46 environmental stimuli. Emerging experimental data indicates that a given gene in the eukaryotic genome  
47 is controlled by a high number of TFs (*e.g.* Xu et al., 2013; Kaufmann et al., 2010a). In plants, techniques  
48 including Chromatin Immuno-Precipitation (ChIP, Kaufmann et al, 2010b; Nagel et al., 2015), DAP-seq  
49 (O'Malley et al., 2016), TARGET (Medici et al., 2015; Para et al., 2014; Bargmann et al., 2013; Doidy et  
50 al, 2016), or Y1H (Brady et al., 2011; Gaudinier et al., 2011; Taylor-Teeple et al., 2015) are consistent  
51 with this potential high number of regulators, define some of the inner features of Gene Regulatory  
52 Networks (GRNs) topology. Knowing for instance that an eukaryotic genome possesses ~2500 TFs, this  
53 presupposes that ~250,000 regulatory connections are pointing towards 30,000 genes. Thus, in average,  
54 a gene is likely to be under the direct influence of ~6 to 40 TFs. Hence complex interactions between TFs  
55 could determine the amplitude of the responses to different stress conditions. Despite its primary  
56 importance, so far our capacity to predict how specific TFs interact and form functional networks to  
57 regulate gene expression level is limited. Very few computational tools are able to predict TFs  
58 combinatorial effects so far. It is worth noting that while the current computational techniques are mainly  
59 based on the detection of co-occurrence of cis-regulatory elements in promoters as well as study of  
60 protein-protein interaction or Chip-Seq data none of these techniques use gene expression *per se* to extract  
61 such potential cooperation (GuhaThakurta and Stormo, 2001, Nagamine et al., 2005, Chang et al., 2006,  
62 Yu et al., 2006, Datta and Zhao, 2008, Qin et al., 2014).

63 In the last few years, attention has been stepped up to develop and use computational tools to  
64 decode complex TFs regulatory network involved in the regulation of complex biological process such as  
65 the coordination of nutrient signalling pathways (Rouached and Rhee, 2017). For instance, an intriguing  
66 coordination between the homeostasis of a macronutrient (phosphate, Pi) and micronutrient (zinc, Zn)  
67 respectively has been recognized in plants: Pi accumulation in the shoots is increased by Zn deficiency (-  
68 Zn) (for review, Bouain et al., 2014; Kisko et al., 2015). Nevertheless, despite its fundamental importance

69 the molecular basis of the over-accumulation of Pi in -Zn conditions remains elusive. In Arabidopsis, the  
70 Pi transporter *PHO1;H3* was identified as an important player in the coordination of Pi and Zn  
71 homeostasis (Khan et al., 2014). *PHO1;H3* transcript abundance specifically increases in roots upon -Zn  
72 treatment (Khan et al., 2014). Plants that do not express *PHO1;H3* accumulate more Pi in the shoots than  
73 wild-type plants. *PHO1;H3* is proposed to be a negative regulator of Pi translocation to the shoot in  
74 response to -Zn (Khan et al., 2014). With these characteristics, *PHO1;H3* constitutes an entry point to  
75 extend our knowledge on the molecular network regulating Pi-Zn signalling crosstalk in plants.

76 In this study, we developed a computational tool, named TransDetect, able to predict TFs  
77 combination controlling a given gene. We used it to identify TFs involved in the regulation of the  
78 expression of *PHO1;H3*. A reverse genetic approach was then used to functionally validate the role of the  
79 identified TFs in regulating: (i) *PHO1;H3* expression (ii) Pi accumulation in shoots in presence or  
80 absence of Zn. The interaction between the TF themselves and with *PHO1;H3* promoter was assessed  
81 using yeast two- and one-hybrid assays, respectively. Overall, this work leads us to validate the  
82 TransDetect method, and to provide a backbone for the establishment of a new regulatory network for Pi  
83 accumulation under -Zn conditions in plants. The uncovered molecular network defines an independent  
84 path from the well-established Pi-starvation signalling pathway (Bari et al., 2006; Lin et al., 2008).

## 85 **Results**

### 86 **TransDetect: a new algorithm identifying potential regulating pair of TFs**

87 In this study, we first undertook a computational approach designed to extract, from transcriptomic data,  
88 potential information concerning TF interaction in the control of a particular gene. The scheme of the  
89 algorithm, written in R (<https://www.r-project.org/>), is depicted in Figure 1. The whole process starts with  
90 the selection by the user of a particular gene (here *PHO1;H3*). Then, the script selects all the TFs having  
91 a putative binding site in the target promoter as described in Katari et al., (2010). This step can be easily  
92 bypassed in order to infer interactions of TFs that may not interact directly with the target gene or interact

93 with a non-canonical DNA sequence. Following this, the algorithm enters into an iterative process (Figure  
94 1). Each pair of TFs expression in a first transcriptomic dataset (named data1) is used to fit the target  
95 expression following the equation:  $\text{Target}_{\text{data1}} = \alpha\text{TF1}_{\text{data1}} + \beta\text{TF2}_{\text{data1}} + \gamma\text{TF1}_{\text{data1}}*\text{TF2}_{\text{data1}} + \varepsilon$ . In this  
96 equation,  $\text{Target}_{\text{data1}}$ ,  $\text{TF1}_{\text{data1}}$ ,  $\text{TF2}_{\text{data1}}$  represents the Target, TF1 and TF2 expression in the first dataset  
97 respectively  $\text{TF1}_{\text{data1}}*\text{TF2}_{\text{data1}}$  represents the potential combinatorial interaction of TF1 and TF2  
98 expressions;  $\alpha$ ,  $\beta$ ,  $\gamma$  represents the coefficients of the linear modeling and  $\varepsilon$  the non-explained variance.  
99 Each model is then evaluated based on two criteria. The first one is that both TF1 and TF2 have to explain  
100 the target expression additionally or in combination. This is performed by filtering on p-values  $< 0.001$   
101 on [the  $\alpha$  and the  $\beta$  coefficients] OR the  $\gamma$  alone. Any model recording the effect of only one TF is  
102 discarded since the rationale is that it can be retrieved by simple correlation network studies. The second  
103 criterion is the capacity of the fitted model to predict the target expression in a second external dataset  
104 (named data2, see Figure 1) that was not used to fit the model.

105 We thus use the fitted model coefficients to predict (without fitting) the Target expression, in the new  
106 conditions (data2), following the equation  $\text{Target}_{\text{data2}} = \alpha\text{TF1}_{\text{data2}} + \beta\text{TF2}_{\text{data2}} + \gamma\text{TF1}_{\text{data2}}*\text{TF2}_{\text{data2}} + \varepsilon$ . It  
107 then evaluates the quality of the prediction by generating the  $R^2$  value for a linear regression between  
108 observed and predicted values (on the second dataset).

109 This process is iterated for all the potential TF pairs. This is necessary to find out the “best” pairs of TFs  
110 that: (i) fit the training dataset and (ii) predict gene expression in the external dataset. These models are  
111 selected if they maximize the sum of the  $R^2$  values (default threshold 15 for the sum of the  $R^2$  0.75 for  
112 simple  $R^2$ ) in the fitted and in the predicted dataset. Thus it is possible to detect potential coordination of  
113 a gene expression by pairs of TFs. Finally each model (pair of TF) is saved. The TFs are finally ranked  
114 based of the number of times they have been found to participate in a model having passed the different  
115 criteria.

116 **MYB15, MYB84 and bHLH35 regulate *PHO1;H3* expression under zinc deficiency**

117 TransDetect was used to identify TFs regulating the expression of the Pi transporter *PHO1;H3* in  
118 Arabidopsis roots. The root transcriptomic dataset used for the learning step (52 data points/Affy Chips)  
119 and the root transcriptomic dataset used for the validation (69 data points/Affy Chips) are publically  
120 available (Brady et al, 2007; Dinneny et al, 2008; Azevedo et al, 2016). Our analysis identified a total of  
121 165 TFs organized in pairs (Table S1) as potential regulators of *PHO1;H3*. The potential TFs network  
122 constructed based on TransDetect analysis (Figure 2) illustrates the candidate TFs pairs to regulate  
123 *PHO1;H3*, and show number of TFs are involved in more than one connection. In this study, the candidate  
124 TFs were ranked based on the number of appearances in significant models. The top ten TFs were  
125 considered for further analyses (Figure 3A)

126 *PHO1;H3* transcript abundance is known to increase in response to  $-Zn$  condition (Khan et al, 2014). We  
127 therefore hypothesized that mutations of these TFs would affect the expression of *PHO1;H3* in response  
128 to  $-Zn$ . For each of the ten selected TFs *PHO1;H3* transcript accumulation was assessed by quantitative  
129 RT-PCR in roots of WT Col-0 plants and of two different T-DNA insertion mutant lines grown in the  
130 presence or absence of Zn for 18 days. As expected (Khan et al, 2014),  $-Zn$  treatment caused a two-fold  
131 increase of *PHO1;H3* transcripts in WT Col-0 plants (Figure 3A). Interestingly, among the twenty  
132 considered mutant lines the loss of function of members of the MYB15/MYB84 and MYB15/bHLH35  
133 TFs pairs affect the *PHO1;H3* expression in  $-Zn$  conditions. Mutation of the two R2R3-MYB TFs *MYB15*  
134 (At3g23250) or *MYB84* (At3g49690) lead to an increase in *PHO1;H3* transcript accumulation in  $-Zn$   
135 (Figure 3 A). This result suggests that these two TFs are negatively regulating *PHO1;H3* expression in  
136 response to a  $-Zn$ . In contrast, mutations in *bHLH35* (At5g57150) lead to a decrease in *PHO1;H3*  
137 transcript accumulation (Figure 3A), revealing a positive regulatory role of *bHLH35* (activator) on  
138 *PHO1;H3* expression in  $-Zn$  condition. *PHO1;H3* transcript accumulation was not significantly altered  
139 in plants harbouring a mutation in any of the other 7 TFs (Figure 3A). It is noteworthy that among these  
140 three TFs only *MYB15* was significantly induced in WT plants grown for 18 days in  $-Zn$  (Figure 3B).

141 We then tested whether *MYB15*, *MYB84* and *bHLH35* could interact with the promoter of *PHO1;H3*

142 (*pPHO1;H3*), using a yeast one-hybrid assay. Interestingly, MYB15 and MYB84 interacted with  
143 *pPHO1;H3* fragments, but bHLH35 did not (Figure 3C). It thus cannot be excluded that MYB15 and  
144 MYB84 regulate *PHO1;H3* expression independently from each other. However, bHLH35 may interact  
145 with *pPHO1;H3* through a partnership with another protein. Indeed, it cannot be ruled out that bHLH35  
146 controls *pPHO1;H3* activity through the activation of an intermediate TF that was not identified using  
147 TransDetect.

#### 148 **MYB15, MYB84 and bHLH35 influence Pi accumulation in the shoot under zinc deficiency**

150 *PHO1;H3* is known to regulate the accumulation of Pi in the shoot of Zn deficient plants (Khan et al,  
151 2014). When grown in Zn-free medium, *pho1;h3* mutant plants display a higher shoot Pi accumulation  
152 than wild-type plants, indicating that PHO1;H3 reduces Pi translocation to the shoot in response to -Zn  
153 (Khan et al, 2014). To check whether changes in *PHO1;H3* transcript accumulation in *myb15*, *myb84*,  
154 and *bhlh45* mutant backgrounds would result in changes in Pi accumulation under Zn deficiency, we  
155 determined the Pi concentration in shoots of WT (Col-0), *myb15*, *myb84*, and *bhlh35* mutant lines grown  
156 in either +Zn or -Zn conditions for 18 days. As expected, Pi concentration was increased in the shoots of  
157 WT plants grown under -Zn (Figure 3D). Then, while mutations in *myb15* and *myb84* resulted in reduced  
158 Pi accumulation in shoots, mutations in *bhlh35* caused an increase in Pi accumulation (Figure 3D). These  
159 variations in shoot Pi concentration were consistent with the variations of *PHO1;H3* transcript abundance.  
160 Our results thus demonstrate the involvement of MYB15, MYB84 and bHLH35 in the regulation of Pi  
161 accumulation in the shoot of plants grown in -Zn conditions.

#### 162 **MYB15/MYB84 and MYB15/bHLH35 interactions are involved in the plant response to -Zn**

163 Since the MYB15/MYB84 and MYB15/bHLH35 TFs pairs were predicted by TransDetect to  
164 cooperatively regulate *PHO1;H3* expression (Figure 4A-B), we tested whether the TFs constituting these  
165 pairs interact physically. Using a yeast two-hybrid assay, we found that MYB15 has the ability to



166 physically interact with MYB84 and with bHLH35 (Figure 4C). We then generated *myb15/myb84* and  
167 *myb15/bhlh35* double KO mutants in Arabidopsis by crossing single mutant lines. Interestingly, when  
168 grown in -Zn, the *myb15/myb84* double mutant showed an increase of the *PHO1;H3* expression level and  
169 a decrease of Pi accumulation in the shoots compared to *myb15*, *myb84* or WT plants (Figure 4D-E). As  
170 already mentioned MYB15 and bHLH35 have opposite effects on both *PHO1;H3* expression and Pi  
171 accumulation. When grown in -Zn, the *myb15/bhlh35* double mutant showed an accumulation of  
172 *PHO1;H3* transcript (Figure 4D) and Pi concentration in shoots similar to what was observed in the  
173 *myb15* single mutant (Figure 4E). These results are indicative that the three TFs likely belong to the same  
174 molecular pathway regulating *PHO1;H3* in response to -Zn. It is likely that in this pathway MYB15 acts  
175 downstream bHLH35 (Figure 4D-E).

#### 176 **ICE1 regulates Pi accumulation under Zn deficiency in a MYB15 dependent manner.**

177 The role of MYB15 in regulating Pi accumulation is new. However, MYB15 has been shown to physically  
178 interact with the MYC-like bHLH TF ICE1 (INDUCER OF CBP EXPRESSION 1) to regulate the plant  
179 response to cold stress (Miura et al, 2007; Agarwal et al, 2006). Through this interaction, ICE1 suppresses  
180 the activity of MYB15 (Miura et al, 2007). It is noteworthy that lowering the stringency of our  
181 TransDetect analysis revealed a potential effect of MYB15 and ICE1 as TFs pair on the expression of  
182 *PHO1;H3* (Figure 5A). We thus tested whether ICE1, individually or cooperatively with MYB15, could  
183 be involved in the regulation of *PHO1;H3* expression. First *ICE1* transcript level was found to be  
184 significantly ~2 fold induced under -Zn (Figure 5B). Then a KO mutation of *ice1* lead to a decrease in  
185 *PHO1;H3* transcript accumulation (Figure 5C) and coupled with an increase in shoot Pi concentration  
186 under -Zn when compared to WT plants (Figure 5D). The *myb15/ice1* double mutant displayed increased  
187 *PHO1;H3* transcript accumulation (Figure 5C) and a decreased Pi concentration in shoots in response to  
188 -Zn, in a similar range as what was observed in the single *myb15* mutant (Figure 5D). Our results thus  
189 indicate that MYB15 most probably acts downstream ICE1 to control Pi accumulation under -Zn *via*

190 *PHO1;H3*.

191 **Discussion.**

192  
193 Gene expression data is increasing with the use of transcriptomic technologies, requiring the development  
194 of methods for their efficient analysis. Combination with functional genomics approaches these  
195 computational tools can help to gain new insight on the molecular mechanisms of complex phenomenon  
196 such as the regulation of ions homeostasis in plants (Mongon et al, 2017; Rouached and Rhee, 2017). In  
197 this context, we developed and used an algorithm, TransDetect, to identify regulators (TFs) and to build  
198 a GRNs that control the expression of a Pi transporter, *PHO1;H3*, in response to -Zn conditions.

199 In Arabidopsis, the *PHO1;H3* gene was demonstrated to be involved in the coordination of Pi and Zn  
200 homeostasis (Khan et al, 2014). The expression of *PHO1;3* is induced in response to -Zn treatments in  
201 roots (Khan et al, 2014). Mutation of *PHO1;H3* causes an overaccumulation of Pi in Arabidopsis shoots  
202 (Khan et al, 2014). Therefore, *PHO1;H3* gene was proposed to plays a negative regulatory role of Pi  
203 transfer from root to shoots in -Zn conditions (Khan et al, 2014). Nevertheless, the GRN that regulates  
204 the expression of *PHO1;H3* in response to -Zn still unknown. Using TransDetect algorithm, we identified  
205 a list of candidate TFs pair for the regulation of the *PHO1;H3* expression. Among the top 10 candidates  
206 revealed by TransDetect, we tested 20 mutant lines (2 mutant per TFs) among which 3 display interesting  
207 phenotypes related to *PHO1;H3* expression (Figure 3 A-B). We believe that this relatively high level of  
208 success could be explained as follow: in general, algorithms focusing on correlation between genes do  
209 not provide any information concerning causality. Say that if 2 genes A and B are highly correlated, it  
210 does not imply if A→B or if B→A. Dynamic aspects can sort between the 2 situations (Krouk et al, 2013).  
211 Unfortunately, the vast majority of the transcriptome in databases are not kinetics. We would like to  
212 mention here that, by the way TransDetect is working, it might extract some directionally information  
213 from static data. To clearly explain this, consider the ideal case where 2 TFs (TFA and TFB) control a  
214 Target gene following a logic gate (Figure 6). In this particular case,  $\gamma$  coefficient of the linear model will  
215 be highly significant because it is the combination of TFA and TFB expression that is necessary to fully

216 explain Target transcript level. On the other hand, it is not possible to infer TFA by a linear combination  
217 of Target and TFB, nor to explain TFB by a linear combination of Target and TFA. Thus, the term of the  
218 equation  $\gamma_{TFA*TFB}$  intrinsically possess some directionality explanatory power. It is important to note  
219 that this directionality will occur only when some interactions between the explanatory variables exist.  
220 Since the TransDeTect algorithm favours models having a significant interaction term between TFs, we  
221 believe that this particularity might explain its availability to infer actual regulators. Thus, we propose  
222 that TransDetect is suitable to discover TFs that coordinate the expression of any gene or set of genes of  
223 interest. It worth noting that although microarray data were used in this study, TransDetect algorithm  
224 could also use value of mRNA level obtained from RNA-seq experiments. While ICE1 and MYB15 was  
225 not detected as TFs pair using our set-up of fitting and predicting dataset, it was detected by lowering the  
226 threshold. Therefore, testing different fitting and predicting dataset set-up on one hand, and changing  
227 threshold on another had, could help reaching conclusion on possible detection TFs pairs. In the frame of  
228 this study, beside *PHO1;H3*, the use of TransDetect and based on TF ranking (counting the number of  
229 times that a particular TF appears in a model), which is a crucial step in the algorithm, known TFs and  
230 their targets could be retrieved. For instance, TransDetect retrieved TF and target gene involved in the  
231 regulation of root development (e.g. DNA BINDING WITH ONE FINGER 53 (DOF53, At5g60200) its  
232 targets *REVOLUTA (REV, At5g60690)* (Brady et al, 2011), secondary cell wall synthesis (e.g.  
233 *ARABIDOPSIS THALIANA HOMOLOG OF E2F C TF (E2Fc, At1g47870)* and its target *ASCULAR*  
234 *RELATED NAC-DOMAIN PROTEIN 7 (VND7, At1g71930)* (Taylor-Teeple et al, 2015), and iron  
235 transport (*POPEYE (PYE, At3g47640)* and its target *IRON-REGULATED TRANSPORTER 1 (IRT1,*  
236 *At4g19690)*) (Long et al, 2010) (Supplementary Figure 1). These data further support the utility of  
237 TransDetect to detect TFs. The validation of detected TFs require *in planta* and biochemical testing, which  
238 would include the analysis of expression profiles of the target genes in different plant genetic backgrounds  
239 (eg wild-type plants, knock-out mutants /or overexpressing lines). As performed in this work,  
240 transcriptionally linked TFs and promoter of target genes could be further tested for their possible direct

241 interaction.

242

243 Current understanding of adaptive mechanisms regulating Pi homeostasis in plants comes from

244 investigations conducted mainly in *Arabidopsis thaliana* under Pi limitation. From twenty years of

245 research only a handful of TFs (for review Jain et al, 2012) and only one complete Pi signalling pathway,

246 the “PHR1- miR399-PHO2” pathway (Bari et al, 2006; Lin et al, 2008) have been discovered.

247 Nevertheless, as aforementioned, Pi accumulation in shoot is altered when plants are challenged by Zn

248 limitation, and this alteration is not dependent from the “PHR1-miR399-PHO2” regulatory pathway

249 (Khan et al, 2014). These observations indicate the existence of specific regulatory pathway(s) underlying

250 this Zn-Pi relationship (Khan et al, 2014). Unfortunately, available tools do not offer the possibility to

251 identify the TF pairs involved in such nutrient homeostasis coordination. Thanks to TransDetect used to

252 search for TFs controlling Pi homeostasis through the examination of the publically available

253 transcriptomic data sets with *PHO1;H3* as target gene we identified four new TF (MYB15, MYB84,

254 bHLH35 ICE1) displaying a striking phenotype with regards to both *PHO1;H3* expression and shoot Pi

255 accumulation in plant grown under -Zn. Considering that only a handful of TFs involved in the

256 transcriptional control of plant response to Pi or Zn deficiency were described before this study (Assunção

257 et al, 2010; Jain et al, 2012; Khan et al, 2014), one can consider that in this regard the success rate of this

258 strategy - combining TransDetect together with functional genomics approaches - is high. More

259 importantly, integration of these data provide blueprint for defining novel regulatory pathway controlling

260 Pi homeostasis in plants. Using the phenotypic data obtained from the characterisation of single and

261 double mutant TF mutants effect on the expression of *PHO1;H3* it was possible to propose a new

262 regulatory transcriptional module regulating Pi accumulation in shoot of Zn-deficient plants (Figure 7).

263 In this module, MYB15, MYB84 are likely to play a negative regulatory role on the expression of

264 *PHO1;H3*, while ICE1 bHLH35 plays a positive regulatory role upstream MYB15 (Figure 7). This work

265 thus lead to the identification of new key players that act in the -Zn signalling pathways to control the

266 expression of *PHO1;H3* and Pi accumulation in plants, which is indeed independent of the PHR1-  
267 miR399-PHO2 pathway constituents.

268  
269 In conclusion, this work identified four new TFs acting to regulate Pi accumulation in Arabidopsis in  
270 response to Zn deficiency. Prediction using TransDetect was validated using three different strategies  
271 First, molecular and genetic evidences showing the involvement of the selected TFs in modulating  
272 *PHO1;H3* expression in response to  $-Zn$ . Second, yeast-one and two-hybrid experiments shows that these  
273 TFs can interact with the *PHO1;H3* promoter, that they are able to form TF pairs. Finally, these TF pairs  
274 are involved in the regulation of Pi accumulation in plants under  $-Zn$  conditions. The method developed  
275 in the frame of this work should benefit to other studies aiming at identifying TFs cooperatively regulating  
276 a gene(s) expression, and to dissect regulatory pathway(s) controlling an important biological  
277 phenomenon.

## 278 **Materials Methods**

### 280 **Algorithm**

281 The TransDetect algorithm as been written in R (<https://wwwr-project.org/>) and it follows the exact logic  
282 described in Figure 1. The R code is available at  
283 <https://sites.google.com/site/gabrielkroukresearch/transdetect>. The running time is about 20 minutes per  
284 target gene on a desktop Apple Mac Pro computer with parallelized computation on 32 CPUs. The  
285 transcriptomic dataset used is from the Benfey lab (Brady et al, 2007; Dinneny et al, 2008; Azevedo et al,  
286 2016) and has been spited arbitrarily into fitting (52 chips) and predicting dataset (69 chips).

### 287 **Plant materials and growth conditions**

288 The *Arabidopsis thaliana* mutants used in all experiments were in the Columbia (Col-0) genetic  
289 background. The previously described *ice1-2* (At3g26744) mutant (Denay et al, 2014) was provided by  
290 Dr Gwyneth Ingram (ENS, Lyon, France). T-DNA insertion mutant lines for considered TFs At5g57150  
291 (line1: N516841 and line2: N536664); At3g23250 (line1: N651976 and line2: N491226); At3g49690

292 (line1: N641918 and line2: N612398); At4g37790 (N585964, N2100629); At2g46510 (N587068,  
293 N867699); At1g31050 (N595172; N545538); At5g04760 (N2101152, N2104259); At4g31800  
294 (N550079, N871514); At4g24060 (N652104, N504243) and At3g50060 (N567655, N555373) were from  
295 Nottingham Arabidopsis Stock Centre (Alonso et al, 2003). The presence of a T-DNA insertion within  
296 the TF gene and absence of transcripts of the mutated TFs were checked using the appropriate PCR  
297 strategy using PCR primers listed in Table S2. Double mutant lines *myb15/myb84*, *myb15/bhlh35*, and  
298 *ice1/myb15* were generated through crossing. Homozygosity of the generated double mutants was  
299 confirmed through appropriate PCR strategy as performed for the identification of the single mutants.  
300 Plants were germinated grown in vertical position on 1% agar-solidified media (A1296, Sigma) The  
301 complete nutrient medium contained 05 mM KNO<sub>3</sub>, 1 mM MgSO<sub>4</sub>, 1 mM KH<sub>2</sub>PO<sub>4</sub>, 025 mM Ca(NO<sub>3</sub>)<sub>2</sub>,  
302 100 μM NaFeEDTA, 30 μM H<sub>3</sub>BO<sub>3</sub>, 10 μM MnCl<sub>2</sub>, 1 μM CuCl<sub>2</sub>, 15 μM ZnSO<sub>4</sub>, 01 μM (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>,  
303 50 μM KCl, pH 5.7. Zn-free medium was made by removing the only source of Zn (ZnSO<sub>4</sub>), by washing  
304 the agar Seeds were sown on the plates stratified at 4 °C in the dark for 3 d. Plates were then transferred  
305 in a growth chamber for 18 d, day 1 of growth being defined as the first day of exposure of stratified seeds  
306 to light. Plants were grown under long-day conditions (16/8h light/dark cycle, 250 μmol·m<sup>-2</sup>·s<sup>-1</sup>,  
307 24/20°C).

#### 308 309 **Phosphate quantification**

310 Shoots and roots were collected separately. Shoots were weighed and ground into powder in liquid  
311 nitrogen, then incubated at 70 °C for ½ hour. The determination of Pi concentrations in these tissues was  
312 performed using the Ames methods (1996). For every measurement, three to five biological replicates (3  
313 plants per sample) were performed, leading to three to five corresponding samples.

#### 314 315 **Real-time quantitative reverse-transcription PCR**

316 Roots of 18-day-old plants grown on different medium composition in the presence or absence of Zn were  
317 collected for gene expression analysis. Total RNA was extracted from 100 mg frozen roots using Plant

318 RNeasy extraction kit (Qiagen) and RQ1 RNase-free DNase (Promega). Two  $\mu\text{g}$  of total RNA were used  
319 to synthesize cDNA using poly-A oligos. Real-time quantitative reverse-transcription PCR (RT-qPCR)  
320 was performed with a Light Cycler 480 Real-Time PCR System using SYBR green dye technology  
321 (Roche) as described previously (Rouached et al, 2011). The *PHO1;H3* transcript abundance were  
322 quantified using quantitative real time PCR using specific primers listed in Table S2, which showed an  
323 efficiency (E) of  $100\% \pm 3\%$ . E was determined after the analysis of serial 1:10 dilutions of a plasmidic  
324 solution of each target gene by using the equation  $E = [(10^{-1/s}) - 1] \cdot 100$ . In this equation “s” represent the  
325 slope of the linear regression of the threshold cycle ( $C_T$ ) values per the  $\log_{10}$  values of DNA copy numbers  
326 used for PCR reactions (Rouached et al, 2008). Relative transcripts levels was quantified using the  
327 comparative threshold cycle ( $C_T$ ) method (Livak Schmittgen, 2001). For every data point,  $C_T$  value was  
328 the average of the  $C_T$  values obtained from the triplicate PCR analysis. For each gene, the relative amount  
329 of calculated mRNA was normalized to the level of the control gene *Ubiquitin10* (*UBQ10*: At4g05320)  
330 and expressed as relative values against wild-type plants grown in reference treatment (RT: +Zn) medium.  
331 For example, relative gene expression of the *PHO1;H3* genes  $\Delta C_{T,PHO1;H3}$  was expressed following  
332 normalization against the average of the  $C_T$  values obtained for the gene used for stardization:  $\Delta C_{T,PHO1;H3}$   
333 =  $C_{T,PHO1;H3} - C_{T,UBQ10}$  (Livak Schmittgen, 2001). For treatment of interest (TOI: -Zn) was compared to  
334 a reference treatment (RT: +Zn), the relative expression of a *PHO1;H3* gene was expressed as a  $\Delta\Delta C_t$   
335 value calculated as follows:  $\Delta\Delta C_t = \Delta C_{T,TOI} - \Delta C_{T,RT}$  (Livak Schmittgen, 2001). The fold change in  
336 relative gene expression was determined as  $2^{-\Delta\Delta C_t}$ . Using this method, +Zn values were normalized to 1.  
337 The methodology apply for the analysis of relative expression of the other genes: *MYB15*, *ICE1*, *BHLH35*  
338 and *MYB84*.

### 339 **Yeast experiments**

340 All the PCR products were obtained using high-fidelity Phusion DNA polymerase. The constructs were  
341 sequenced to ensure their integrity. All primers used for yeast one-hybrid (Y1H) and two-hybrid (Y2H)

342 experiments are described in table S2. For Y1H experiments bHLH35 (At5g57150) cDNA was PCR-  
343 amplified from a pool of Columbia (Col-0) cDNA using the cbHLH35-B1 and cbHLH35-B2 primers,  
344 introduced into the pDONR207 vector (BP recombination, Gateway®), and then recombined into the  
345 pDEST22 vector (LR recombination, Gateway®) allowing the expression of bHLH35 fused to the GAL4  
346 activation domain (AD) in yeast pDEST22. Clones containing MYB15 (At3g23250) and MYB84  
347 (At3g49690) were obtained from a previous study (Kelemen et al, 2015). In order to assess if bHLH35,  
348 MYB15 and MYB84 could interact with the different MYB (seven) and bHLH (one) putative binding site  
349 present on the *PHO1;3* promoter, each one was separately cloned as hexamers into the pHis-LIC vector  
350 (Kelemen et al, 2015). Subsequent interaction assays were carried out as described in (Dubos et al, 2014).  
351 For Y2H experiments, bHLH35, MYB15 MYB84 were LR recombined into pDEST32 allowing fusion  
352 with the GAL4 DNA binding domain (BD). Each pDEST22 and pDEST32 vector containing either  
353 bHLH35, MYB15 or MYB84 were transformed alone or in two-by-two combination into yeast (AH109  
354 strain, Clontech). Subsequent steps were carried out accordingly to manufacturer's instructions using the  
355 ADE2 HIS3 reporter genes (Clontech).

### 356 357 **Statistical analysis**

358 Statistical differences between genotypes were calculated using t-test analyses and ANOVA with  
359 subsequent post hoc tests using Graphpad Prism (GraphPad Software Inc, San Diego, CA, USA) or  
360 Microsoft Excel (Microsoft, USA).

### 361 362 **Supplemental Data**

363 **Table S1** AGI number occurrence in TransDetect analysis for *PHO1;H3* (At1g14040).  
364

365 **Table S2** List of primers used in this work.

366 **Supplemental Figure 1.** TransDetect prediction of regulators for *REVOLUTA*, *ASCULAR RELATED*  
367 *NAC-DOMAIN PROTEIN 7* and *IRON-REGULATED TRANSPORTER 1*.



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370 Stanford, CA, USA) for very helpful discussions.

371

372 **Figures Legends**

373 **Figure 1. Scheme of TransDetect algorithm organization.** The algorithm is built on a inference  
374 iterative process. First the target gene transcript level is fitted by a linear combination of the transcript  
375 levels of two transcription factors (TFs). The resulting model is kept only if the two TFs significantly  
376 participate in the fit. The learnt coefficients are then used to predict the transcript levels of the target gene  
377 in an independent dataset. If the model is able to properly fit the transcript levels of the target gene in the  
378 first dataset and predict them in the second dataset, the corresponding TF pair is kept. A final list of  
379 selected TF pairs is generated and TFs are ranked based on the number of times they appear in this list.

380  
381 **Figure 2. TransDetect network potentially influencing *PHO1;H3* gene expression.** Each node  
382 represent a potential *PHO1;H3* regulator based on the TransDetect criteria defined in the text. If a pair of  
383 TF is predicted to explain *PHO1;H3* expression it is linked by an edge. The edge width is proportional to  
384 the sum of the  $R^2$  for the fit and predict processes (values are ranging from 16 to 176). The most influential  
385 factors are likely to be the most connected.

386  
387 **Figure 3. MYB15, MYB84 and bHLH35 regulate both the expression of *PHO1;H3* and the**  
388 **accumulation of Pi in the shoot under zinc deficiency. A-** Relative *PHO1;H3* (At1g14040) transcript  
389 accumulation in the roots of wild-type plants (Col-0) and mutant lines harbouring loss-of-function  
390 mutations in the following transcription factors: At5g57150 (*bHLH35*), At3g23250 (*MYB15*), At3g49690  
391 (*MYB84*), At4g37790 (*HAT22*), At2g46510 (*bHLH17*), At1g31050 (*bHLH111*), At5g04760 (*MYB-type*),  
392 At4g31800 (*WRKY18*), At4g24060 (*Dof46*) and At3g50060 (*MYB77*). Plants were grown for 18 days in  
393 the presence (+Zn) or absence (-Zn) of zinc. *PHO1;H3* transcript abundance was measured by qRT-PCR  
394 normalized against *UBQ10* (At4g05320). **B-** *MYB15*, *MYB84* and *bHLH35* transcripts accumulation in  
395 response to Zn deficiency. Relative *MYB15*, *MYB84* and *bHLH35* transcript accumulation was quantified  
396 in roots of wild-type plants (Col-0) grown for 18 days in the presence or absence of Zn by qRT-PCR and  
397 normalized against *UBQ10*. **C-** Yeast one-hybrid assay. Sequences of the Arabidopsis *PHO1;H3*

398 promoter fused to the *HIS3* auxotrophic marker were stably transformed into yeast. These different yeast  
399 strains were then co-transfected with MYB15, MYB84 or bHLH35. Left panel, growth of the different  
400 yeast strains on control media deprived of tryptophan (-W), allowing the selection of yeast cells  
401 expressing the selected TFs. Right panel, growth of the different yeast strains on selective media deprived  
402 of tryptophan and histidine (-W -H). **D-** Pi concentrations measured in the shoots of wild type, *myb15*,  
403 *myb84*, or *bhlh35* plants grown for 18 days in the presence or absence of Zn. For A, C and D panels,  
404 central lines in the boxes show the medians; box limits indicate the 25th and 75th percentiles; whiskers  
405 extend 15 times the interquartile range from the 25th and 75th percentiles Letters a, b and c indicate  
406 significantly different values at  $p < 0.05$  determined by one-way ANOVA and Tukey HSD.

407  
408 **Figure 4. Interactions between MYB15 and MYB84 and between MYB15 and bHLH35 influence**  
409 **the expression of *PHO1;H3* Pi accumulation under zinc deficiency.** **A, B-** TransDetect prediction of  
410 correlation between the expression of the *MYB15 / MYB84* and *MYB15 / bHLH35* TF pairs and the  
411 *PHO1;H3* transcript level with  $R^2=0.75$  and  $R^2=0.84$  respectively. **C-** Yeast two-hybrid assay bHLH35,  
412 MYB15 and MYB84 were fused with either the GAL4 DNA binding domain (BD) or the GAL4 activation  
413 domain (AD) into appropriate expression vectors, which were then transferred into yeast. The different  
414 yeast strains were plated on non-selective medium (NS) or on selective media deprived of histidine (-  
415 His), adenine (-Ade) or both simultaneously (-His-Ade). **D-** Relative *PHO1;H3* transcript accumulation  
416 in roots of wild type (Col-0), *bhlh35*, *myb15*, *myb84*, *myb15/myb84* *myb15/bhlh35* mutant plants grown  
417 for 18 days in the absence of zinc (-Zn) compared to +Zn. *PHO1;H3* transcript abundance was measured  
418 by qRT-PCR and normalized against *UBQ10*. **E-** Shoot Pi concentrations measured in wild-type (Col-0),  
419 *bhlh35*, *myb15*, *myb84*, *myb15xmyb84* and *myb15xbhlh35* mutant plants grown on either +Zn or -Zn for  
420 18 days. For D and E panels, Box central lines show the medians; box limits indicate the 25th and 75th  
421 percentiles; whiskers extend 15 times the interquartile range from the 25th and 75th percentiles Letters a,  
422 b and c indicate significantly different values at  $p < 0.05$  determined by one-way ANOVA and Tukey HSD.

423  
424 **Figure 5. The ICE1 / MYB15 transcription factor pair regulates both the expression of *PHO1;H3***  
425 **the accumulation Pi under -Zn. A-** TransDetect's prediction of the correlation between the expression  
426 of the TF pair MYB15 and ICE1 and the *PHO1;H3* expression ( $R^2=0,73$ ). **B-** *ICE1* transcript  
427 accumulation. Expression of *ICE1* was quantified in wild-type (Col-0) seedlings grown for 18 days in  
428 presence (+Zn) or absence (-Zn) of zinc. *ICE1* transcript abundance was measured by qRT-PCR  
429 normalized against *UBQ10*. **C-** *PHO1;H3* transcript accumulation. Expression of *PHO1;H3* gene was  
430 quantified in wild type (Col-0), *ice1*, *myb15* and *myb15/ice1* seedlings grown for 18 days in +Zn or -  
431 Zn. *PHO1;H3* transcript abundance was measured by qRT-PCR and normalized against *UBQ10*. **D-** Pi  
432 accumulations. Pi concentrations were measured from shoots of wild type (Col-0), *ice1*, *myb15*, and  
433 *myb15/ice1* seedlings grown for 18 days in presence +Zn or -Zn. For B, C and D, Box center lines show  
434 the medians; box limits indicate the 25th and 75th percentiles; whiskers extend 15 times the interquartile  
435 range from the 25th and 75th percentiles. Letters a, b and c indicate significantly different values at  $p<005$   
436 determined by one-way ANOVA and Tukey HSD.

437  
438 **Figure 6. Idealized model to explain how TransDetect extract directionality in static data. A-** Two  
439 transcription factors TFA and TFB positively control the expression of a Target gene TA following a  
440 AND logic-gate. **B-** TA expression is induced only when TFA and TFB expression are both upregulated.  
441 **C-** Linear modelling of TA expression. Considering the ideal case where 2 transcription factors (TFA and  
442 TFB) control a target gene TA following a AND logic-gate. In this particular case,  $\gamma$  coefficient of the  
443 linear model will be highly significant because it is the combination of TFA and TFB expression that is  
444 necessary to fully explain TA expression. On the other hand, it is not possible to infer TFA by a linear  
445 combination of TA and TFB, nor to explain TFB by a linear combination of TA and TFA. Thus the term  
446 of the equation  $\gamma TFA * TFB$  intrinsically possess some directionality explanatory power in this case where  
447 both TFs interact in the control of TA.

448  
449 **Figure 7. Schematic representation of the MYB15, MYB84, bHLH35 and ICE1 regulatory module**  
450 **controlling *PHO1;H3* gene expression and Pi accumulation in shoots under zinc deficiency.**  
451 Phosphate increases in the shoots of plants exposed to zinc deficiency. *PHO1;H3* plays a negative  
452 regulatory role in this process. Red solid lines indicate connections between MYB15, MYB84, bHLH35  
453 and ICE1. Negative and positive regulatory effects of these transcription factors on *PHO1;H3* expression  
454 under zinc deficiency are indicated by flat-ended dashed lines and arrowheads, respectively. a indicates  
455 previous knowledge on ICE1 and MYB15 physical interaction.

456 **Supplementary Figure 1. TransDetect prediction of regulators for *REVOLUTA*, *ASCULAR***  
457 ***RELATED NAC-DOMAIN PROTEIN 7* and *IRON-REGULATED TRANSPORTER 1*.** TransDetect  
458 was used to predict regulators for the following target genes A- *REVOLUTA* (*REV*, At5g60690), B-  
459 *ASCULAR RELATED NAC-DOMAIN PROTEIN 7* (*VND7*, At1g71930), and C- *IRON-REGULATED*  
460 *TRANSPORTER 1* (*IRT1*, At4g19690). Among number of TFs predicted, TransDetect retrieved known  
461 regulators for these genes, namely DNA BINDING WITH ONE FINGER 53 (*DOF53*, At5g60200) for  
462 *REV*, ARABIDOPSIS THALIANA HOMOLOG OF E2F C TF (*E2Fc*, At1g47870) for *VND7*, and  
463 POPEYE (*PYE*, At3g47640) for *IRT1*.

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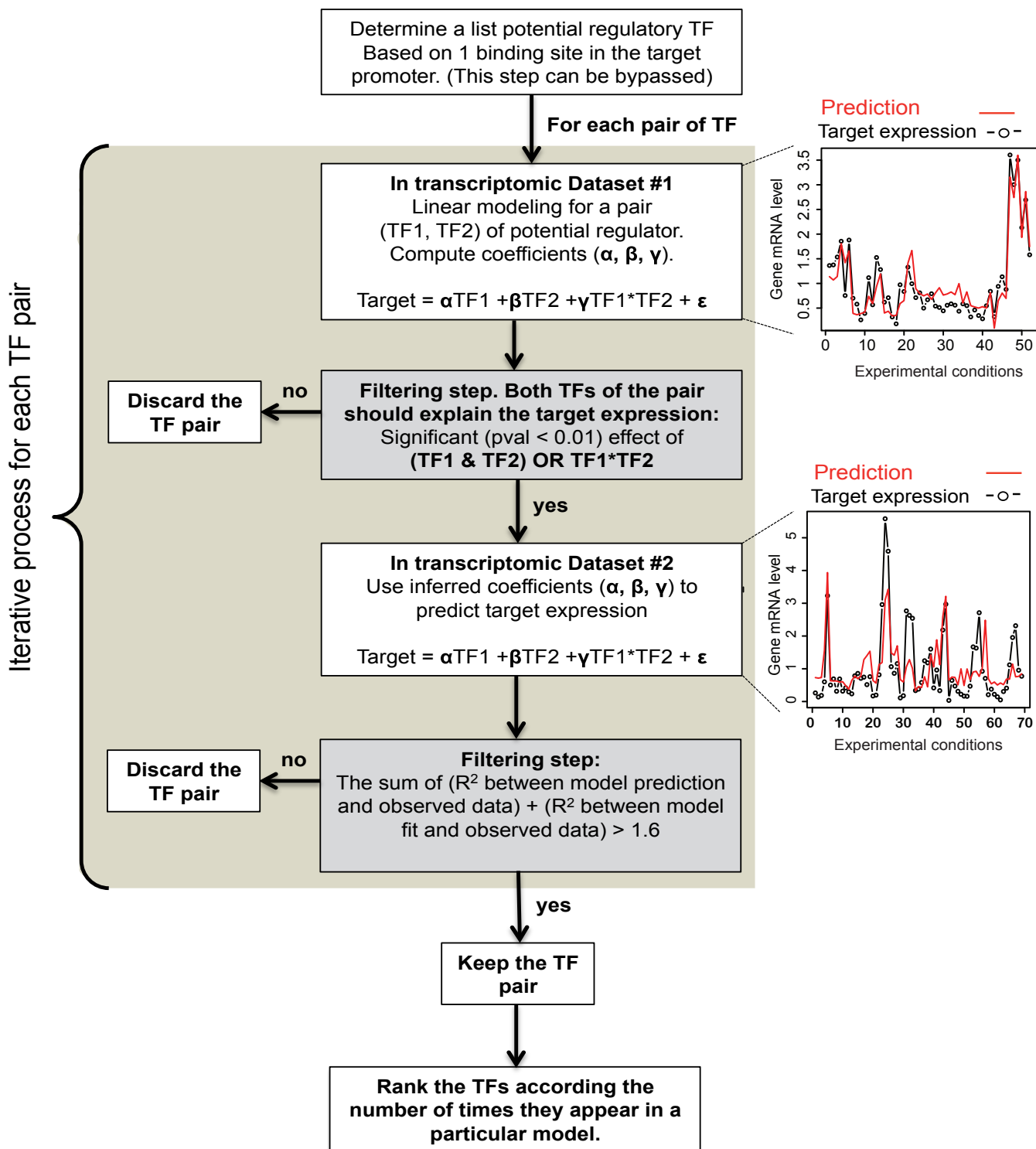
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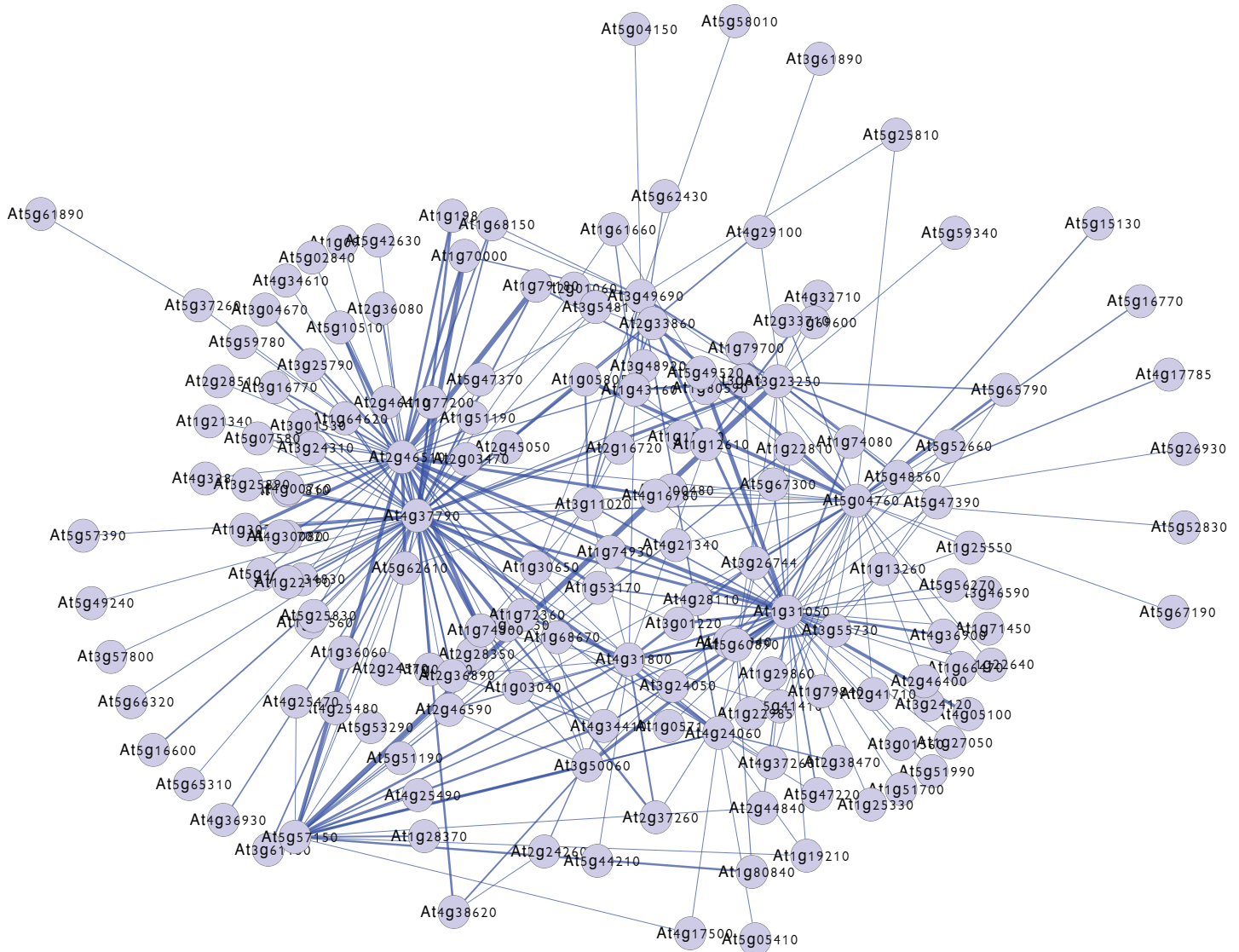


Figure 1



**Figure 1. Scheme of TransDetect algorithm organization. The algorithm is built on an inference iterative process.** First the target gene transcript level is fitted by a linear combination of the transcript levels of two transcription factors (TFs). The resulting model is kept only if the two TFs significantly participate in the fit. The learnt coefficients are then used to predict the transcript levels of the target gene in an independent dataset. If the model is able to properly fit the transcript levels of the target gene in the first dataset and predict them in the second dataset, the corresponding TF pair is kept. A final list of selected TF pairs is generated and TFs are ranked based on the number of times they appear in this list.

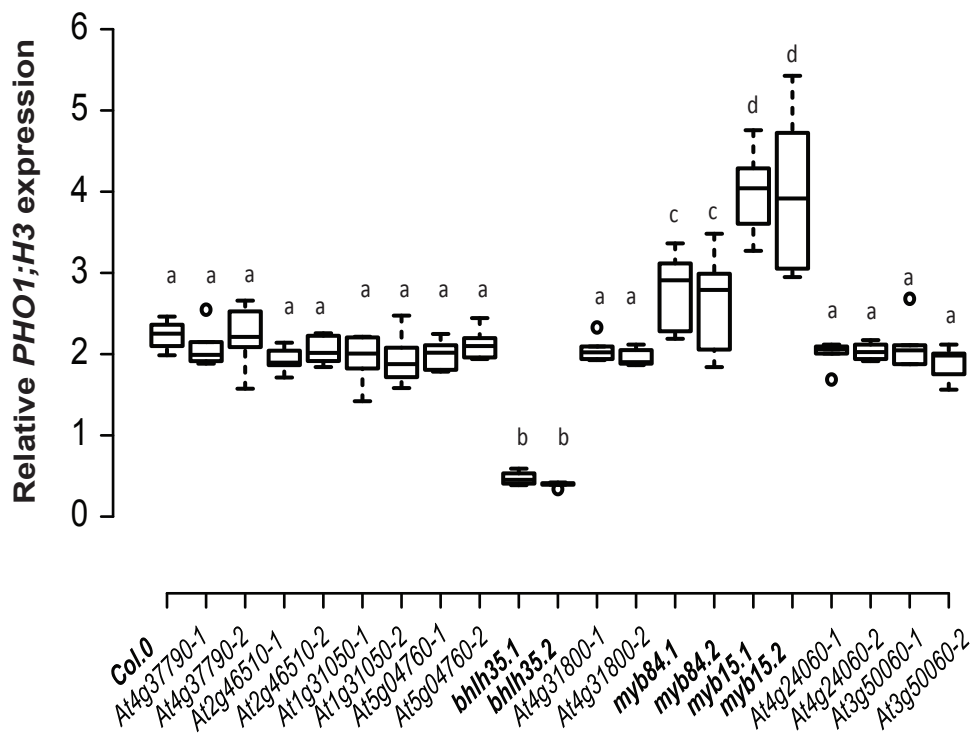
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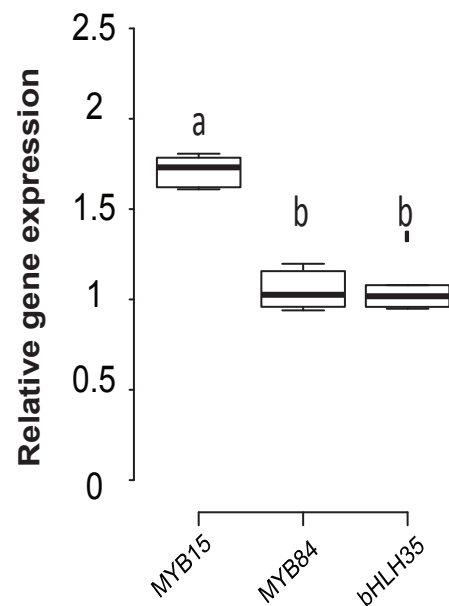
**Figure 2. TransDetect network potentially influencing *PHO1;H3* gene expression.** Each node represent a potential *PHO1;H3* regulator based on the TransDetect criteria defined in the text. If a pair of TF is predicted to explain *PHO1;H3* expression it is linked by an edge. The edge width is proportional to the sum of the R2 for the fit and predict processes (values are ranging from 16 to 176). The most influential factors are likely to be the most connected.

Figure 3

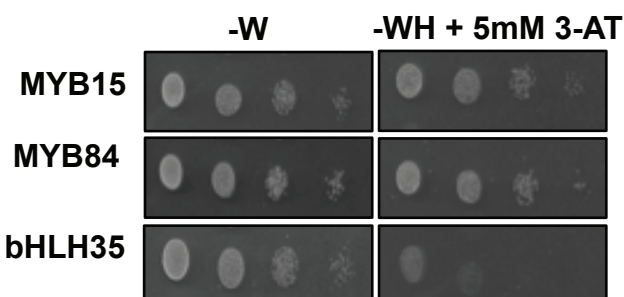
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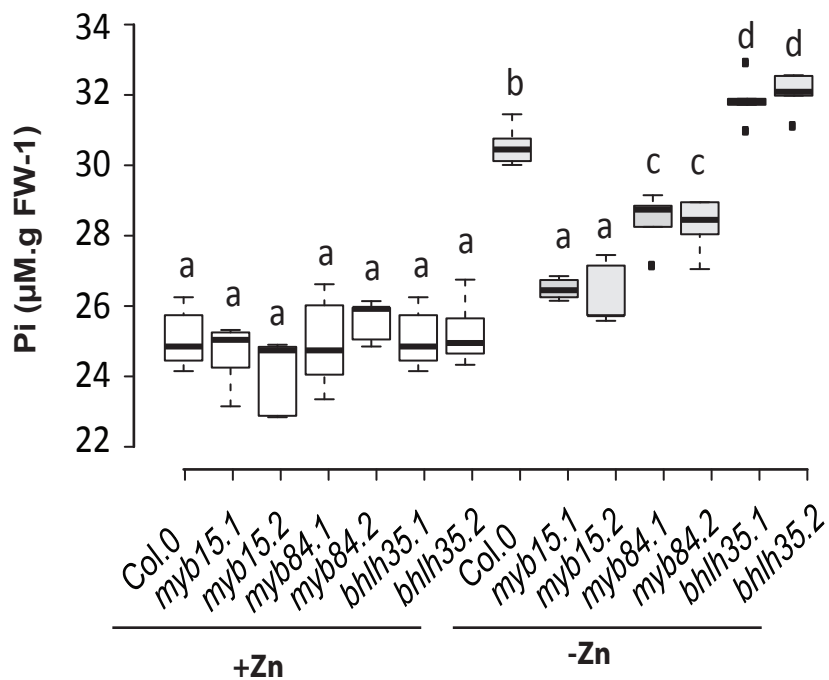
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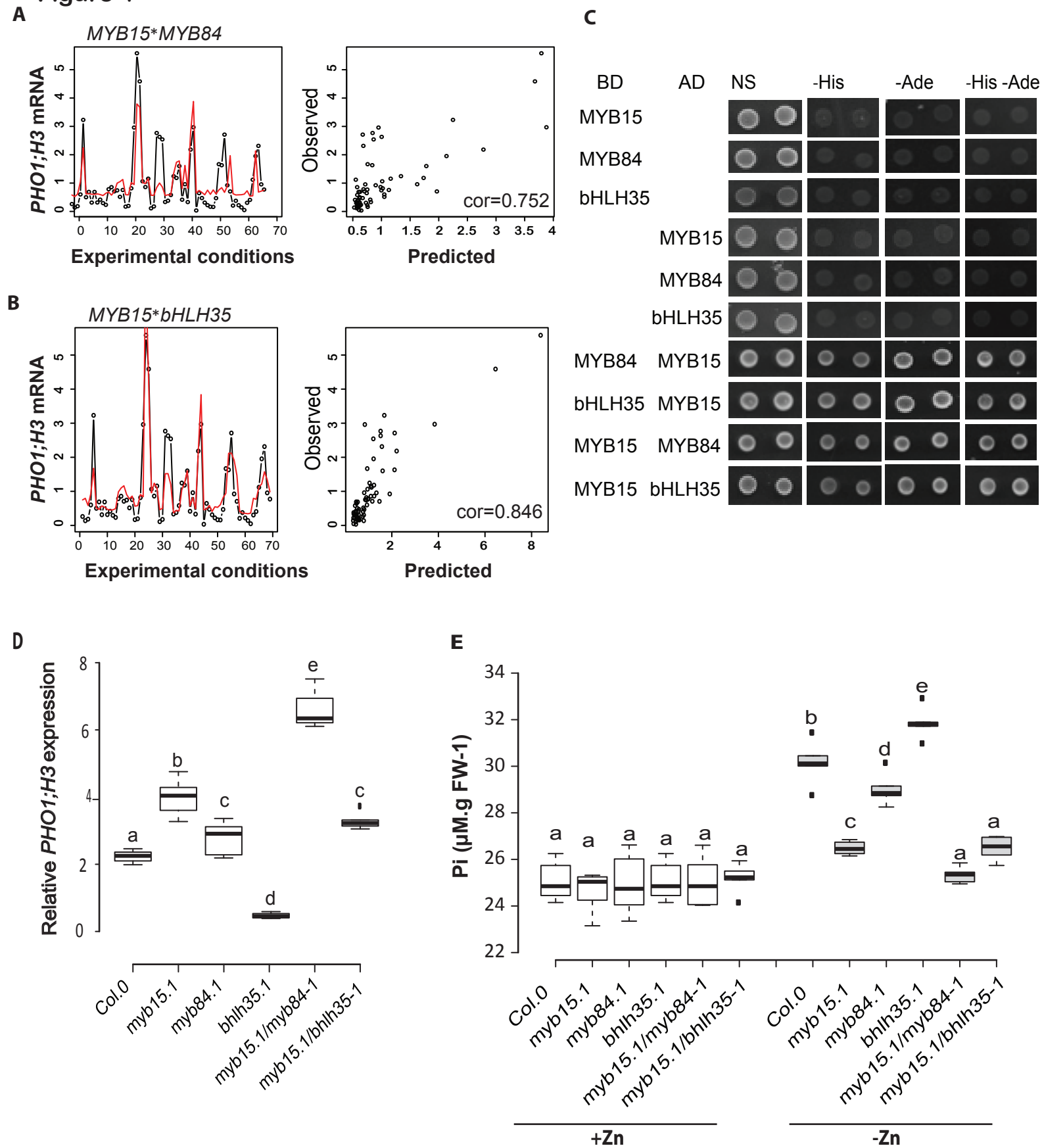
D



**Figure 3. MYB15, MYB84 and bHLH35 regulate both the expression of *PHO1;H3* and the accumulation of Pi in the shoot under zinc deficiency.**

A- Relative *PHO1;H3* (At1g14040) transcript accumulation in the roots of wild-type plants (Col-0) and mutant lines harbouring loss-of-function mutations in the following transcription factors: At5g57150 (*bHLH35*), At3g23250 (*MYB15*), At3g49690 (*MYB84*), At4g37790 (*HAT22*), At2g46510 (*bHLH17*), At1g31050 (*bHLH111*), At5g04760 (*MYB*-type), At4g31800 (*WRKY18*), At4g24060 (*Dof46*) and At3g50060 (*MYB77*). Plants were grown for 18 days in the presence (+Zn) or absence (-Zn) of zinc. *PHO1;H3* transcript abundance was measured by qRT-PCR normalized against *UBQ10* (At4g05320). B- *MYB15*, *MYB84* and *bHLH35* transcripts accumulation in response to Zn deficiency. Relative *MYB15*, *MYB84* and *bHLH35* transcript accumulation was quantified in roots of wild-type plants (Col-0) grown for 18 days in the presence or absence of Zn by qRT-PCR and normalized against *UBQ10*. C- Yeast one-hybrid assay. Sequences of the Arabidopsis *PHO1;H3* promoter fused to the HIS3 auxotrophic marker were stably transformed into yeast. These different yeast strains were then co-transformed with *MYB15*, *MYB84* or *bHLH35*. Left panel, growth of the different yeast strains on control media deprived of tryptophan (-W), allowing the selection of yeast cells expressing the selected TFs. Right panel, growth of the different yeast strains on selective media deprived of tryptophan and histidine (-W-H). D- Pi concentrations measured in the shoots of wild type, *myb15*, *myb84*, or *bhlh35* plants grown for 18 days in the presence or absence of Zn. For A, C and D panels, central lines in the boxes show the medians; box limits indicate the 25th and 75th percentiles, whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles. Letters a, b and c indicate significantly different values at  $p < 0.05$  determined by one-way ANOVA and Tukey HSD.

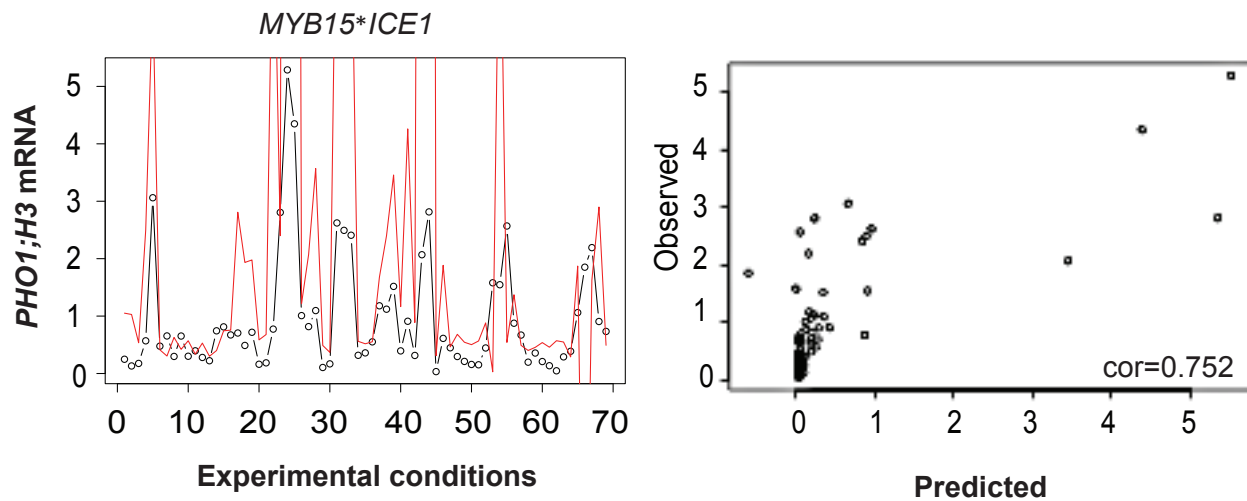
**Figure 4**



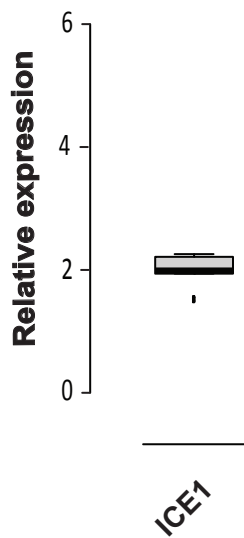
**Figure 4: Interactions between MYB15 and MYB84 and between MYB15 and bHLH35 influence the expression of *PHO1;H3* and Pi accumulation under zinc deficiency.** A, B- TransDetect's prediction of correlation between the expression of the MYB15 / MYB84 and MYB15 / bHLH35 TF pairs and the *PHO1;H3* transcript level with  $R^2=0.75$  and  $R^2=0.84$  respectively. C- Yeast two-hybrid assay. bHLH35, MYB15 and MYB84 were fused with either the GAL4 DNA binding domain (BD) or the GAL4 activation domain (AD) into appropriate expression vectors, which were then transferred into yeast. The different yeast strains were plated on non-selective medium (NS) or on selective media deprived of histidine (-His), adenine (-Ade) or both simultaneously (-His-Ade). D- Relative *PHO1;H3* transcript accumulation in roots of wild type (Col-0), *bhlh35*, *myb15*, *myb84*, *myb15/myb84* and *myb15/bhlh35* single and double mutant plants grown for 18 days in the absence vs. presence of zinc (-Zn/+Zn). *PHO1;H3* transcript abundance was measured by qRT-PCR and normalized against *UBQ10*. E- Shoot Pi concentrations measured in wild-type (Col-0), *bhlh35*, *myb15*, *myb84*, *myb15xmyb84* and *myb15xbhlh35* single and double mutant plants grown on either +Zn or -Zn for 18 days. For D and E panels, Box central lines show the medians; box limits indicate the 25th and 75th percentiles; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles. Letters a, b and c indicate significantly different values at  $p < 0.05$  determined by one-way ANOVA and Tukey HSD.

Figure 5

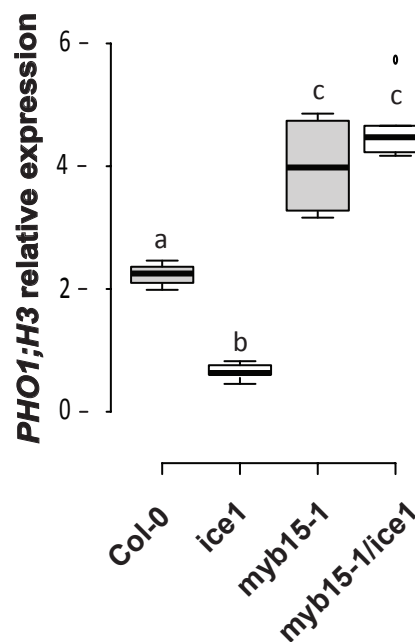
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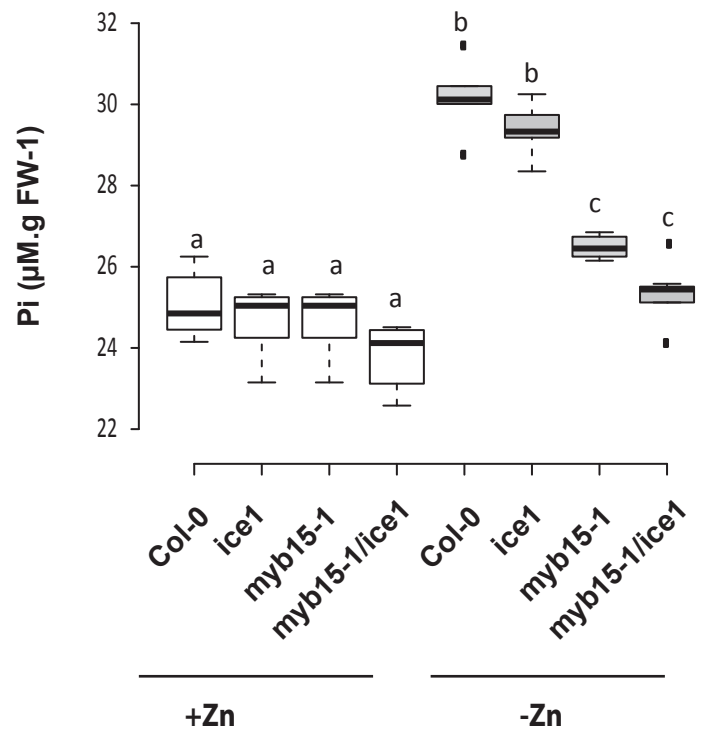
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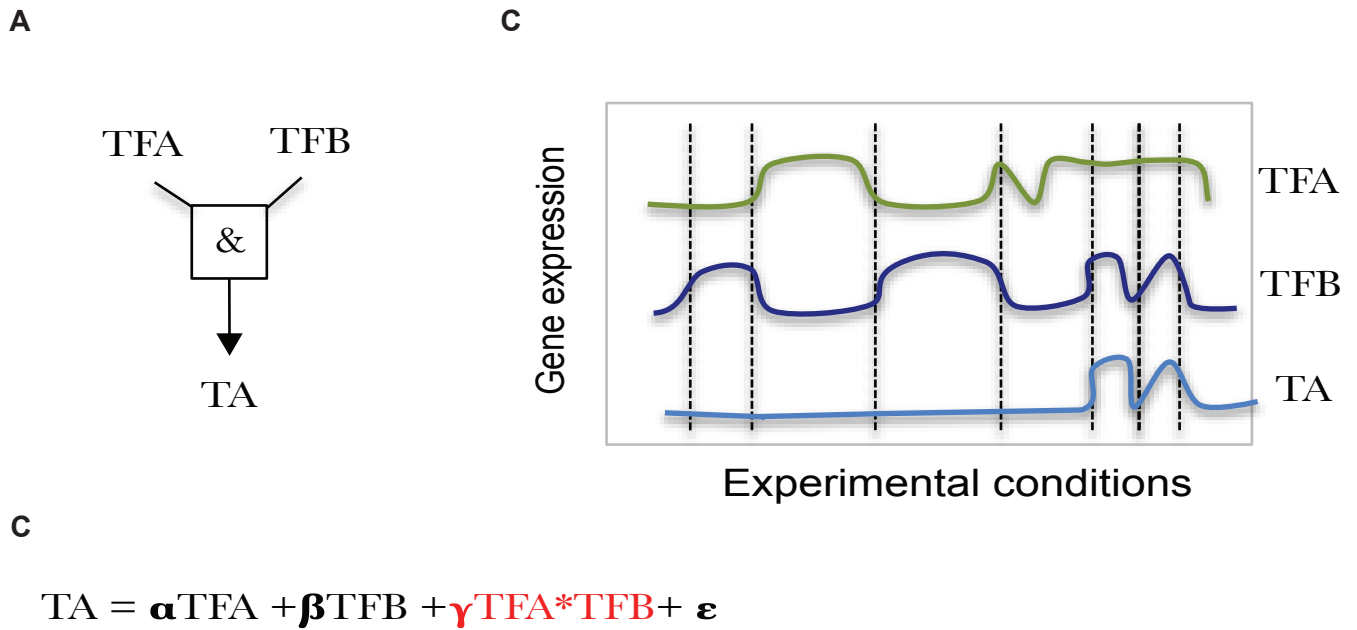


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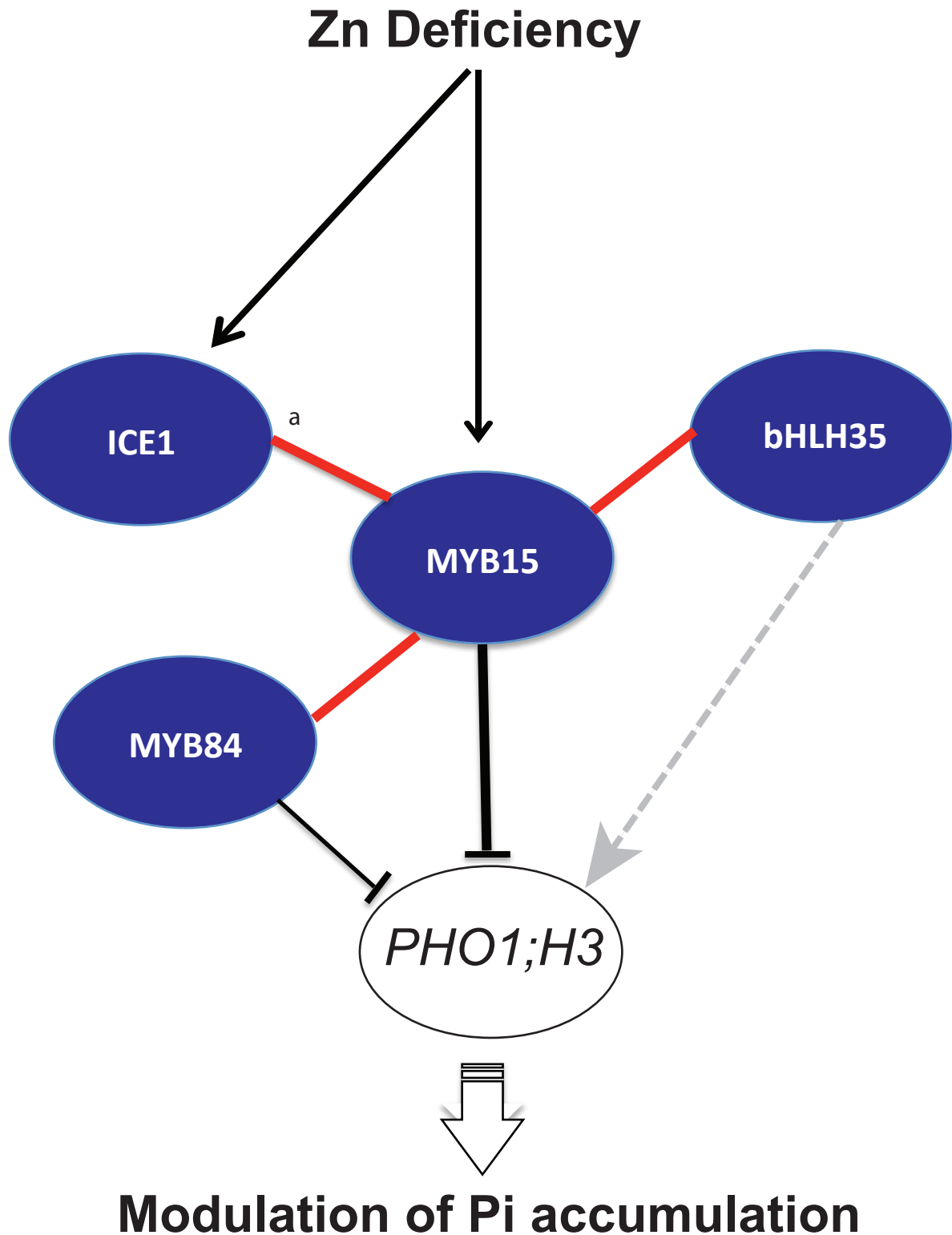
**Figure 5. The ICE1 / MYB15 transcription factor pair regulates both the expression of *PHO1;H3* the accumulation Pi under  $-Zn$ .** A- TransDetect's prediction of the correlation between the expression of the TF pair MYB15 and ICE1 and the *PHO1;H3* expression ( $R^2=0,73$ ). B- *ICE1* transcript accumulation. Expression of *ICE1* was quantified in wild-type (Col-0) seedlings grown for 18 days in presence (+Zn) or absence (-Zn) of zinc. *ICE1* transcript abundance was measured by qRT-PCR normalized against *UBQ10*. C- *PHO1;H3* transcript accumulation. Expression of *PHO1;H3* gene was quantified in wild type (Col-0), *ice1*, *myb15* and *myb15/ice1* seedlings grown for 18 days in +Zn or -Zn. *PHO1;H3* transcript abundance was measured by qRT-PCR and normalized against *UBQ10*. D- Pi accumulations. Pi concentrations were measured from shoots of wild type (Col-0), *ice1*, *myb15*, and *myb15/ice1* seedlings grown for 18 days in presence +Zn or -Zn. For B, C and D, Box center lines show the medians; box limits indicate the 25th and 75th percentiles; whiskers extend 15 times the interquartile range from the 25th and 75th percentiles. Letters a, b and c indicate significantly different values at  $p<005$  determined by one-way ANOVA and Tukey HSD.

Figure 6



**Figure 6. Idealized model to explain how TransDetect extract directionality in static data.** A- Two transcription factors TFA and TFB positively control the expression of a target gene TA following an AND logic-gate. B- TA expression is induced only when TFA and TFB expression are both upregulated. C- Linear modelling of TA expression. Considering the ideal case where 2 transcription factors (TFA and TFB) control a target gene TA following an AND logic gate. In this particular case,  $\gamma$  coefficient of the linear model will be highly significant because it is the combination of TFA and TFB expression that is necessary to fully explain TA expression. On the other hand it is not possible to infer TFA by a linear combination of TA and TFB, nor to explain TFB by a linear combination of TA and TFA. Thus the term of the equation  $\gamma TFA * TFB$  intrinsically possess some directionality explanatory power in this case where both TFs interact in the control of TA.

Figure 7



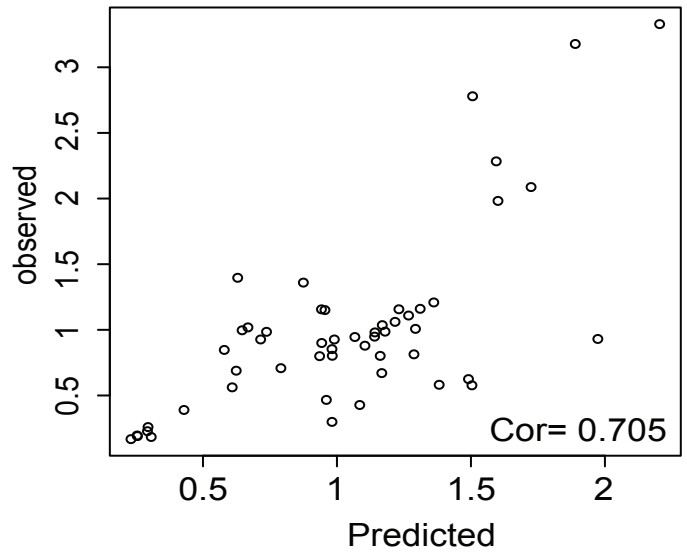
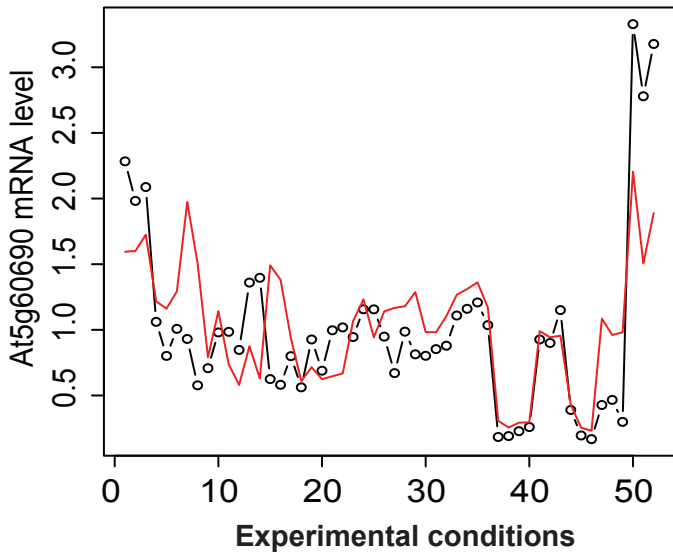
**Figure 7. Schematic representation of the MYB15, MYB84, bHLH35 and ICE1 regulatory module controlling *PHO1;H3* gene expression and Pi accumulation in shoots under zinc deficiency.** Phosphate increases in the shoots of plants exposed to zinc deficiency. *PHO1;H3* plays a negative regulatory role in this process. Red solid lines indicate connections between MYB15, MYB84, bHLH35 and ICE1. Negative and positive regulatory effects of these transcription factors on *PHO1;H3* expression under zinc deficiency are indicated by flat-ended dashed lines and arrowheads, respectively. a indicates previous knowledge on ICE1 and MYB15 physical interaction.



Supplementray Figure 1

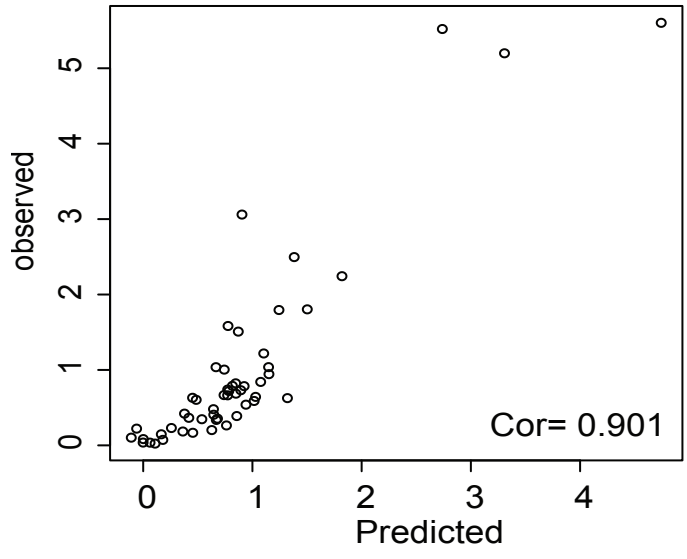
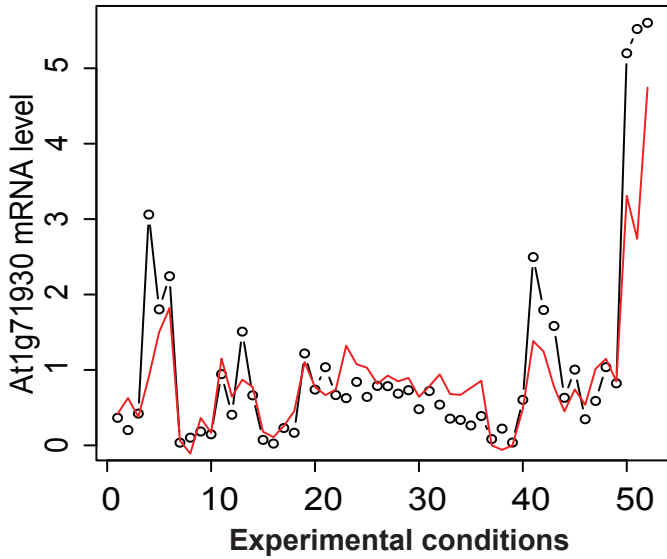
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A



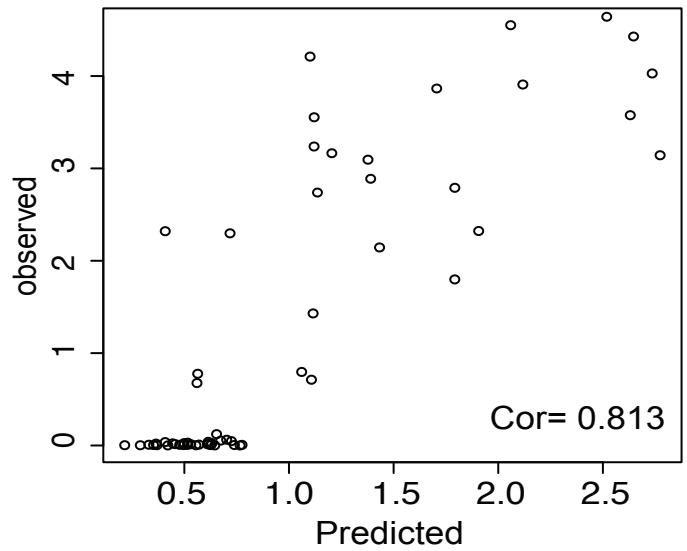
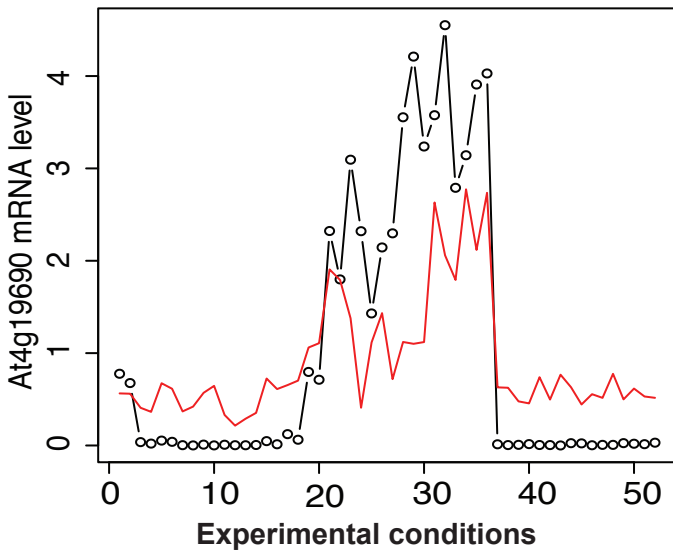
B

At1g12260 \* At1g47870



C

At3g47640 \* At1g18400



**Supplementary Figure 1. TransDetect prediction of regulators for REVOLUTA, ASCULAR RELATED NAC-DOMAIN PROTEIN 7 and IRON-REGULATED TRANSPORTER 1.** TransDetect was used to predict regulators for the following target genes A- *REVOLUTA* (*REV*, At5g60690), B- *ASCULAR RELATED NAC-DOMAIN PROTEIN 7* (*VND7*, At1g71930), and C- *IRON-REGULATED TRANSPORTER 1* (*IRT1*, At4g19690). Among number of TFs predicted, TransDetect retrieved known regulators for these genes, namely DNA BINDING WITH ONE FINGER 5.3 (DOF5.3, At5g60200) for *REV*, ZINC FINGER PROTEIN 11 (ZFP11, At2g34710) for *VND7*, and POPEYE (PYE, At3g47640) for *IRT1*.  
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