Plant Physiology Preview. Published on January 24, 2014, as DOI:10.1104/pp.113.229740

Running head: Alteration of Pi starvation responses by SAP11

Address correspondence to:

Jun-Yi Yang

Institute of Biochemistry, National ChungHsing University, 250 Kuokuang Road

Taichung 40227, Taiwan

886-4-22840468 ext 232

jyang@nchu.edu.tw

Research area: Signaling and Response

Transgenic Plants that Express the Phytoplasma Effector SAP11 Show Altered Phosphate Starvation and Defense Responses

Yen-Ting Lu[1], Meng-Ying Li[1], Kai-Tan Cheng, Choon Meng Tan, Li-Wen Su, Wei-Yi Lin, Hsien-Tzung Shih, Tzyy-Jen Chiou and Jun-Yi Yang

Institute of Biochemistry, National ChungHsing University, Taichung 40227, Taiwan (Y.-T.L., M.-Y.L., K.-T.C., C.M.T., L.-W.S., J.-Y.Y.); Ph.D. Program in Microbial Genomics, National ChungHsing University, Taichung 40227, Taiwan (C.M.T.); Department of Applied Zoology, Agricultural Research Institute, Taichung 413, Taiwan (H.T.S.); Agricultural Biotechnology Research Center, Academia Sinica, Taipei 115, Taiwan (W.-Y.L., T.-J.C.); Institute of Biotechnology, National ChungHsing University, Taichung 40227, Taiwan (J.-Y.Y.); NCHU-UCD Plant and Food Biotechnology Center, National ChungHsing University, Taichung 40227, Taiwan (J.-Y.Y.); Agricultural Biotechnology Center, National ChungHsing University, Taichung 40227, Taiwan (J.-Y.Y.)

[1] These authors contributed equally to this work.

One-sentence summary:

Expression of a bacterial effector alters plant responses to phosphorus nutrient and a bacterial pathogen.

Financial source: This work was supported by the National Science Council (NSC 100-2321-B-005-007-MY3, NSC 101-2911-I-005-301 and NSC 102-2911-I-005-301) and Ministry of Education (ATU plan), Taiwan.

Corresponding author: Jun-Yi Yang, jyang@nchu.edu.tw

ABSTRACT

Phytoplasmas have the smallest genome among bacteria and lack many essential genes required for biosynthetic and metabolic functions, making them unculturable, phloem-limited plant pathogens. In this study, we observed that transgenic *Arabidopsis thaliana* expressing the secreted effector protein $SAP11_{AYWB}$ of the Aster Yellows phytoplasma strain Witches' Broom (AY-WB) shows an altered root architecture, similarly to the disease symptoms of phytoplasma-infected plants, by forming hairy roots. This morphological change is paralleled by an accumulation of cellular Pi and an increase in the expression levels of Pi starvation-induced genes and miRNAs. In addition to the Pi starvation responses, we found that $SAP11_{AYWB}$ suppresses salicylic acid-mediated defense responses and enhances the growth of a bacterial pathogen. These results contribute to an improved understanding of the role of phytoplasma effector SAP11 and provide new insights for understanding the molecular basis of plant–pathogen interactions.

INTRODUCTION

Nutrients are essential for the growth of both host cells and pathogens. Host cells obtain nutrients for their own metabolism, but they also provide a favorable environment for pathogens to exploit. To defend against the growth of pathogens, host cells can develop a strategy to sequester key nutrients from the pathogens (Radtke and O'Riordan, 2006; Hood and Skaar, 2012). However, nutritional deficiencies also impair immune responses and alter the host susceptibility to pathogens. In general, successful pathogens have evolved various ways of manipulating host nutrition by modulating the uptake and utilization of nutrients in order to satisfy their requirements. Global transcriptome analysis has been shown that intracellular bacteria, such as *Listeria monocytogenes*, can upregulate genes required for nutritional stresses in mammalian cells (Chatterjee et al., 2006). In the case of *Xanthomonas oryzae*, the transcriptional activator-like type III effector pthXol can activate the expression of *OsSWEET11* in controlling cellular sugar efflux (Chen et al., 2010). Thus, it is not surprising that nutritional immunity is a complex process, and nutrition is an important factor in regulating the interaction between a host and a pathogen.

Recently, a study on huanglongbing disease through small RNA profiling analysis revealed that the infection of *Candidatus Liberibacter*, an intracellular bacterial pathogen, specifically induced miR399 in citrus plants (Zhao et al., 2013). MiR399 is induced by phosphate (Pi) starvation and has the ability to regulate the homeostasis of Pi by repressing the expression of *PHOSPHATE 2* (*PHO2*) (Aung et al., 2006; Chiou et al., 2006). *PHO2* encodes a ubiquitin E2 enzyme required for the degradation of PHT1 Pi transporters and PHOSHATE1 (PHO1) involved in Pi uptake and translocation Pi starvation signaling (Liu et al., 2012; Huang et al., 2013). The Pi deficiency symptom associated with huanglongbing disease implies that the availability of Pi is an important factor for the disease development caused by *Candidatus*

Liberibacter. Indeed, the application of Pi reduces the disease symptoms of huanglongbing (Zhao et al., 2013).

Pi is required for the synthesis of nucleic acids and phospholipids, and it also plays an important function in energy metabolism and signaling transduction (Shen et al., 2011). Although Pi is an essential nutrient for plant growth and development, the amount of available Pi in the soil is limited (Raghothama, 1999; Jain et al., 2012). As a result, plants have evolved a way to improve their Pi acquisition by forming mutualistic symbiosis with arbuscular mycorrhizal (AM) fungi (Bucher et al., 2009; Ohkama-Ohtsu and Wasaki, 2010). Colonization by AM fungi can modify the plants' root architecture and enhance the uptake of mineral nutrients, in particular Pi (Gu et al., 2011; Volpe et al., 2013). Interestingly, several studies also reported that AM fungi reduce the disease symptoms caused by phytoplasmas (Lingua et al., 2002; Romanazzi et al., 2009; Kamińska et al., 2010; Sampò et al., 2012).

Similar to *Candidatus Liberibacter*, phytoplasmas are intracellular bacterial pathogens and depend on sap-feeding insects for transmission (Lee et al., 2000). Phytoplasmas cause various disease symptoms, including witches' broom, phyllody, virescence, bunchy roots, and stunting and yellowing of plants (Hogenhout et al., 2008). Owing to the significant reduction in the genome of phytoplasmas, they cannot be cultured *in vitro* and are restricted to phloem sieve cells with a rich growth environment (Oshima et al., 2004; Bai et al., 2006). Although phytoplasmas depend on their plant host for survival, they are able to manipulate the plant's physiology through their secreted effectors (Sugio et al., 2011a).

Recent studies have shown that the protein 11 ($SAP11_{AYWB}$) secreted by the Aster Yellows phytoplasma strain Witches' Broom (AY-WB) contains a nuclear localization signal (NLS) for nuclear-targeting outside phloem cells (Bai et al., 2008). *Arabidopsis* plants that overexpress $SAP11_{AYWB}$ display crinkled leaves that resemble transgenic plants overexpressing miR319 (Sugio et al., 2011b). MiR319 has been shown to target transcripts of

106 *TEOSINTE BRANCHED1*/*CYCLOIDEA*/*PROLIFERATING CELL FACTOR1* (*TCP*) genes,
107 which are involved in the control of cell proliferation in leaf morphogenesis and the
108 biosynthesis of jasmonate (JA) (Ori et al., 2007; Schommer et al., 2008). Although
109 $SAP11_{AYWB}$ has not been shown to interfere with miRNA expression, it has been reported that
110 it can destabilize TCP transcription factors through direct interaction (Sugio et al., 2011b).
111 Because TCPs can directly regulate the expression of *LIPOXGENASE 2* (*LOX2*), a key
112 component in the biosynthesis of JA, destabilization of TCPs leads to a decrease in JA
113 (Schommer et al., 2008). In agreement with the findings that JA plays an important role in
114 defense responses against insect herbivores, impairment of JA biosynthesis in
115 $SAP11_{AYWB}$-overexpressing plants leads to an increase in the progeny of *Macrosteles*
116 *quadrilineatus* (Kessler et al., 2004; Sugio et al., 2011b).
117 In this study, we have shown that the expression of phytoplasma effector $SAP11_{AYWB}$ in
118 *Arabidopsis* triggers phosphate starvation responses and suppresses plant defense responses
119 against a bacterial pathogen. These findings advance our understanding of the molecular
120 mechanism underlying the disease symptoms elicited by the secreted effectors and provide
121 new insights into the interaction between host plants and phytoplasmas.
122
123 **RESULTS**
124 **Expression of $SAP11_{AYWB}$ Alters miRNA Accumulation Involved in Pi Starvation and**
125 **Auxin Responses in *Arabidopsis***
126 The phenotypical similarities in the leaf morphologies of $SAP11_{AYWB}$-overexpressing
127 plants (*35S::SAP11*$_{AYWB}$) and miR319a-overexpressing (*35S::miR319a*) plants (Sugio et al.,
128 2011b) prompted us to examine whether the expression of $SAP11_{AYWB}$ can regulate the
129 accumulation of miRNAs in host cells (Fig. 1A). Expression of $SAP11_{AYWB}$ was confirmed
130 by using the anti-$SAP11_{AYWB}$ antibody (Fig. 1B). Then, total RNA was extracted from plants

grown on half-strength Murashige and Skoog (1/2× MS) medium for small RNA northern blotting assays. Here, we compared the accumulation of miR159, miR160, miR164, miR167, miR319, miR399, and miR393 in *Arabidopsis* wild-type (WT) Col-0 and *35S::SAP11*$_{AYWB}$ transgenic lines. Interestingly, the accumulation of miR319 and miR159, miR164, and miR167 was not affected by the expression of SAP11$_{AYWB}$ (Fig. 1C). However, a decrease of miR393 and an induction of miR399 and miR160 were detected in *35S::SAP11*$_{AYWB}$ transgenic lines (Fig. 1C). MiR399 plays a key role in regulating Pi starvation responses, whereas miR393 and miR160 are involved in auxin responses. In generally, auxin is regulated upon Pi depletion in order to change root architecture with the aim of increasing Pi uptake (Péret et al., 2011). Thus, auxin has been shown to play an important role in stimulating root architecture during Pi starvation responses (Nacry et al., 2005; Jain et al., 2007; Pérez-Torres et al., 2008). Because miR393 and miR160 are involved in auxin responses, it is possible that those miRNAs have indirect effects on the root architecture alterations required for Pi uptake. Our results suggest that Pi starvation and auxin responses are altered in 35S::SAP11$_{AYWB}$ transgenic plants.

Pi Starvation Responses and Pi Homeostasis Are Altered in SAP11 Transgenic *Arabidopsis* Plants

To investigate whether Pi starvation responses have been altered in *35S::SAP11*$_{AYWB}$ transgenic plants, the expression levels of Pi starvation-induced genes were examined using quantitative RT-PCR (qRT-PCR). RNA samples were extracted from seedlings grown in 1/2× MS (Pi-sufficient) medium, and typical Pi starvation-induced genes such as *INDUCED BY PHOSPHATE STARVATION 1* (*IPS1*), *IPS2*, *SPX DOMAIN GENE 1* (*SPX1*), *SPX3*, *PHOSPHATE STARVATION-INDUCED GENE 2* (*PS2*), *PS3*, and *PHOSPHATE TRANSPORTER 1;4* (*PHT1;4*) were examined. *IPS1*, *IPS2*, *SPX1*, *SPX3*, *PS2*, *PS3*, and

PHT1;4 were all elicited in *35S::SAP11$_{AYWB}$* transgenic plants, in comparison with WT plants (Fig. 2A). To examine the levels of cellular Pi, *Arabidopsis* seedlings grown in hydroponic solution were collected. A higher amount of Pi was detected in the aerial parts of *35S::SAP11$_{AYWB}$* transgenic plants grown in 0.01 mM KH_2PO_4 (Pi-deficient) and 0.25 mM KH_2PO_4 (Pi-sufficient) hydroponic solutions than in WT plants (Fig. 2B). These results indicate that Pi starvation responses and Pi homeostasis are altered in *35S::SAP11$_{AYWB}$* transgenic plants.

Expression of SAP11$_{AYWB}$ Reduces the Accumulation of Anthocyanin and Alters the Root Architecture in *Arabidopsis*

Pi is a major limiting factor for the growth and development of plants. Thus, plants have evolved a series of morphological and physiological modifications triggered by Pi deficiency, such us an increase in root/shoot ratio, proliferation of lateral roots, and accumulation of anthocyanin (Rouached et al., 2010). To examine the effects of Pi deficiency, variations in the development of *35S::SAP11$_{AYWB}$* transgenic lines grown on 0.01 mM and 0.25 mM KH_2PO_4 medium were compared. As a control, WT plants showed a significant reduction in plant size and displayed red/purple color in leaf when grown on 0.01 mM KH_2PO_4 medium (Fig. 3A). Compared to WT plants, *35S::SAP11$_{AYWB}$* transgenic plants showed a growth-inhibiting phenotype on both 0.01 mM and 0.25 mM KH_2PO_4 media (Fig. 3A). However, the accumulation of anthocyanin was strongly reduced in *35S::SAP11$_{AYWB}$* transgenic plants grown on 0.01 mM KH_2PO_4 medium (Fig. 3B). We further investigated the root architecture of *35S::SAP11$_{AYWB}$* transgenic plants. Compared to WT plants, *35S::SAP11$_{AYWB}$* transgenic plants grown on both 0.01 mM and 0.25 mM KH_2PO_4 media showed a clear inhibition of primary roots, a significant proliferation of adventitious roots, and an elongation of lateral roots (Fig. 3C). Taken together, the molecular alterations associated with the expression of

SAP11$_{AYWB}$ were correlated with the morphological changes observed in the *35S::SAP11$_{AYWB}$* transgenic plants.

Genome-Wide Identification of Differentially Expressed mRNAs Altered by the Expression of SAP11$_{AYWB}$ in *Arabidopsis*

To examine the genome-wide expression profile of mRNAs in response to the expression of SAP11$_{AYWB}$ in *Arabidopsis*, the *35S::SAP11$_{AYWB}$* transgenic line #13 with higher expression of SAP11$_{AYWB}$ was selected for a comparative transcriptome analysis (Fig. 4A). Here, total RNA from *35S::SAP11$_{AYWB}$* transgenic seedlings and WT seedlings grown on 1/2× MS medium were extracted for next-generation sequencing. In summary, a total of 26.2 million reads mapped to the *Arabidopsis* genome were generated after quality trimming from two cDNA libraries (Supplemental Table S1). To identify differentially expressed genes altered by SAP11$_{AYWB}$, the expression levels of mapped genes from two samples were quantified and analyzed using DESeq, a differential gene expression data analysis program (Anders and Huber, 2010). A total of 395 differentially expressed genes (p □ 0.01) were identified in the *35S::SAP11$_{AYWB}$* transgenic plants. To narrow down the differentially expressed genes, a criterion of $p < 0.001$ was selected, and 163 differentially expressed genes were identified in the *35S::SAP11$_{AYWB}$* transgenic plants (Supplemental Data S1). Among them, 59 genes were upregulated and 104 genes were downregulated.

Functional annotations on the 59 upregulated genes revealed that a total of 18 Pi starvation-induced genes, including *MYB DOMAIN PROTEIN 90* (*MYB90*), *IPS1*, *GLUCOSE-6-PHOSPHATE/PHOSPHATE TRANSLOCATOR 2* (*GPT2*), *SULFATE TRANSPORTER 1;3* (*SULTR1;3*), *SPX3, MONOGALACTOSYLDIACYLGLYCEROL SYNTHASE 3* (*MGD3*), *BETA-AMYLASE 5* (*BAM5*), *AT5G09570*, *PS2*, *PHOSPHOETHANOLAMINE/PHOSPHOCHOLINE PHOPHATASE 1* (*PEPC1*),

206 *AT5G20790*, *AT1G23110*, *IPS2*, *SPX1*, *PHOSPHOLIPASE D P2* (*PLDP2*), *PS3*,
207 *SENESCENCE-RELATED GENE 3* (*SRG3*), and *PURPLE ACID PHOSPHATASE 17*
208 (*PAP17*), were highly elicited by $SAP11_{AYWB}$ (Table 1). Notably, a total of 7 Pi
209 starvation-induced genes were found in the top 10 list of $SAP11_{AYWB}$-elicited genes. Further
210 comparison revealed that more than 20% of the $SAP11_{AYWB}$-elicited genes (p □ 0.001)
211 overlapped with the differentially expressed genes (more than 2-fold upregulation) identified
212 by Muller et al. (2007) and Liu et al. (2011) under Pi starvation conditions (Fig. 4B). In
213 addition to the Pi starvation responses, a small subset of genes involved in sugar metabolic
214 processes and iron-deficiency responses were also elicited by $SAP11_{AYWB}$ (Table 1). To
215 evaluate the quality of the RNA-Seq data, $SAP11_{AYWB}$-elicited genes were randomly selected
216 for validation by using the qRT-PCR method. The differentially expressed genes, including
217 *GPT2*, *BAM5*, *MGD3*, *PLDP2*, *SWEET1*, *MYB90*, *TPPI*, *SRG3*, *CYTOCHROME P450*
218 *FAMILY 707 SUBFAMILY A POLYPEPTIDE 2* (*CYP707*A2), and *FERRIC REDUCTION*
219 *OXIDASE 5* (*FRO5*), were all elevated in the *35S::SAP11$_{AYWB}$* transgenic plants (Fig. 4C).
220 Taken together, these results indicate that Pi starvation-induced genes are highly upregulated
221 by the expression of $SAP11_{AYWB}$ in *Arabidopsis*.
222
223 **Genome-Wide Identification of Differentially Expressed miRNAs Altered by the**
224 **Expression of $SAP11_{AYWB}$ in *Arabidopsis***
225 To investigate the genome-wide expression profile of miRNAs in response to the
226 expression of $SAP11_{AYWB}$ in *Arabidopsis*, RNA samples were collected from seedlings grown
227 on 1/2× MS medium. Two small RNA libraries from *35S::SAP11$_{AYWB}$* transgenic line #13 and
228 WT plants were constructed for deep-sequencing. In summary, a total of 34.5 million reads
229 mapped to the *Arabidopsis* genome were generated after quality trimming from two cDNA
230 libraries (Supplemental Table S2). Among them, about 9.3 million reads were mapped to

Arabidopsis miRNAs deposited in miRBase (Kozomara and Griffiths-Jones, 2011). The expression levels of known miRNAs in two samples were normalized, and the differentially expressed miRNAs with fold-change >2 or <1/2 were identified (Fig. 5; Supplemental Data S2). Compared to WT plants, *35S::SAP11$_{AYWB}$* transgenic plants showed significant upregulation of miR160, miR2111, miR394, miR399, miR5639, miR5655, miR827, miR850, and miR863. In contrast, miR172, miR396, miR5020, miR5629, miR5645, and miR5648 showed significant downregulation in the *35S::SAP11$_{AYWB}$* transgenic plants. Among them, miR2111, miR399, and miR827 have been shown to be upregulated by Pi starvation (Hsieh et al., 2009; Pant et al., 2009) and miR399 and miR827 play important roles in regulating Pi homeostasis (Liu et al., 2012; Huang et al., 2013; Lin et al., 2013); miR160 and miR396 are required for regulating the auxin signaling response and cell proliferation during leaf and root development (Gutierrez et al., 2009; Pulido and Laufs, 2010; Debernardi et al., 2012; Bazin et al., 2013); miR172 has been shown to regulate the transition from the vegetative to reproductive phases (Aukerman and Sakai, 2003; Wu et al., 2009); and miR394 is required for the regulation of leaf morphology and shoot meristem stem-cell maintenance (Song et al., 2012; Knauer et al., 2013). However, miR5020b, miR5629, miR5639, miR5645d, miR5648, miR5655, and miR850 are newly identified miRNAs with unknown functions.

We further evaluated the results of small RNA sequencing by using small RNA northern blotting. Here, several miRNAs were randomly selected for validation, and the results were consistent with the genome-wide miRNA expression profiling (Fig. 4D). Moreover, we investigated the expression level of *PHO2* in *35S::SAP11$_{AYWB}$* transgenic line #13, because miR399 has been shown to downregulate *PHO2* by directly targeting its transcripts (Aung et al., 2006; Chiou et al., 2006). Consistent with the upregulation of miR399, a significant downregulation of *PHO2* was observed in *35S::SAP11$_{AYWB}$* transgenic line #13 (Fig. 4E). Taken together, these results indicate that miRNAs involved in Pi-starvation and

auxin-signaling pathways are altered by the expression of $SAP11_{AYWB}$ in *Arabidopsis*.

Role of MiR319a in $SAP11_{AYWB}$-Triggered Pi Starvation Responses

Although $SAP11_{AYWB}$ did not interfere with the accumulation of miR319 (Fig. 1C and 4D), the crinkled leaves that appeared in *35S::SAP11$_{AYWB}$* transgenic plants resembled those in the *35S::miR319a* transgenic plants (Fig. 1A and 6A), which prompted us to examine whether miR319a has an effect on the $SAP11_{AYWB}$-triggered Pi starvation responses. We investigated the accumulation of miR159, miR164, miR319, miR393, and miR399 in *35S::miR319a* transgenic plants grown on 1/2× MS medium. An increase in the amount of miR159, miR164, and miR399 was observed with the overexpression of miR319 in *Arabidopsis* (Fig. 4D). However, the amount of increase for miR399 was much lower in *35S::miR319a* transgenic plants than in *35S::SAP11$_{AYWB}$* transgenic line #13. Consistently, the expression levels of Pi starvation-induced genes were much lower in *35S::miR319a* transgenic plants, in comparison with *35S::SAP11$_{AYWB}$* transgenic line #13 (Fig. 4C and E). We further investigated whether miR319a has an effect on the root architecture. Compared to *35S::SAP11$_{AYWB}$* transgenic line #13, *35S::miR319a* transgenic plants grown on 0.01 mM KH_2PO_4 medium showed no proliferation of adventitious roots and strong inhibition of primary roots (Fig. 6B). The root architecture in *35S::miR319a* transgenic plants was more similar to that in WT plants (Fig. 6B). Because $SAP11_{AYWB}$ does not affect miR319 expression levels and the root architecture of miR319 transgenic plants are more similar to wild type, it is unlikely that miR319 is involved in the $SAP11_{AYWB}$-triggered Pi starvation responses. However, the transcript levels of Pi starvation-induced genes and miR399 were lightly upregulated in *35S::miR319a* transgenic plants. Therefore, TCP transcripts targeted by miR319 may have minimal contribution to the phosphate starvation responses.

SAP11$_{AYWB}$-Triggered Pi Starvation Responses Are Mainly Dependent on *PHR1*

Molecular mechanisms underlying Pi starvation responses remain largely unknown (Rouached et al., 2010). Only a few transcription factors have been identified as important players in the Pi-starvation signaling pathway. Among them, *PHOSPHATE STARVATION RESPONSE 1* (*PHR1*) that encodes a MYB transcription factor plays a key role in responding to the Pi deficiency in *Arabidopsis* (Rubio et al., 2001). However, there was no significant difference in the transcript level of *PHR1* between WT and SAP11$_{AYWB}$ transgenic plants (Fig. 4E). When we analyzed the potential cis-regulatory elements on the Pi-starvation induced genes regulated by SAP11$_{AYWB}$ using PlantPAN, a navigator for plant promoter analysis (Chang et al., 2008), we found that 14 out of 18 Pi-starvation induced genes contain PHR1 binding sites in their promoter regions (Table 3). Thus, to understand whether the *PHR1*-dependent pathway contributes to the SAP11$_{AYWB}$-triggered Pi starvation responses, *35S::SAP11$_{AYWB}$* was introduced into a *phr1* mutant. With the expression of SAP11$_{AYWB}$, *35S::SAP11$_{AYWB}$*/*phr1* transgenic plants showed a wavy-margin phenotype in the leaves (Fig. 7A). However, the Pi starvation-induced genes, including *IPS1*, *IPS2*, *SPX1*, *SPX3*, *PS2*, *PS3*, and *PHT1;4*, were all suppressed when SAP11$_{AYWB}$ was expressed under a *phr1* mutant background (Fig. 7B). These results indicate that *PHR1* is required for SAP11$_{AYWB}$-triggered Pi starvation responses.

SAP11$_{AYWB}$ Transgenic Plants Show Altered Hormone Synthesis and Signaling and Are More Susceptible to the Bacterial Pathogen *Pseudomonas Syringae*

RNA-Seq analysis of the downregulated genes in *35S::SAP11$_{AYWB}$* #13 transgenic line revealed that a subset of genes, including *ELICITOR-ACTIVATED GENE 3-1* (*ELI3-1*), *At3g05730* (defensin-like family protein), *PATHOGENESIS-RELATED GENE 1* (*PR1*), and *LOX2*, were highly suppressed (Table 2). The RNA-seq data was validated using the

qRT-PCR method (Fig. 8A and B). The downregulation of *LOX2* is consistent with the previous studies reported by Sugio et al. (2011b). Interestingly, *LOX2* is involved in JA synthesis, whereas *PR1* and *ELI3-1* are salicylic acids (SA)-responsive genes (Kiedrowski et al., 1992; Spoel et al., 2003). These results suggest that $SAP11_{AYWB}$ not only interferes with JA synthesis but also suppresses SA-signaling responses. We found that $SAP11_{AYWB}$ repressed the expression of miR393, an Flg22-induced miRNA that participates in basal resistance against bacterial pathogens (Navarro et al., 2006). Thus, repression of miR393 and *PR1* in 35S::$SAP11_{AYWB}$ transgenic plants prompted us to investigate whether the expression of $SAP11_{AYWB}$ can suppress innate immunity against bacterial pathogens in *Arabidopsis*. To this end, we monitored the expression levels of genes involved in the SA-mediated defense-signaling network under the treatment of 1 mM SA. Genes involved in SA biosynthesis (*EDS5* and *SID2*), SA accumulation (*FMO1*, *WIN3*, and *PAD4*), as well as the standard marker genes for SA-mediated response (*PR1* and *AIG1*), were all repressed in *35S::*$SAP11_{AYWB}$ #13 transgenic line (Fig. 8A). We further investigated the effect of $SAP11_{AYWB}$ on the multiplication of *Pseudomonas syringae* pv. *tomato* strain DC3000 (*Pto* DC3000) in *Arabidopsis*. In this study, we used the *35S::*$SAP11_{AYWB}$ #12 and #29 transgenic lines for bacterial inoculation assays because the leaves of *35S::*$SAP11_{AYWB}$ #13 were too crinkled and small for inoculation. Compared with WT plants, both *35S::*$SAP11_{AYWB}$ #12 and #29 transgenic lines showed an increase in bacterial growth (Fig. 8C). Taken together, these results indicate that the expression of $SAP11_{AYWB}$ suppresses the host's innate immunity and makes the plants more susceptible to a bacterial infection.

DISCUSSION

$SAP11_{AYWB}$ has been shown to mediate the destabilization of *Arabidopsis* TCPs, which leads to the downregulation of *LOX2*, a key component in the biosynthesis of JA (Schommer

et al., 2008; Sugio et al., 2011b). In this study, our RNA-Seq analysis also showed a significant decrease in *LOX2* transcripts in *35S::SAP11$_{AYWB}$* transgenic plants (Table 2). In addition, we showed that the expression of miR319 was not changed in the *SAP11$_{AYWB}$*-overexpressing plants (Fig. 1C and 4D). These data are in agreement with previous published results showing that SAP11$_{AYWB}$ degrades TCPs (Sugio et al., 2011b), and suggest that the SAP11$_{AYWB}$-mediated suppression of TCPs' activities probably occurs only through post-translational regulation. In contrast to miR319, a significant increase in the amount of miR399, miR2111, and miR827,which have been shown to be upregulated by Pi starvation (Hsieh et al., 2009; Pant et al., 2009), was observed in *35S::SAP11$_{AYWB}$* transgenic plants (Fig. 1C; Supplemental Data S2). Among them, miR399 and miR827 play positive roles in regulating Pi uptake and translocation (Chiou et al., 2006; Liu et al., 2012; Huang et al., 2013; Lin et al., 2013). Consistent with this, *35S::SAP11$_{AYWB}$* transgenic plants accumulated a higher amount of Pi in cells and displayed reduced symptoms in the Pi-deficient condition, e.g., a lower level of anthocyanin accumulation (Fig. 2B and 3B). These phenomena were paralleled by an increase in the transcript levels of Pi starvation-induced genes, e.g., *IPS1*, *IPS2*, *SPX1*, *SPX3*, *PS2*, *PS3*, and *PHT1;4* (Fig. 2A).

Although Pi homeostasis is altered in *35S::SAP11$_{AYWB}$* transgenic plants, the molecular mechanism of SAP11$_{AYWB}$-triggered Pi starvation responses remains unclear and has yet to be clarified. In this study, we show that the Pi starvation responses triggered by SAP11$_{AYWB}$ are mainly dependent on the pathway regulated by *PHR1* (Fig. 7B). PHR1, a MYB transcription factor, plays a crucial role as a central hub in the transcriptional activation of genes involved in Pi transport and translocation, root architecture remodeling, anthocyanin accumulation, and sugar metabolic processes (Busto et al., 2010; Chiou and Lin, 2011). Thus, more than 75% of the Pi-starvation induced genes regulated by SAP11$_{AYWB}$ contain the potential *cis*-regulatory elements for PHR1 binding in the promoter region (Table 3). These results

might provide some information in elucidating the functional relationships among $SAP11_{AYWB}$ and Pi starvation responses. In *Arabidopsis*, there are 24 TCPs divided into two classes based on sequence similarities (Aguilar-Martinez et al., 2007). Recently, Sugio et al. (2011b) showed that class II CIN-TCPs were selectively destabilized by $SAP11_{AYWB}$. Class II CIN-TCPs contains 8 members, but only 5 of them can be targeted by miR319 (Ori et al., 2007). In this study, we found that the transcript levels of Pi starvation-induced genes and miR399 were not changed significantly in *35S::miR319a* transgenic plants (Fig. 4C to E), suggesting that miR319-mediated regulation may have minimal contribution to the $SAP11_{AYWB}$-triggered Pi starvation responses. Nevertheless, because only 5 transcripts out of 8 CIN-TCPs are targeted by miR319, involvement of other class II CIN-TCP members in Pi starvation responses cannot be excluded. On the other hand, whether the class II CYC/TB1-TCPs, the potential targets of $SAP11_{AYWB}$, is also involved in Pi starvation responses of *35S::*$SAP11_{AYWB}$ transgenic plants requires further investigation.

Phytoplasmas have the smallest genome among bacteria, and many essential genes required for standard metabolic functions are lost, including ATP synthase subunits (Oshima et al., 2004; Chung et al., 2013). Thus, phytoplasmas might uptake essential substances from the cytoplasm of surrounding host cells. Here, we showed that overexpression of $SAP11_{AYWB}$ could trigger Pi starvation responses and alter the Pi homeostasis in host cells (Fig. 2A and B). Pi is an essential nutrient required for various basic biological functions, e.g., synthesis of nucleic acids and phospholipids, regulation of energy metabolism, and signal transduction cascades (Raghothama, 1999; Shen et al., 2011). Although the molecular mechanism of $SAP11_{AYWB}$-triggered Pi starvation responses remains elucidated, it might alter the physiology of plant cells into a metabolically rich environment, which might be able to facilitate the growth of phytoplasmas.

Pi deficiency is typically accompanied with the accumulation of Fe and sugar (Jain et al.,

2007; Ward et al., 2008; Rouached et al., 2010). Similarly, our RNA-Seq analysis uncovers transcriptomic variations not only in genes involved in Pi starvation responses, but also in genes related to sugar metabolic processes and iron-deficiency responses, e.g., *GPT2*, *BAM5*, *TPPI*, *SWEET1*, *bHLH100*, *bHLH38*, and *bHLH39* (Table 1). These molecular changes might play a role in the cross-regulation between Pi starvation, sucrose signaling, and Fe responses. Because sugar and Fe are also essential nutrients for the growth and development of pathogens (Chen et al., 2010; Hood and Skaar, 2012), upregulation of genes involved in sugar metabolic processes and iron-deficiency responses might provide a better environment for the growth of phytoplasmas in plant cells.

The importance of Pi in the development of disease symptoms could be revealed in huanglongbing disease, in which an increase in the expression levels of miR399 and Pi starvation-induced genes was observed in citrus plants infected by *Candidatus Liberibacter* (Zhao et al., 2013). Noticeably, the disease symptoms of *Candidatus Liberibacter*-infected citrus were reduced after the application of Pi. In the case of phytoplasmas, although the correlation between Pi and the disease symptoms remains unclear, the disease symptoms in phytoplasma-infected plants could be reduced by the application of AM fungi (Lingua et al., 2002; Romanazzi et al., 2009; Kamińska et al., 2010; Sampò et al., 2012). AM fungi are symbiotic organisms that are able to enhance the uptake of Pi through upregulation of Pi starvation responses in host plants (Branscheid et al., 2010; Gu et al., 2010; Volpe et al., 2013). Thus, it will be interesting to explore whether the application of Pi could reduce the phytoplasma-mediated disease symptoms in the future. Although it is reasonable to assume that the availability of Pi in host cells might affect the disease symptoms in phytoplasma-infected plants, other factors might alter the disease symptoms as well. For example, the plant responses elicited by AM fungi, such as SA- and JA-defense responses (Gutjahr and Paszkowski, 2009), might participate in counteracting the colonization of

406 phytoplasmas, leading to the reduction of disease symptoms.

407 In addition to the regulation of Pi uptake, we found that the expression of $SAP11_{AYWB}$ is
408 able to suppress host's innate immunity by repressing and delaying the SA-mediated defense
409 responses (Table2; Fig. 8A). As a result, $SAP11_{AYWB}$-overexpressing plants become more
410 susceptible to bacterial infections (Fig. 8C). Because miR393 plays an important role in
411 modulating plant defense responses against bacterial pathogens through the repression of
412 auxin signaling pathway (Navarro et al., 2006; Katiyar-Agarwal and Jin, 2010), the changes
413 of host's innate immunity in *35S::SAP11$_{AYWB}$* transgenic plants might correlate with the
414 downregulation of miR393. However, the role of class II TCPs in *35S::SAP11$_{AYWB}$* transgenic
415 plants remains unclear. The suppression of host's innate immunity might be possible to be an
416 indirect consequence caused by the $SAP11_{AYWB}$-mediated destabilization of TCPs.

417 Root architecture remodeling induced by Pi starvation requires the regulation of auxin
418 signaling (Nacry et al., 2005; Jain et al., 2007; Pérez-Torres et al., 2008; Péret et al., 2011). In
419 *35S::SAP11$_{AYWB}$* transgenic plants, the formation of adventitious roots, elongation of lateral
420 roots, and reduction of primary roots was associated with the differential expression of
421 miRNAs involved in auxin signaling (Fig. 3C and 6B; Supplemental Data S2). These
422 miRNAs include miR160, miR393 and miR396, which target transcripts encoding the auxin
423 response factors, auxin receptors, and growth-regulating factors, respectively (Mallory et al.,
424 2005; Parry et al., 2009; Debernardi et al., 2012). Although the molecular changes in auxin
425 signaling triggered by $SAP11_{AYWB}$ remain largely unknown, the phenotypic alterations in root
426 architecture resemble to the disease symptoms appeared in the roots of several
427 phytoplasma-infected plants (Del Serrone et al., 2001; Lee et al., 2006).

428 Our findings provide useful information for the activities of $SAP11_{AYWB}$ in plant immune
429 responses and suggest that the expression of $SAP11_{AYWB}$ not only suppresses the JA signaling
430 responses against insect vectors but also downregulates the SA-mediated defense responses

against bacterial pathogens. These studies not only contribute to the understanding of molecular mechanisms underlying the interaction between phytoplasmas and plants, but also provide new insights into development of the strategy in controlling phytoplasma diseases.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

Arabidopsis ecotype Col-0 was used for the generation of transgenic plants, and bacterial inoculation. *Nicotiana benthamiana* was used for agroinfiltration. Plants were grown at 21°C in a semicontrolled walk-in chamber with a 16:8-h photoperiod for *Agrobacterium* transformation and Pi measurement; a 12:12-h photoperiod was used for SA treatment and bacterial inoculation.

Generation of Transgenic *Arabidopsis* Plants

A synthetic DNA template with the codon-optimized version of $SAP11_{AYWB}$ was used for PCR amplification. A DNA fragment encoding $SAP11_{AYWB}$ without the signal peptide was amplified using AccuPrime pfx DNA polymerase (Invitrogen) and subcloned into the pBA002 vector under the control of the *cauliflower mosaic virus* (*CaMV*) 35S promoter. After verification by DNA sequencing, plasmid DNAs were introduced into *Agrobacterium tumefaciens* strain ABI by using the freeze-thaw method and transformed into *Arabidopsis* by using the floral-dip method. To obtain homozygous transgenic lines expressing $SAP11_{AYWB}$, seeds were screened on 1/2× MS medium containing Basta (25 μg ml^{-1}) and carbenicillin (100 μg ml^{-1}) and examined with western blotting using specific anti-SAP11 antibodies.

Root Architecture Investigation and Anthocyanin Measurement

Arabidopsis seeds were germinated on 1/2× MS medium with 1% sucrose and 1% agar.

After incubation for 3 days, seedlings were transferred to the half-strength modified Hoagland nutrient solution containing 1% sucrose and 1% agar with the indicated concentration of KH_2PO_4. For root architecture investigation, seedlings were placed vertically to allow root growth along the surface of the agar, and the root architecture was observed at 14 days after germination. For anthocyanin measurement, seedlings were harvested at 13 days after germination and the anthocyanin content was determined as described with modifications (Saijo et al., 2009). Briefly, seedlings were homogenized in the extraction buffer (Propanol:HCl:H_2O = 18:1:81) and immersed into boiling water for 1.5 minutes. After centrifugation, the supernatant was collected for measuring the absorbance at 535 and 650 nm. The relative anthocyanin amount was calculated by the equation: (A535-2XA650)/fresh weight (g).

Pi Measurement

Pi contents were measured according to the method described by Chiou et al. (2006). Briefly, *Arabidopsis* seeds were germinated on agar plate with half-strength modified Hoagland medium containing 0.25 mM KH_2PO_4 for 11 days. The seedlings were then transferred to hydroponic culture. Before Pi starvation treatment, seedlings were grown on 0.25 mM KH_2PO_4. Fresh tissues were homogenized with extraction buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA, 100 mM NaCl, 1 mM-mercaptoethanl, and 1 mM phenylmethylsulfonyl fluoride) and then mixed with 1% glacial acetic acid. After centrifugation at 13,000 × *g* for 5 min, Pi assay solution (0.35% NH_4MoO_4, 0.86 NH_2SO_4, and 1.4% ascorbic acid) was added to the supernatant and then incubated at 42°C for 30 min. Pi content was measured using Tecan Infinite 200 PRO at A_{820}.

Small RNA Northern Blotting

For miRNA analysis, 12 μg of total RNA was fractionated on a 15% polyacrylamide gel containing 8 M urea and then transferred to a Hybond-N^+ membrane (GE Biosciences). Oligonucleotide probes were end-labeled with [γ-^{32}P]ATP by using T4 polynucleotide kinase (New England Biolabs). Hybridization was performed overnight at 42°C with the ULTRAHyb-Oligo hybridization buffer (Ambion). Signals were detected using autoradiography.

Antibody Production and Western Blotting

To generate recombinant proteins, a PCR product encoding $SAP11_{AYWB}$ without the signal peptide was subcloned into the SUMO-pET vector to generate an N-terminal His-SUMO fusion construct. After verification by DNA sequencing, plasmid DNA was introduced into *Escherichia coli* BL21 (DE3) cells, and His-SUMO-$SAP11_{AYWB}$ protein was purified using Ni^{2+}-NTA resin (Qiagen), according to the manufacturer's instruction. After being cleaved with *Ulp*1 to remove the His-SUMO tag, recombinant $SAP11_{AYWB}$ protein was purified for antibody production in a rabbit. The polyclonal antibody against $SAP11_{AYWB}$ was obtained by affinity purification using a polyvinylidene difluoride membrane as a coupling matrix. To detect $SAP11_{AYWB}$ in *Arabidopsis* transgenic plants, total extracts were prepared by directly adding 2.5× SDS sample buffer into plant samples after grinding. Amersham ECL western blotting reagents were used for the reactions, and the chemiluminescence signals were captured using an ImageQuant LAS 4000 mini (GE Healthcare).

RNA-Seq Analysis

To perform the RNA-Seq analysis, 3-week-old *35S::SAP11$_{AYWB}$* transgenic plants grown in 1/2× MS medium were used, and next-generation sequencings were performed using Hiseq 2000 (Illumina) with total RNA samples extracted by the RNeasy plant mini kit (Qiagen). For

transcriptome analysis, sequence reads were aligned using CLC bio, and gene expression levels were normalized as reads per kilobase of exon model per million mapped reads (RPKM). To identify differentially expressed genes in *35S::SAP11*$_{AYWB}$ transgenic plants, DEseq (Anders and Huber, 2010) analysis was performed, and differentially expressed genes with $P < 0.05$ were selected for further analysis.

qRT-PCR

For qRT-PCR analysis, *Arabidopsis* total RNA was extracted using the Trizol reagent (Invitrogen), and complementary DNAs were synthesized using Superscript III first strand synthesis supermix (Invitrogen), according to the manufacturer's instructions. The RNA expression analyses were performed using the KAPA SYBR fast qPCR kit (Kapa Biosystems) and Illumina Eco real-time PCR system. The expression levels of the selected genes were determined by normalizing to the reference gene *Actin2*. Experiments were repeated at least three times.

SA Treatments and Bacterial Inoculations

To examine the immune responses in *35S::SAP11*$_{AYWB}$ transgenic plants, 4- to 5-week-old plants grown in the soil were treated with 1 mM SA with foliar sprays. After spraying, the aerial parts of the plants were harvested at indicated times and frozen immediately in liquid nitrogen. For bacterial inoculations, *Pseudomonas syringae* pv. *tomato* DC3000 (*Pto* DC3000) was cultured in NY broth (3 g of beef extract, 5 g of peptone, and 3 g of yeast extract in 1 L of water) at 28°C. The 4- to 5-week-old *35S::SAP11*$_{AYWB}$ transgenic plants were inoculated with the bacterial suspension (5×10^5 CFU ml^{-1}). The inoculated plants were maintained at 21°C, and bacterial populations were determined at indicated time intervals by using NY agar plates containing rifampicin (50 μg ml^{-1}). Experiments were repeated at least three times.

ACKNOWLEDGEMENTS

We thank Dr. Yuval Eshed (Weizmann Institute of Science, Rehovot, Israel) for providing us with *35S::miR319a* seeds; Dr. Nai-Chun Lin (National Taiwan University, Taipei, Taiwan) for the *Pto* DC3000 strain.

LITERATURE CITED

Aguilar-Martinez JA, Poza-Carrion C, Cubas P (2007) *Arabidopsis BRANCHED1* acts as an integrator of branching signals within axillary buds. Plant Cell **19**: 58-72

Anders S, Huber W (2010) Differential expression analysis for sequence count data. Genome Biol **11**: R106

Aukerman MJ, Sakai H (2003) Regulation of flowering time and floral organ identity by a microRNA and its *APETALA2*-like target genes. Plant Cell **15**: 2730-2741

Aung K, Lin S-I, Wu C-C, Huang Y-T, Su C-l, Chiou T-J (2006) pho2, a phosphate overaccumulator, is caused by a nonsense mutation in a microRNA399 target gene. Plant Physiol **141**: 1000-1011

Bai X, Zhang J, Ewing A, Miller SA, Jancso Radek A, Shevchenko DV, Tsukerman K, Walunas T, Lapidus A, Campbell JW, Hogenhout SA (2006) Living with genome instability: the adaptation of phytoplasmas to diverse environments of their insect and plant hosts. J Bacteriol **188**: 3682-3696

Bai X, Correa VR, Toruño TY, Ammar E-D, Kamoun S, Hogenhout SA (2008) AY-WB phytoplasma secretes a protein that targets plant cell nuclei. Mol Plant-Microbe Interact **22**: 18-30

Bazin J, Khan GA, Combier J-P, Bustos-Sanmamed P, Debernardi JM, Rodriguez R, Sorin C, Palatnik J, Hartmann C, Crespi M, Lelandais-Brière C (2013) MiR396

556 affects mycorrhization and root meristem activity in the legume *Medicago truncatula*.
557 Plant J **74**: 920-934
558 **Branscheid A, Sieh D, Pant BD, May P, Devers EA, Elkrog A, Schauser L, Scheible W-R,**
559 **Krajinski F** (2010) Expression pattern suggests a role of miR399 in the regulation of the
560 cellular response to local Pi increase during arbuscular mycorrhizal symbiosis. Mol
561 Plant-Microbe Interact **23**: 915-926
562 **Bucher M, Wegmüller S, Drissner D** (2009) Chasing the structures of small molecules in
563 arbuscular mycorrhizal signaling. Cur Opin Plant Biol **12**: 500-507
564 **Bustos R, Castrillo G, Linhares F, Puga MI, Rubio V, Pérez-Pérez J, Solano R, Leyva A,**
565 **Paz-Ares J (2010)** A central regulatory system largely controls transcriptional activation
566 and repression responses to phosphate starvation in *Arabidopsis*. PLoS Genet **6**: e1001102
567 **Chang WC, Lee TY, Huang HD, Huang HY and Pan RL** (2008) PlantPAN: Plant
568 Promoter Analysis Navigator, for identifying combinatorial cis-regulatory elements with
569 distance constraint in plant gene group. BMC Genomics **9**: 561
570 **Chatterjee SS, Hossain H, Otten S, Kuenne C, Kuchmina K, Machata S, Domann E,**
571 **Chakraborty T, Hain T** (2006) Intracellular gene expression profile of listeria
572 monocytogenes. Infect Immun **74**: 1323-1338
573 **Chen L-Q, Hou B-H, Lalonde S, Takanaga H, Hartung ML, Qu X-Q, Guo W-J, Kim J-G,**
574 **Underwood W, Chaudhuri B, Chermak D, Antony G, White FF, Somerville SC,**
575 **Mudgett MB, Frommer WB** (2010) Sugar transporters for intercellular exchange and
576 nutrition of pathogens. Nature **468**: 527-532
577 **Chiou T-J, Aung K, Lin S-I, Wu C-C, Chiang S-F, Su C-l** (2006) Regulation of phosphate
578 homeostasis by microRNA in *Arabidopsis*. Plant Cell **18**: 412-421
579 **Chiou T-J, Lin S-I** (2011) Signaling network in sensing phosphate availability in plants. Ann.
580 Rev. Plant Biol. **62**: 185-206

581 **Chung W-C, Chen L-L, Lo W-S, Lin C-P, Kuo C-H** (2013) Comparative analysis of the
582 peanut witches'-broom phytoplasma genome reveals horizontal transfer of potential mobile
583 units and effectors. PLoS ONE **8**: e62770
584 **Debernardi JM, Rodriguez RE, Mecchia MA, Palatnik JF** (2012) Functional
585 specialization of the plant mir396 regulatory network through distinct microrna–target
586 interactions. PloS Genet **8**: e1002419
587 **Del Serrone P, Marzachì C, Bragaloni M, Galeffi P** (2001) Phytoplasma infection of
588 tomato in central Italy. Phytopathol Mediterr **40**: 137-142
589 **Gu M, Xu K, Chen A, Zhu Y, Tang G, Xu G** (2010) Expression analysis suggests potential
590 roles of microRNAs for phosphate and arbuscular mycorrhizal signaling in *Solanum*
591 *lycopersicum*. Physiol Plant **138**: 226-237
592 **Gu M, Chen A, Dai X, Liu W, Xu G** (2011) How does phosphate status influence the
593 development of the arbuscular mycorrhizal symbiosis? Plant Signal Behav **6**: 1300-1304
594 **Gutierrez L, Bussell JD, Păcurar DI, Schwambach J, Păcurar M, Bellini C** (2009)
595 Phenotypic plasticity of adventitious rooting in *Arabidopsis* is controlled by complex
596 regulation of AUXIN RESPONSE FACTOR transcripts and microRNA abundance. Plant
597 Cell **21**: 3119-3132
598 **Gutjahr C, Paszkowki U** (2009) Weights in the balance: JA and SA signaling in
599 root-biotroph interactions. MPMI **22**: 763-772
600 **Hogenhout SA, Oshima K, Ammar E-D, Kakizawa S, Kingdom HN, Namba S** (2008)
601 Phytoplasmas: bacteria that manipulate plants and insects. Mol Plant Pathol **9**: 403-423
602 **Hood MI, Skaar EP** (2012) Nutritional immunity: transition metals at the pathogen–host
603 interface. Nat Rev Micro **10**: 525-537
604 **Hsieh L, Lin S, Shih A, Chen J, Lin W, Tseng C, Li W, Chiou T** (2009) Uncovering small
605 RNA-mediated responses to phosphate-deficiency in *Arabidopsis* by deep sequencing.

606 Plant Physiol **151**: 2120-2132
607 **Huang T-K, Han C-L, Lin S-I, Chen Y-J, Tsai Y-C, Chen J-W, Lin W-Y, Chen P-M, Liu**
608 **T-Y, Chen Y-S and Chiou T-J** (2013) Identification of downstream components of
609 ubiquitin-conjugating enzyme PHOSPHATE2 by quantitative membrane proteomics in
610 *Arabidopsis* roots. Plant Cell **25**: 4044-4060
611 **Jain A, Poling MD, Karthikeyan AS, Blakeslee JJ, Peer WA, Titapiwatanakun B,**
612 **Murphy AS, Raghothama KG** (2007) Differential effects of sucrose and auxin on
613 localized phosphate deficiency-induced modulation of different traits of root system
614 architecture in *Arabidopsis*. Plant Physiol **144**: 232-247
615 **Jain A, Nagarajan V, Raghothama K** (2012) Transcriptional regulation of phosphate
616 acquisition by higher plants. Cell Mol Life Sci **69**: 3207-3224
617 **Kamińska M, Klamkowski K, Berniak H, Sowik I** (2010) Response of mycorrhizal
618 periwinkle plants to aster yellows phytoplasma infection. Mycorrhiza **20**: 161-166
619 **Katiyar-Agarwal S, Jin H** (2010) Role of small RNAs in host-microbe interactions. Annu
620 Rev Phytopathol **48**: 225-246
621 **Kessler A, Halitschke R, Baldwin IT** (2004) Silencing the jasmonate cascade: Induced plant
622 defenses and insect populations. Science **305**: 665-668
623 **Kiedrowski S, Kawalleck P, Hahlbrock K, Somssich IE, Dangl JL** (1992) Rapid activation
624 of a novel plant defense gene is strictly dependent on the *Arabidopsis* RPM1 disease
625 resistance locus. EMBO J **11**: 4677-4684
626 **Knauer S, Holt Anna L, Rubio-Somoza I, Tucker Elise J, Hinze A, Pisch M, Javelle M,**
627 **Timmermans Marja C, Tucker Matthew R, Laux T** (2013) A protodermal miR394
628 signal defines a region of stem cell competence in the *Arabidopsis* shoot meristem. Dev
629 Cell **24**: 125-132
630 **Kozomara A, Griffiths-Jones S** (2011) miRBase: integrating microRNA annotation and

631 deep-sequencing data. Nucleic Acids Res **39**: D152-D157
632 **Kuo H-F, Chiou T-J** (2011) The role of microRNAs in phosphorus deficiency signaling.
633 Plant Physiol **156**: 1016-1024
634 **Lee I-M, Davis RE, Gundersen-Rindal DE** (2000) Phytoplasma: phytopathogenic
635 mollicutes1. Annu Rev Microbiol **54**: 221-255
636 **Lee I-M, Bottner KD, Munyaneza JE, Davis RE, Crosslin JM, du Toit LJ, Crosby T**
637 (2006) Carrot purple leaf: A new spiroplasmal disease associated with carrots in
638 Washington State. Plant Dis **90**: 989-993
639 **Lin W-Y, Huang T-K, Chiou T-J** (2013) NITROGEN LIMITATION ADAPTATION, a
640 target of microRNA827, mediates degradation of plasma membrane-localized phosphate
641 transporters to maintain phosphate homeostasis in *Arabidopsis*. Plant Cell **25**: 4061-4074
642 **Lingua G, D'Agostino G, Massa N, Antosiano M, Berta G** (2002) Mycorrhiza-induced
643 differential response to a yellows disease in tomato. Mycorrhiza **12**: 191-198
644 **Liu T-Y, Aung K, Tseng C-Y, Chang T-Y, Chen Y-S, Chiou T-J** (2011) Vacuolar $Ca2^{+}/H^{+}$
645 transport activity is required for systemic phosphate homeostasis involving shoot-to-root
646 signaling in *Arabidopsis*. Plant Physiol **156**: 1176-1189
647 **Liu T-Y, Huang T-K, Tseng C-Y, Lai Y-S, Lin S-I, Lin W-Y, Chen J-W, Chiou T-J** (2012)
648 PHO2-dependent degradation of PHO1 modulates phosphate homeostasis in *Arabidopsis*.
649 Plant Cell **24**: 2168-2183
650 **Mallory AC, Bartel DP, Bartel B** (2005) MicroRNA-directed regulation of *Arabidopsis*
651 *AUXIN RESPONSE FACTOR17* is essential for proper development and modulates
652 expression of early auxin response genes. Plant Cell **17**: 1360-1375
653 **Muller R, Morant M, Jarmer H, Nilsson L, Nielsen T** (2007) Genome-wide analysis of the
654 *Arabidopsis* leaf transcriptome reveals interaction of phosphate and sugar metabolism.
655 Plant Physiol **143**: 156 -171

Nacry P, Canivenc G, Muller B, Azmi A, Van Onckelen H, Rossignol M, Doumas P (2005) A role for auxin redistribution in the responses of the root system architecture to phosphate starvation in *Arabidopsis*. Plant Physiology **138:** 2061-2074

Navarro L, Dunoyer P, Jay F, Arnold B, Dharmasiri N, Estelle M, Voinnet O, Jones JDG (2006) A plant miRNA contributes to antibacterial resistance by repressing auxin signaling. Science **312**: 436-439

Ohkama-Ohtsu N, Wasaki J (2010) Recent progress in plant nutrition research: cross-talk between nutrients, plant physiology and soil microorganisms. Plant Cell Physiol **51**: 1255-1264

Ori N, Cohen AR, Etzioni A, Brand A, Yanai O, Shleizer S, Menda N, Amsellem Z, Efroni I, Pekker I, Alvarez JP, Blum E, Zamir D, Eshed Y (2007) Regulation of *LANCEOLATE* by miR319 is required for compound-leaf development in tomato. Nat Genet **39**: 787-791

Oshima K, Kakizawa S, Nishigawa H, Jung H-Y, Wei W, Suzuki S, Arashida R, Nakata D, Miyata S-i, Ugaki M, Namba S (2004) Reductive evolution suggested from the complete genome sequence of a plant-pathogenic phytoplasma. Nat Genet **36**: 27-29

Pant BD, Musialak-Lange M, Nuc P, May P, Buhtz A, Kehr J, Walther D, Scheible W-R (2009) Identification of nutrient-responsive *Arabidopsis* and rapeseed microRNAs by comprehensive real-time polymerase chain reaction profiling and small RNA sequencing. Plant Physiol **150**: 1541-1555

Parry G, Calderon-Villalobos LI, Prigge M, Peret B, Dharmasiri S, Itoh H, Lechner E, Gray WM, Bennett M, Estelle M (2009) Complex regulation of the TIR1/AFB family of auxin receptors. Proc Natl Acad Sci USA **106**: 22540-22545

Péret B, Clément M, Nussaume L, Desnos T (2011) Root developmental adaptation to phosphate starvation: better safe than sorry. Trends Plant Sci **16**: 442-450

Pérez-Torres C-A, López-Bucio J, Cruz-Ramírez A, Ibarra-Laclette E, Dharmasiri S, Estelle M, Herrera-Estrella L (2008) Phosphate availability alters lateral root development in *Arabidopsis* by modulating auxin sensitivity via a mechanism involving the TIR1 auxin receptor. Plant Cell **20**: 3258-3272

Pulido A, Laufs P (2010) Co-ordination of developmental processes by small RNAs during leaf development. J Exp Bot **61**: 1277-1291

Radtke AL, O'Riordan MXD (2006) Intracellular innate resistance to bacterial pathogens. Cell Microbiol **8**: 1720-1729

Raghothama KG (1999) Phosphate acquisition. Annu Rev Plant Physiol Plant Mol Biol **50**: 665-693

Romanazzi G, Musetti R, Marzachì C, Casati P (2009) Induction of resistance in the control of phytoplasma diseases. Petria **19**: 113-129

Rouached H, Arpat AB, Poirier Y (2010) Regulation of phosphate starvation responses in plants: signaling players and cross-talks. Mol Plant **3**: 288-299

Rubio V, Linhares F, Solano R, Martin A, Iglesias J, Leyva A, Paz-Ares J (2001) A conserved MYB transcription factor involved in phosphate starvation signaling both in vascular plants and in unicellular algae. Genes Dev **15**: 2122 – 2133

Saijo Y, Tintor N, Lu X, Rauf P, Pajerowska-Mukhtar K, Häweker H, Dong X, Robatzek S, Schulze-Lefert P (2009) Receptor quality control in the endoplasmic reticulum for plant innate immunity. EMBO J **28**: 3439-3449

Sampò S, Massa N, D'Agostino U, Bosco D, Marzachì C, Berta G (2012) Effects of two AM fungi on phytoplasma infection in the model plant *Chrysanthemum carinatum* Agric Food Sci **21**: 39-51

Schommer C, Palatnik JF, Aggarwal P, Chételat A, Cubas P, Farmer EE, Nath U, Weigel D (2008) Control of jasmonate biosynthesis and senescence by miR319 targets.

706 PLoS Biol **6**: e230
707 **Shen J, Yuan L, Zhang J, Li H, Bai Z, Chen X, Zhang W, Zhang F** (2011) Phosphorus
708 dynamics: from soil to plant. Plant Physiol **156**: 997-1005
709 **Song JB, Huang SQ, Dalmay T, Yang ZM** (2012) Regulation of leaf morphology by
710 microRNA394 and its target *LEAF CURLING RESPONSIVENESS*. Plant Cell Physiol **53**:
711 1283-1294
712 **Spoel SH, Koornneef A, Claessens SMC, Korzelius JP, Van Pelt JA, Mueller MJ,**
713 **Buchala AJ, Métraux J-P, Brown R, Kazan K, Van Loon LC, Dong X, Pieterse CMJ**
714 (2003) NPR1 modulates cross-talk between salicylate- and jasmonate-dependent defense
715 pathways through a novel function in the cytosol. Plant Cell **15**: 760-770
716 **Sugio A, MacLean AM, Kingdom HN, Grieve VM, Manimekalai R, Hogenhout SA**
717 (2011a) Diverse targets of phytoplasma effectors: from plant development to defense
718 against insects. Annu Rev Phytopathol **49**: 175-195
719 **Sugio A, Kingdom HN, MacLean AM, Grieve VM, Hogenhout SA** (2011b) Phytoplasma
720 protein effector SAP11 enhances insect vector reproduction by manipulating plant
721 development and defense hormone biosynthesis. Proc Natl Acad Sci USA **108**:
722 E1254–E1263
723 **Volpe V, Dell'Aglio E, Giovannetti M, Ruberti C, Costa A, Genre A, Guether M,**
724 **Bonfante P** (2013) An AM-induced, *MYB*-family gene of *Lotus japonicus* (*LjMAMI*)
725 affects root growth in an AM-independent manner. Plant J **73**: 442-455
726 **Ward JT, Lahner B, Yakubova E, Salt DE, Raghothama KG** (2008) The effect of iron on
727 the primary root elongation of *Arabidopsis* during phosphate deficiency. Plant Physiol **147**:
728 1181-1191
729 **Wu G, Park MY, Conway SR, Wang J-W, Weigel D, Poethig RS** (2009) The sequential
730 action of miR156 and miR172 regulates developmental timing in *Arabidopsis*. Cell **138**:

731 750-759

732 **Zhao H, Sun R, Albrecht U, Padmanabhan C, Wang A, Coffey MD, Girke T, Wang Z,**

733 **Close TJ, Roose M, Yokomi RK, Folimonova S, Vidalakis G, Rouse R, Bowman KD,**

734 **Jin H** (2013) Small RNA profiling reveals phosphorus deficiency as a contributing factor

735 in symptom expression for Citrus Huanglongbing disease. Mol Plant **6**: 301-310

736

737

738 **FIGURE LEGENDS**

739 **Figure 1.** Expression of $SAP11_{AYWB}$ regulates the accumulation of miRNAs involved in Pi-
740 and auxin-signaling responses in *Arabidopsis*. (A) Comparison of leaf morphologies between
741 WT (Col-0) and transgenic plants carried $SAP11_{AYWB}$ or miR319a driven by *CaMV* 35S
742 promoter. Scale bar: 8 mm. (B) Examination of the translated product of $SAP11_{AYWB}$ by
743 western blotting using specific antibody against $SAP11_{AYWB}$. Asterisk indicates the
744 cross-reacting band appeared in all samples. Anti-tubulin was used for loading control. (C)
745 Comparisons of the expression levels of miRNAs in WT and *35S::*$SAP11_{AYWB}$ transgenic lines
746 by small RNA northern blotting. The RNAs staining bands were used as a loading control.
747 The values of band intensities were measured using ImageJ.

748

749 **Figure 2.** $SAP11_{AYWB}$ elicits the expression of Pi starvation-induced genes and increases the
750 accumulation of Pi in *Arabidopsis*. (A) The mRNA levels of genes triggered by Pi starvation
751 responses in WT (Col-0) plants and *35S::*$SAP11_{AYWB}$ transgenic lines were examined by
752 quantitative RT-PCR (qRT-PCR), and normalized to *Actin2*. The relative expression levels of
753 each gene in WT plants were set at 1. (B) The levels of Pi concentration in the aerial parts of
754 WT and *35S::*$SAP11_{AYWB}$ transgenic lines grown in hydroponic solutions with 0.01 mM or
755 0.25 mM KH_2PO_4 were measured. Statistically significant differences were determined using
756 Student's *t* test (* $p < 0.05$, ** $p < 0.005$ for transgenic plants versus the WT).

757

758 **Figure 3.** Expression of $SAP11_{AYWB}$ in *Arabidopsis* alters the phenotypes in anthocyanin
759 accumulation and root architecture. Comparisons of plant size (A), anthocyanin accumulation
760 (B), and root morphology (C) between WT (Col-0) plants and *35S::*$SAP11_{AYWB}$ transgenic
761 lines grown on horizontal (A) or vertical (C) plates containing 0.01 mM or 0.25 mM KH_2PO_4.

762 Statistically significant differences were determined using Student's *t* test (* $p < 0.05$, ** $p <$
763 0.005 for transgenic plants versus the WT). Scale bars: 15 mm. FW: fresh weight.
764
765 **Figure 4.** Comparison of gene expression profiles. (A) Comparison of the expression levels
766 of $SAP11_{AYWB}$ in *Arabidopsis 35S::SAP11$_{AYWB}$* transgenic lines by western blotting using
767 specific antibodies against $SAP11_{AYWB}$. Asterisk indicates the cross-reacting band appeared in
768 all samples. (B) Venn diagrams show the comparisons of $SAP11_{AYWB}$-elicited genes (p □
769 0.001) with the Pi starvation response (PSR) genes (up-regulation, 2-fold higher). PSR (I)
770 indicates the data set collected by Muller et al. (2007) from leaf samples in response to Pi
771 starvation. PSR (II) and PSR (III) indicate the data set collected by Liu et al. (2011) from leaf
772 and root samples, respectively, in response to Pi starvation. (C) The mRNA levels of
773 differentially expressed genes identified in the *35S::SAP11$_{AYWB}$* transgenic line #13 were
774 examined by qRT-PCR, and normalized to *Actin2*. The relative expression levels of each gene
775 in WT (Col-0) plants were set at 1. (D) Comparisons of the expression levels of miRNAs by
776 small RNA northern blotting. The RNAs staining bands were used as a loading control. (E)
777 The mRNA levels of genes involved in Pi starvation responses were examined by qRT-PCR,
778 and normalized to *Actin2*. The relative expression levels of each gene in WT (Col-0) plants
779 were set at 1. Statistically significant differences were determined using Student's *t* test (* $p <$
780 0.05, ** $p < 0.005$ for transgenic plants versus the WT). The values of band intensities were
781 measured using ImageJ.
782
783 **Figure 5.** Comparison of miRNA expression profiles. Scatter plot shows the related
784 expression levels of known miRNAs in *Arabidopsis* WT plants (X-axis) and *35S::SAP11$_{AYWB}$*
785 transgenic line #13 (Y-axis), in which the scales indicate the expression intensity of
786 normalized miRNAs. Red points refer to miRNAs whose expression levels were 2-fold

higher in *35S::SAP11$_{AYWB}$* transgenic line #13 as compared with WT plants. Green points refer to miRNAs whose expression levels were 2-fold lower in *35S::SAP11$_{AYWB}$* transgenic line #13 as compared with WT plants. Blue points refer to miRNAs whose expression levels were not higher or lower than 2-fold in the *35S::SAP11$_{AYWB}$* transgenic line #13 as compared with WT plants.

Figure 6. Phenotypic comparisons in leaf morphology and root architecture between *35S::miR319a* and *35S::SAP11$_{AYWB}$* transgenic plants. (A) Leaf morphologies of *Arabidopsis* seedlings grown on 1/2× MS medium. Scale bar: 8 mm. (B) Root architectures of *Arabidopsis* seedlings grown on the vertical plate containing 0.01 mM KH_2PO_4. Scale bar: 15 mm.

Figure 7. *PHR1* is required for SAP11$_{AYWB}$-triggered Pi starvation responses in *Arabidopsis*. (A) Leaf morphologies of *Arabidopsis* transgenic plants overexpressing SAP11$_{AYWB}$ in the background of a *phr1* mutant. The expression level of SAP11$_{AYWB}$ was detected by western blotting using specific antibodies against SAP11$_{AYWB}$. Asterisk indicates the cross-reacting band appeared in all samples. Scale bar: 8 mm. (B) The mRNA levels of genes involved in Pi starvation responses among WT (Col-0), *phr1* mutant, and *35S::SAP11$_{AYWB}$/phr1* transgenic plants were examined by qRT-PCR, and normalized to *Actin2*. The relative expression levels of each gene in WT plants were set at 1. Statistically significant differences were determined using Student's *t* test (* $p < 0.05$, ** $p < 0.005$ for WT versus *phr1*; + $p > 0.05$ for *phr1* versus *35S::SAP11$_{AYWB}$/phr1*).

Figure 8. SAP11$_{AYWB}$ suppresses the innate immunity against bacterial pathogens in *Arabidopsis*. RNA samples were extracted from SA-treated WT (Col-0) and *35S::SAP11$_{AYWB}$*

transgenic plants harvested at indicated times. The mRNA levels of genes involved in SA-mediated defense signaling network (A) and JA-signaling responses (B) were examined by qRT-PCR, and normalized to *Actin2*. The relative expression levels of each gene in WT plants without SA treatment were set at 1. (C) Bacterial growth of *Pseudomonas syringae* pv. *tomato* DC3000 (*Pto* DC3000) in WT and *35S::SAP11*$_{AYWB}$ transgenic plants were measured to examine the effects of SAP11$_{\mathrm{AYWB}}$ on the resistance of *Arabidopsis* against bacterial pathogens. Hand-infiltrated leaves were collected at the indicated times for measuring the *in planta* growth of *Pto* DC3000. Statistically significant differences were determined using Student's *t* test (* $p < 0.05$, ** $p < 0.005$ for transgenic plants versus the WT).

821

822 **TABLES**

Table 1. Classification and annotation of the differentially expressed genes ($p < 0.001$) elicited by SAP11$_{AYWB}$

Category		AGI[b] No.	Base Mean[a]		Fold change[c]	Short description	Reference[d]
Rank	*Name*		WT	*35S::SAP11 AYWB*			
Pi starvation response							
1st	*MYB90*	At1g66390	1.11	60.23	54.14	Myb domain protein 90	B, D
2nd	*IPS1*	At3g09922	18.91	620.26	32.8	Induced by phosphate starvation 1	
3rd	*GPT2*	At1g61800	7.79	175.29	22.51	Glucose-6-phosphate/phosphate translocator 2	B, D
5th	*SULTR1;3*	At1g22150	5.56	82.7	14.87	Sulfate transporter 1;3	A, C
6th	*SPX3*	At2g45130	5.56	79.11	14.22	SPX domain gene 3	B, C, D
8th	*MGDC*	At2g11810	30.04	374.86	12.48	Monogalactosyldiacylglycerol synthase type C	A, B, C, D
10th	*BAM5*	At4g15210	13.35	161.81	12.12	Beta-amylase 5	A, B, C
11th		At5g09570	7.79	89.89	11.54	Cox19-like CHCH family protein	C
14th	*PS2*	At1g73010	67.86	737.13	10.86	Phosphate starvation-induced gene 2	A, B, C, D
15th	*PEPC1*	At1g17710	46.72	468.34	10.02	PhosphoEthanolamine/PhosphoCholine phophatase 1	A, B, C, D
21th		At5g20790	84.55	747.01	8.84	Unknown protein	A, B, C, D
24th		At1g23110	28.92	232.82	8.05	Unknown protein	A
35th	*IPS2*	At5g03545	199.13	1141.65	5.73	Induced by phosphate starvation 2	
36th	*SPX1*	At5g20150	284.78	1629.77	5.72	SPX domain gene 1	A, B, C, D
45th	*PLDP2*	At3g05630	73.42	317.32	4.32	Phospholipase D P2	A, B, C, D
48th	*PS3*	At3g47420	543.98	2238.34	4.11	Phosphate starvation-induced gene 3	A, B, C, D
52th	*SRG3*	At3g02040	327.05	1253.11	3.83	Senescence-related gene 3	A, B, C, D
56th	*PAP17*	At3g17790	263.65	944.78	3.58	Purple acid phosphatase 17	A, B, C, D
Sugar metabolic processes							
3rd	*GPT2*	At1g61800	7.79	175.29	22.51	Glucose-6-phosphate/phosphate translocator 2	
10th	*BAM5*	At4g15210	13.35	161.81	12.12	Beta-amylase 5	
16th	*TPPI*	At5g10100	13.349	133.04	9.97	Trehalose-6-phosphate phosphatase I	
44th	*SWEET1*	At1g21460	350.42	1552.46	4.43	Nodulin MtN3 family protein	
Iron-deficiency responses							
20th	*bHLH100*	At2g41240	20.02	191.47	9.56	Basic helix-loop-helix protein 100	
25th	*bHLH38*	At3g56970	32.26	249.01	7.72	Basic helix-loop-helix protein 38	
29th	*bHLH39*	At3g56980	35.6	251.7	7.07	Basic helix-loop-helix protein 39	

[a] Base mean: the number of reads divided by the size factor (normalized constant) of sample.

[b] AGI: *Arabidopsis* Genome Initiative

[c] Fold change: *35S::SAP11 AYWB* base mean/WT base mean

[d] Reference: Pi starvation-induced genes (> 2 fold change) reported by Muller et al. (2007) (A), liu et al. (2011) (B: shoot, C: root) and Morcuende et al. (2007) (D).

823

824

Table 2. Classification and annotation of the top 10 differentially expressed genes ($p < 0.001$) suppressed by $SAP11_{AYWB}$

Category		AGI[b] No.	Base Mean[a]		Fold	
Rank	*Name*		WT	*35S::SAP11$_{AYWB}$*	change[c]	Short description
1st		At3g16670	814.29	0.89	914.9	Pollen Ole e 1 allergen and extensin family protein
2nd	*ELI3-1*	At4g37980	1311.55	2.69	487.6	Elicitor-activated gene 3-1
3rd		At3g05730	2143.65	8.09	264.9	Defensin-like (DEFL) family protein
4th	*PR1*	At2g14610	150.17	0.89	168.7	Pathogenesis-related gene 1
5th		At2g29300	518.39	5.393	96.1	NAD(P)-binding Rossmann-fold superfamily protein
6th		At3g19620	109.01	1.79	60.9	Glycosyl hydrolase family protein
7th		At4g12900	58.95	1.79	32.9	Gamma interferon responsive lysosomal thiol (GILT) reductase family protein
8th	*LOX2*	At3g45140	2688.74	86.29	31.2	Lipoxygenase 2
9th		At2g29290	80.09	3.59	22.3	NAD(P)-binding Rossmann-fold superfamily protein
10th		At2g32160	75.64	3.59	21.1	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein

[a] Base mean: the number of reads divided by the size factor (normalized constant) of sample.

[b] AGI: *Arabidopsis* Genome Initiative

[c] Fold change: WT base mean/*35S::SAP11$_{AYWB}$* base mean

825

826

827

Table 3. The potential sequence of PHR1-binding site found at the upstream region of Pi-starvation induced genes regulated by $SAP11_{AYWB}$

Gene	AGI[a] number	Sequence (Position[b])
IPS1	At3G09922	GCATATTC (-581); GCATATTC (-547)
GPT2	At1G61800	GTATATTC (-125)
SULTR1;3	At1G22150	GGATATTC (-439)
SPX3	At2G45130	GAATATGC (-212); GCATATCC (-89)
	At5G09570	GGATATAC (-1656); GAATATTC (-142)
PS2	At1G73010	GGATATTC (-693); GAATATTC (-308)
PEPC1	At1G17710	GAATATTC (-765); GAATATTC (-370)
	At5G20790	GAATATGC (-1325)
	At1G23110	GAATATGC (-346); GAATATGC (-171)
IPS2	At5G03545	GTATATGC (-687); GCATATTC (-149)
SPX1	At5G20150	GAATATTC (-824); GGATATTC (-67); GAATATTC (-43)
PLDP2	At3G05630	GAATATTC (-698); GGATATTC (-664); GCATATAC (-635); GCATATAC (-244)
SRG3	At3G02040	GTATATGC (-334); GAATATTC (-137); GAATATCC (-73); GAATATTC (-73)
PAP17	At3G17790	GAATATCC (-131)

[a] AGI: *Arabidopsis* Genome Initiative

[b] Position: the position in the 5'-upstream region is given with respect to the ATG strat codon.

828

829