BOTRYTIS-INDUCED KINASE1, a plasma membrane-localized receptor-like protein kinase, is a negative regulator of phosphate homeostasis in Arabidopsis thaliana

Huijuan Zhang¹², Lei Huang², Yongbo Hong² and Fengming Song²*

Abstract

Background: Plants have evolved complex coordinated regulatory networks to cope with deficiency of phosphate (Pi) in their growth environment; however, the detailed molecular mechanisms that regulate Pi sensing and signaling pathways are not fully understood yet. We report here that the involvement of Arabidopsis BIK1, a plasma membrane-localized receptor-like protein kinase that plays critical role in immunity, in Pi starvation response.

Results: qRT-PCR analysis revealed that expression of BIK1 was induced by Pi starvation and GUS staining indicated that the BIK1 promoter activity was detected in root, stem and leaf tissues of plants grown in Pi starvation condition, demonstrating that BIK1 is responsive to Pi starvation stress. The bik1 plants accumulated higher Pi content in root and leaf tissues and exhibited altered root architecture such as shorter primary roots, longer and more root hairs and lateral roots, as compared with those in the wild type plants, when grown under Pi sufficient and deficient conditions. Increased anthocyanin content and acid phosphatase activity, reduced accumulation of reactive oxygen species and downregulated expression of Pi starvation-induced genes including PHR1, WRKY75, AT4, PHT1;2 and PHT1;4 were observed in bik1 plants grown under Pi deficient condition. Furthermore, the expression of PHO2 was downregulated while the expression of miRNA399a and miRNA399d, which target to PHO2, was upregulated in bik1 plants, compared to the wild type plants, when grown under Pi deficient condition.

Conclusion: Our results demonstrate that BIK1 is a Pi starvation-responsive gene that functions as a negative regulator of Pi homeostasis in Arabidopsis.

Keywords: BOTRYTIS-INDUCED KINASE1 (BIK1), Phosphate starvation stress, Phosphate starvation response, Root architecture

Background

Phosphate (Pi) is one of the indispensable macronutrients to plants for growth, development and reproduction. Pi deficiency (~Pi) is one of the main limiting factors for increasing crop yield and improving quality because of low bioavailability of Pi in soil [1–4]. To cope with Pi deficiency, plants develop a series of tightly controlled adaptive responses including external developmental alterations of increasing Pi absorption and internal metabolic, physiological and biochemical alterations of reducing Pi usage [4–6]. To increase Pi uptake under Pi depletion stress, plants adapt to modulate the root architecture bearing more and longer lateral roots as well as denser root hairs, which enable the roots to explore the Pi resources in soil [7–12]. To reduce Pi consumption under Pi deficiency condition, plants often modulate metabolisms to maintain intracellular Pi homeostasis by reducing metabolic consumption of Pi [1, 3, 13–15] and degrading compounds to release Pi [16]. Furthermore, a series of physiological
and biochemical adaptions including the induction and secretion of phosphatases and organic acids and accumulation of protective metabolites such as anthocyanin help augment the availability of both endogenous and exogenous Pi [1, 4, 17, 18].

Genetic, physiological and biochemical studies in Arabidopsis have demonstrated that the acquisition, allocation, and metabolism of Pi are highly regulated processes and require the concerted action of multiple membrane Pi transport systems [19–23]. Among five distinct classes of proteins possessing Pi transport activity, the plastidic Pi translocator group function as antiport systems, whereas the other four Pi transporter families, named the PHOSPHATE TRANSPORTER1 (Pht1), Pht2, Pht3 and Pht4, contribute to the acquisition, allocation and remobilization of Pi [24–27]. Most of the plasma membrane-localized high affinity transporters in the Pht1 family mediate Pi acquisition from external environment [1, 24, 28–30]. Once Pi is transported into root epidemic cells, translocation and allocation of Pi within the plants and cells are key steps in maintaining Pi homeostasis at cell and whole plant levels. The low affinity transporters in the Pht2 and Pht4 families are thought to participate mainly in Pi transfer across internal cellular membranes and thus allocate Pi in different compartments of the cells [25, 27, 31–33]. Pht1:5, a Pht1 family member, PHO1, Pht1;8 and Pht1;9 were shown to play critical roles in systemic regulation of Pi homeostasis, e.g. mobilization of Pi from source to sink organs in accordance with the Pi status of the plant [34–36].

Functional characterization of genes in a number of mutants with altered response to Pi depletion have led to the identification of several different regulatory mechanisms controlling the adaptive responses to Pi starvation. These regulatory mechanisms include transcriptional regulation by transcription factors such as PHR1, WRKY6, WRKY42, WRKY45, WRKY75, ZAT6, bHLH32, ERF070 and MYB62 [37–47], posttranscriptional regulation by microRNAs including miRNA399 [48–53], posttranslational regulation by protein modifications such as sumoylation of SIZ1 [54], phosphorylation of Pht1.1 and activation of MKK9-MPK3/MPK6 module [55, 56], deubiquitination of UBP14 [57] and chromatin histone modification and epigenetic [46, 58]. Furthermore, it was also demonstrated that Pi starvation response cross-talks signaling mediated by different hormones such as auxin [10, 15, 59–61], cytokinin [10, 15, 62, 63], ethylene [9, 10, 64–66] and gibberellin [67]. Although great advance on the adaptive response to Pi starvation has been made during the last decade, the molecular mechanism that regulates these adaptive processes has yet to be elucidated in detail.

The Arabidopsis thaliana BOTRYTIS-INDUCED KINASE1 (BIK1) encodes a plasma membrane-localized receptor-like protein kinase and plays critical roles in Botrytis cinerea resistance [68]. Recent studies have shown that BIK1 interacts with receptors for pathogen- or damage-associated molecular patterns such as FLS2 and PEPRs to regulate immune response against different types of pathogens [69–73] and defense response to insect pests [74]. The bik1 mutant plants showed an altered root architecture [68], similar to morphological phenotypes often seen in mutants with Pi starvation response [75], indicating a possible involvement of BIK1 in Pi starvation response in Arabidopsis. Therefore, we investigated whether BIK1 functions in Pi starvation response and our results demonstrate that BIK1 plays a role in regulation of Pi homeostasis in Arabidopsis.

Results
Responsiveness of BIK1 to Pi starvation
When grown on MS medium under normal conditions, the bik1 plants produced shorter primary roots and longer and significantly more root hairs and lateral roots than WT plants [68], which is reminiscent of the mutants with defects in Pi nutrition [75]. These observations led us to examine whether BIK1 has a function in Pi starvation response. We first examined whether BIK1 is responsive to Pi starvation stress by analyzing the expression patterns of BIK1 in seedlings grown under + Pi and –Pi conditions. As shown in Fig. 1a, expression of BIK1 was detected in roots, shoots and leaves of seedlings grown under + Pi condition and no significant changes in BIK1 expression was observed during the experiment period. However, expression level of BIK1 was markedly induced with similar patterns in roots, shoots and leaves of seedlings after transferring to medium without Pi supplement (Fig. 1a). The transcript levels of BIK1 in seedlings grown under –Pi condition increased at 12 h and peaked at 24 and 48 h after transferring, leading to 7.5–11.2 folds of increases over those in seedlings grown under + Pi condition (Fig. 1a). To gain further information on spatial expression of the BIK1 gene in response to Pi starvation, we generated BIK1pro::GUS transgenic lines and compared the GUS staining patterns in T2 seedlings during Pi starvation stress. Slight GUS staining was observed in leaves and roots of the BIK1pro::GUS seedlings at 0 h after transferred to medium without Pi supplement (Fig. 1b), indicating a basal expression of BIK1 in leaf and root tissues, similar to the results from RT-PCR. At 1 day after transferred to medium without Pi supplement, significant GUS staining was easily seen in roots, shoot and leaves of the BIK1pro::GUS seedlings (Fig. 1b). qRT-PCR analysis showed 7.3 and 9.7 folds of increases in the expression levels of GUS gene in root and leaf tissues of the BIK1pro::GUS
seedlings at 1 day after transferring to \(-\text{Pi}\) condition (Fig. 1c). Notably, GUS staining was clearly observed in vascular tissues of roots, shoots and leaves of the \(\text{BIK1}\text{pro}::\text{GUS}\) seedlings subjected to Pi starvation (Fig. 1b). Together, data from RT-PCR and GUS staining demonstrate that \(\text{BIK1}\) is responsive to Pi starvation.

**Increased Pi concentration in \(\text{bik1}\) plants**

We next examined whether loss of \(\text{BIK1}\) function affects Pi homeostasis in \(\text{bik1}\) plants. Total Pi contents in leaves and roots of \(\text{WT}\) and \(\text{bik1}\) plants grown hydroponically under + Pi (250 \(\mu\)M) and \(-\text{Pi}\) conditions for 27 days were measured. As shown in Fig. 2, a significant increase in total Pi contents was observed in leaves and roots of
*bik1* plants as compared to WT plants under both +Pi and −Pi conditions. When grown under +Pi condition, total Pi contents in roots and leaves of *bik1* plants were 0.43 and 1.12 times higher over those in WT plants, respectively (Fig. 2a and b). Similarly, when grown under −Pi condition, total Pi contents in roots and leaves of *bik1* plants were 0.55 and 1.05 times higher than those of WT plants, respectively (Fig. 2a and b). These data indicate that BIK1 has a function either in Pi uptake or in the transfer of Pi from the roots to the leaves.

**Altered root architecture in *bik1* plants**

Root architecture plays important roles in maintaining Pi homeostasisin plants [75]. We compared the root architecture of WT and *bik1* seedlings grown under Pi normal (+Pi) and starvation (−Pi) conditions. When compared with WT seedlings, the *bik1* seedlings grown under +Pi condition showed shorter primary root and more lateral roots (Fig. 3a, c and d), and this trend was much evident in the *bik1* seedlings grown under −Pi condition (Fig. 3a, c and d). When grown under +Pi and −Pi conditions, the elongation rate of the primary and lateral roots of the *bik1* seedlings was markedly reduced as compared with WT seedlings (Fig. 3e and f). Furthermore, the *bik1* seedlings grown under +Pi and −Pi conditions showed more and longer root hairs than WT seedlings (Fig. 3b, g and h). These data indicate that BIK1 plays an important role in regulating development of root architecture.

**Increased anthocyanin accumulation and acid phosphatase activity in *bik1* plants**

Accumulation of anthocyanin and increased secretion of acid phosphatases are characteristic symptoms in plants under Pi starvation conditions [3]. We thus examined and compared the accumulation of anthocyanin and activity of acid phosphatase between WT and *bik1* plants grown under +Pi and −Pi conditions. When grown on medium under −Pi condition for 5 days, the leaves of the *bik1* seedlings turned to be purple while WT seedlings still kept green (Fig. 4a). The anthocyanin contents in *bik1* seedlings grown under +Pi and −Pi conditions were much higher than those in WT seedlings, showing 32 % and 105 % increase, respectively (Fig. 4b). No visible staining and significant changes in acid phosphatase activity were detected in WT and *bik1* seedlings grown + Pi condition (Fig. 4c and d). When grown under −Pi condition, roots of the *bik1* seedlings are stained deep blue while the staining of roots of WT seedlings is much lighter (Fig. 4c). Similarly, activity of acid phosphatase in roots of *bik1* seedlings is much higher than that in WT seedlings, grown under −Pi condition, showing an increase of ~65 % (Fig. 4d). These results indicate that the *bik1* seedlings secrete higher levels of acid phosphatase than WT seedlings under Pi starvation stress. Collectively, these results suggest that loss of *BIK1* function results in typical Pi starvation responses as revealed by the increased anthocyanin contents and acid phosphatase activity.

**Reduced ROS accumulation in *bik1* plants under Pi starvation condition**

ROS, generated and accumulated in plant response to different types of stresses, has been implicated in many biological processes including biotic and abiotic responses [76, 77]. Therefore, we examined whether mutation in *BIK1* gene affects the balance of ROS and hence leads to accumulation of ROS in plants during Pi starvation stress. In NBT staining of superoxide anion, no significant difference was detected between WT and *bik1* plants grown under +Pi condition; however, significant accumulation of superoxide anion in leaves, stem and roots of WT and *bik1* plants grown under −Pi condition was observed (Fig. 5a). Notably, accumulation of superoxide anion in WT plants was much higher than that in *bik1* plants grown under −Pi condition (Fig. 5a). Quantification of H$_2$O$_2$ contents revealed a significant lower level of H$_2$O$_2$ accumulated in *bik1* plants than that in WT plants when grown under +Pi condition (Fig. 5b). Accumulation of H$_2$O$_2$ in WT and *bik1* plants grown under −Pi condition was markedly increased, leading to 95 and 78 % of increases as compared with those in plants grown under +Pi condition, respectively (Fig. 5b). Under −Pi condition, the content of H$_2$O$_2$ in *bik1* plants was approximately 54 % of that in WT plants (Fig. 5b). These results indicate that loss of *BIK1* function led to reduced accumulation of ROS in *bik1* plants under −Pi starvation stress.
Fig. 3 (See legend on next page.)
Altered expression of Pi starvation-induced genes and miRNA399 in bik1 plants

Expression of some well-characterized Pi starvation-induced genes was analyzed and compared between WT and bik1 seedlings that had been transferred into medium with Pi (+Pi) or without Pi (−Pi) for 5 days. When grown under + Pi condition, the expression levels of AT4[78], WRKY75[39], PHT1;2 and PHT1;4[24, 28] in bik1 seedlings were comparable to those in WT seedlings, while the expression of PHR1[37] in bik1 seedlings was reduced (Fig. 6a). The expression levels of the Pi starvation-induced genes tested were markedly increased in WT and bik1 plants grown under −Pi condition (Fig. 6a). However, significant reductions in the expression levels of these Pi starvation-induced genes was observed in bik1 plants, as compared with those in WT plants, under −Pi condition (Fig. 6a). These results indicate that mutation in BIK1 leads to reduced expression of the Pi starvation-induced genes in plants grown under −Pi condition.

It was found that expression of PHO2, encoding a ubiquitin-conjugating E2 enzyme, was suppressed by miRNA399, which controls Pi homeostasis in plants and whose expression is up-regulated by Pi starvation[48–52]. To examine whether PHO2/miRNA399 links to increased accumulation of Pi in bik1 plants, we analyzed the changes in levels of PHO2 expression and two miRNA399 primary transcripts, miRNA399a and miRNA399d, in bik1 seedlings grown under + Pi and −Pi conditions. The expression level of PHO2 and the transcript levels of miRNA399a and miRNA399d were comparable in WT and bik1 seedlings grown under + Pi condition. However, the expression levels of PHO in WT and bik1 seedlings under −Pi condition were significantly reduced whereas the transcript levels of miRNA399a and miRNA399d in WT and bik1 seedlings grown under −Pi condition were significantly reduced whereas the transcript levels of miRNA399a and miRNA399d in WT and bik1 seedlings grown
Fig. 5 Reduced accumulation of ROS in bik1 plants. Four-week-old seedlings grown under + Pi (250 μM) and –Pi conditions were collected for detection of superoxide anion and measurement of \( \text{H}_2\text{O}_2 \). a NBT staining of superoxide anion in WT and bik1 plants grown under + Pi and –Pi conditions. b Quantification of \( \text{H}_2\text{O}_2 \) contents in WT and bik1 plants grown under + Pi and –Pi conditions. Data presented are the means ± SD from three independent experiments and different letters above the columns indicate significant differences at \( p < 0.05 \) level between WT and bik1 plants grown under same condition.

Fig. 6 Down-regulated expression of Pi starvation-induced genes in bik1 plants. a Expression patterns of Pi starvation-induced genes in WT and bik1 seedlings grown under + Pi and –Pi conditions. Seven-day-old seedlings grown under normal Pi condition were treated with Pi (+Pi, 250 μM) or without Pi (–Pi) for 5 days and total RNA were extracted from root tissues at 5 days after treatment. b Expression patterns of PHO2 and miRNA399 in WT and bik1 seedlings grown under + Pi and –Pi conditions. Seven-day-old seedlings grown under normal Pi condition were transferred to medium supplemented with Pi (+Pi, 250 μM) or without Pi (–Pi) and samples were collected at 48 h after treatment. Expression of Pi starvation-responsive genes and miR399s was analyzed by qRT-PCR using specific primers and data were normalized with the transcript level of UBQ10 as an internal control. Data presented are the means ± SD from three independent experiments and different letters above the columns indicate significant differences at \( p < 0.05 \) level.
under –Pi condition were markedly increased, as compared with the corresponding seedlings under + Pi condition (Fig. 6b). A further reduction in the expression of PHO2 and a further increase in the transcripts of miRNA399a and miRNA399d in bik1 seedlings was observed as compared with those in WT seedlings grown under –Pi condition (Fig. 6b). Together, these data indicate that the PHO2/miRNA399 plays a role in regulating Pi accumulation in bik1 plants under –Pi condition.

Discussion

The maintenance of cellular Pi homeostasis in plants involves complicated regulatory mechanisms. Several studies have demonstrated that posttranslational modifications such as phosphorylation, sumoylation and ubiquitination of regulatory proteins play critical roles in Pi starvation responses [54, 55, 57]. Recently, genome-wide co-expression analysis identifies dozens of protein kinases as important regulators of Pi deficiency-induced root hair remodeling [79, 80]. It was also found that in vivo phosphorylation activation of PPC1 (phosphoenolpyruvate carboxylase 1) is involved in the metabolic adaptations of Pi starvation [81] and activation of MKK9-MPK3/MPK6 enhances phosphate acquisition in Arabidopsis [56]. These findings indicate an important role for protein kinases in regulating Pi starvation response. In this study, we found that BIK1, a plasma membrane-localized receptor-like kinase protein kinase [68], plays an important role in modulating Pi starvation responses and functions as a negative regulator of Pi homeostasis in Arabidopsis plants. Thus, evidence presented in the current study renders BIK1 for a novel function in Pi starvation response, in addition to its previously reported functions in immunity [68–73].

Several lines of evidence presented in this study support that BIK1 functions in Pi starvation response. Earlier study has shown that expression of BIK1 could be induced by infection with B. cinerea as well as treatments with some well-known defense signaling molecules [68]. Our qRT-PCR analysis of BIK1 expression and determination of BIK1 promoter activity in stable BIK1pro::GUS transgenic seedlings demonstrate that BIK1 can be induced by Pi starvation and Pi starvation-induced expression of BIK1 in root and shoot tissues initiated earlier than in leaf tissues (Fig. 1a). The pattern of BIK1 expression induced by Pi starvation is similar to most of the Pi starvation-induced genes identified so far, like WKRY75, ERF070 and ZAT6 [39, 40, 44]. Importantly, promoter activity analysis revealed that GUS staining driven by the BIK1 promoter was initiated in vascular tissues of root (Fig. 1b) that is the primary organ that responds to Pi starvation, further confirming the responsiveness of BIK1 to Pi starvation. Thus, it is likely that BIK1 can respond rapidly to the altered Pi status of plants under Pi starvation condition. It was particularly noteworthy that BIK1 is a plasma membrane-localized receptor-like protein kinase and has been shown to be phosphorylated by other kinases (e.g. BAK1 and FLS2) for its full activity in plant immune responses [69, 70]. In this context, it is thus possible that BIK1 is phosphorylated by unknown kinase during Pi starvation stress and phosphorylated BIK1 acts in Pi starvation response, in addition to its transcriptional regulation upon Pi starvation.

The primary root, lateral root, and root hairs are three main components of the root architecture that is critical to absorption of Pi from soil. Low P availability can drastically alter the root architecture by switching the indeterminate growth to determinate growth to promote lateral root growth [75]. Generally, not only the primary roots but also almost all mature lateral roots enter into the determinate developmental program under low Pi condition [82]. The bik1 seedlings showed a significant change in root architecture and the primary root length of bik1 plants was significantly decreased (Fig. 6). This is similar to a common phenomenon observed that the primary root length is decreased significantly due to the Pi starvation-induced determinate growth in primary root [82]. In addition to the change of the primary root, the bik1 plants showed increased number and length of lateral roots and root hairs (Fig. 6), which increase root surface contacting an increased soil volume to explore Pi availability in soil [75]. Similar root architectures were observed in the bik1 plants under + Pi and –Pi conditions (Fig. 6). Thus, it is likely that the effect of BIK1 on the development of root architecture is independent of the Pi status in plants and Pi availability in soil. Nevertheless, the characteristic root architecture observed in bik1 plants suggests that BIK1 is a negative regulator of lateral root and root hair development. This is in agreement with previous observations that the bik1 plants showed some defects in growth and development, e.g. weak stem strength, early flowering and less seed setting [68]. Together, these data further demonstrate that BIK1 is required for normal plant growth and development [68]. It will be interesting to investigate whether the growth and developmental defects in bik1 plants is due to an activated Pi starvation response and if this is the case, the results obtained will further support a cross-talk between Pi starvation response and plant growth/development. It was previously found that overexpression of ZAT6 retards growth and results in typical Pi starvation responses [40].

Accompanied with high contents of total Pi in roots and leaves of bik1 plants under + Pi and –Pi conditions (Fig. 2) are the significant accumulation of anthocyanin (Fig. 4a and b), increased activity of acid phosphatase (Fig. 4a and b) and alterations in expression of Pi...
starvation-induced genes (Fig. 6). Collectively, these data imply that, whatever grown under + Pi condition or under –Pi condition, a Pi starvation response including those of physiological, molecular and metabolic changes is activated in bik1 plants. Increased activity of acid phosphatases in bik1 plants grown under –Pi condition (Fig. 3b) not only represents a characteristic Pi starvation response [83] but also can release more Pi available for absorption by the roots. This is supported by the findings that mutations in genes encoding for purple acid phosphatases affect markedly the uptake of Pi from exogenous sources [84–86]. Because expression of BIK1 was rapidly induced by Pi starvation (Fig. 1), it seems possible that BIK1 is involved in Pi uptake by regulating the activity of acid phosphatase. Similar observations were also obtained for some Pi starvation response regulators such as PHR1 and ZAT6, which have been shown to affect activity of acid phosphatase and Pi uptake in transgenic plants with altered expression of these genes [40, 85, 87, 88]. Interestingly, expression of Pht1;2 and PhtL1;4, encoding for high-affinity Pi transporters [24, 28], WRKY75, encoding a WRKY transcriptional factor involved in Pi acquisition [39], At4, a member of the Mt4/TPS11 gene family involved in Pi distribution [78], and PHR1, encoding a MYB transcriptional factor involved in Pi starvation response signaling [37], was suppressed significantly in roots of bik1 plants under –Pi condition (Fig. 6a). These data demonstrate that BIK1 has global effect on a set of Pi starvation-induced genes that are involved in Pi acquisition and mobilization. Notably, the expression levels of the tested Pi starvation-responsive gene except PHR1 in bik1 plants were slightly reduced as compared to those in WT plants under + Pi condition (Fig. 6a), indicating a possibility that these Pi starvation-responsive genes may be also affected by mutation of BIK1 itself. Particularly, the expression of PHR1 in bik1 plants grown under + Pi condition was significantly reduced as compared to that in WT plants (Fig. 6a), leading to an open question whether BIK1 acts upstream of PHR1 in regulating the Pi starvation signaling. This can be clarified by phenotyping and analysis of PHR1 expression in BIK1-overexpressing plants and/or detailed examination of biochemical and genetic requirement of BIK1 for Pi starvation response. It is currently difficult to link the function of BIK1 to Pi uptake or its root-to-leaf translocation as Pi uptake and transportation in WT plants is also affected under –Pi condition (Fig. 2). This can further be explained by the reduced expression of these tested Pi starvation-induced genes in root of bik1 plants as compared to those in WT plants under –Pi condition (Fig. 6). Furthermore, altered expression patterns of PHO2 and miRNA399a/d, which are thought to be involved in systemic signaling of Pi starvation response and Pi distribution in plants [49, 52], were also observed in bik1 plants grown under + Pi and –Pi conditions (Fig. 6b). Thus, it is likely that BIK1 is involved in maintaining Pi homeostasis in whole plants. Taken together, these data support an idea that BIK1 is a negative regulator of Pi starvation responses, probably through affecting development of root architecture and a series of physiological and biochemical events related to Pi acquisition, mobilization and translocation. It is reasonable to speculate that, under Pi starvation stress, the bik1 plants may experience a reduced Pi content as the WT plants but they can uptake efficiently Pi from growth environment with their significantly increased root surface area, leading to an increased Pi content in root and leaf tissues of bik1 plants (Fig. 2). However, the detailed mechanism that BIK1 functions in Pi starvation response remains to be explored further. Particularly, characterization of the targets that are phosphorylated by BIK1 will be greatly helpful in elucidating the early signaling events that determine Pi starvation response.

ROS not only are toxic compounds produced in plant response to various stresses but also plays an integral role as signaling molecules in regulation of numerous biological processes such as growth, development, and responses to biotic and/or abiotic stress [89–91]. The involvement of ROS in Pi starvation response has been established recently. In this study, we found that both WT and bik1 plants grown under –Pi condition accumulated more ROS, represented by H2O2 and superoxide anion, than those in plants grown under + Pi condition (Fig. 5). This is similar to the previous observations that H2O2 concentrations in roots increased upon Pi deprivation [92]. Notably, the accumulation of superoxide anion in WT and bik1 plants under + Pi condition was comparable, the accumulation of superoxide anion in bik1 plants was significantly reduced as compared to that in WT plants under –Pi condition (Fig. 5). By contrast, the bik1 plants accumulated lower level of H2O2 than WT plants grown under + Pi condition (Fig. 5), which is similar to the histochemical staining in soil-grown bik1 plants [68]. Thus, it is likely that BIK1 affects the accumulation of superoxide anion and H2O2 in different ways: loss of BIK1 function suppressed, at least partially, the Pi starvation-induced accumulation of superoxide anion while BIK1 regulates directly the accumulation of H2O2 in plants under normal condition. This may also imply different functions of superoxide anion and H2O2 in Pi starvation response. On the other hand, increased accumulation of ROS in plants under –Pi condition might be one of the stress responses as those in response to other abiotic stress [90]. It was recently shown that localization pattern of ROS accumulated in root during Pi starvation stress is critical to shape the root architecture [93–96]. The reduced accumulation of ROS in bik1 plants in relative to those in WT plants (Fig. 5), which is similar to the observation
that loss of BIK1 function impaired the PAMP-induced ROS burst in immunity [97, 98], may attribute to the altered root architecture of bik1 plants (Fig. 3). However, detailed analysis of ROS localization and the possible mechanism regulating ROS generation in root of bik1 plants will provide new insights into the connection between BIK1 and ROS in development of root architecture.

Conclusions
In summary, this study demonstrates that BIK1 is a Pi starvation-responsive gene and functions as a negative regulator of Pi homeostasis in Arabidopsis. This not only renders a novel function for BIK1 but also strengthens our understanding of post-transcriptional regulation during Pi starvation responses in plants. Considering that BIK1 is a plasma membrane-localized receptor-like protein kinase, it thus may play a role in sensing and/or processing of the Pi starvation signal during early stage of Pi starvation stress.

Methods
Plant materials and growth condition
Arabidopsis thaliana ecotype Columbia (Col-0) and bik1 (provided by Dr. Tesfaye Mengiste, Purdue University, USA) mutant [68] were used in this study. Seeds were surface sterilized with 70 % ethanol, washed with sterilized distill water and vernalized at 4 °C for 2 days before germination. Hydroponic, solid medium and liquid medium cultivation were used for different purpose of experiments. For solid medium cultivation, the basic medium used contained 2.06 mM NH₄NO₃, 1.88 mM KNO₃, 0.31 mM MgSO₄, 0.1 mM MnSO₄, 0.03 mM ZnSO₄, 0.1 mM CuSO₄, 0.3 mM CaCl₂, 5.0 mM KI, 0.1 mM CoCl₂, 0.1 mM FeSO₄, 0.1 mM EDTA, 0.1 mM H₃BO₃, 1 mM Na₂MoO₄·2H₂O, 3 g/L sucrose, 10 g/L agar, pH 5.8. When treated for Pi sufficiency (+Pi) and Pi deficiency (−Pi), the medium was supplemented with 1 mM KH₂PO₄, 10 μM KH₂PO₄ and 0.99 mM KCl. For hydroponic cultivation, seedlings at 5 ~ 7-leaf stage were transferred to 1/2 Hoagland solution for short adaption and then transferred to hydroponic solution containing 250 μM Pi or no Pi [99]. For liquid medium cultivation, seeds were dispensed in 1/2 MS medium without agar and rinsed with sterilized distilled water at 7 days. Seedlings were then transferred in MS liquid medium with Pi (1 mM) or without Pi and allowed to grow with shaking at 85 rpm. Plants were grown in a growth room under a 16 h light (100 μmol·s⁻¹·m⁻² photons m⁻² sec⁻¹ of intensity) and 8 h dark cycle at 22 ± 2 °C with 60 % relative humidity.

Measurement of root system architecture
Seven-day-old seedlings grown on 1/2 MS medium were transferred to Petri dishes and treated for + Pi or −Pi for another 7 days. Length of main root, number and length of the lateral root were measured. Total numbers of root hairs in a 5 mm region from root tip were recorded. Data were recorded from 15 individual plants from each treatment.

Anthocyanin analysis
Measurement of anthocyanin was performed as described previously [100]. Briefly, seedlings grown under + Pi or −Pi condition in solid medium were put into 20 mL extraction solution (propanol : HCl : H₂O = 18:1:81, V/V/V) and boiled for 90 s. The mixtures were kept overnight in dark at room temperature, centrifuged for 40 min at 5000× g, and the absorbance (A) was measured at 535 nm and 650 nm. The A₅₃₅ values were corrected with the A₆₅₀ values using formula A₅₃₅ = A₅₃₅ − 2.2 × A₆₅₀. Anthocyanin contents were calculated according to a standard curve prepared with the same protocol.

Quantification of total Pi content
Total Pi content was quantified according to the U.S. Environmental Protection Agency Method 365.2 with minor modifications [39]. Briefly, samples (~50 mg/sample) were flamed to ash after recording of their dry weights and then 100 μL of concentrated HCl was added. Ten microliters of the mixture were drawn and diluted into 790 μL of water, followed by addition of 200 μL assay solution (4.8 mM NH₄MoO₄, 2.5 N H₂SO₄, and 35 μM ascorbic acid). The reactions were incubated at 45 °C for 20 min and the absorbance at 650 nm was measured spectrophotometrically. Contents of total Pi were calculated according to the Pi standard curve prepared with the same procedure.

Quantification and staining of acid phosphatase activity
Activity of acid phosphatase was quantified using the pNPP hydrolysis assay according to a previously described method [101]. Briefly, 30 mg of samples were grounded, transferred to Eppendorf tubes and then spin for 10 min at 2000 × g. Reactions containing 100 μL supernatant, 100 μL p-nitrophenol sodium phosphate and 2.8 mL buffer were kept at 30 °C for 10 min, with shaking occasionally, and terminated by addition of 1 mL 0.5 M NaOH. The absorbance of the reactions was determined spectrophotometrically at 400 nm and the activity of acid phosphatase was calculated from the production of p-nitrophenol. Total protein was estimated using Bradford’s reagent and the total acid phosphatase activity was expressed as mU/mg protein. Staining of acid phosphatase activity was performed as
described by Tomscha et al. [102] with minor modifications. Ten-day-old seedlings grown in liquid medium were rinsed in −Pi medium, transferred to fresh medium supplemented with Pi (1 mM) or without Pi and then covered with a layer of 0.05 % agarose solution containing 0.008 % 5-bromo-4-chloro-3-indolyl phosphate (BCIP) [103].

**Detection and quantification of H$_2$O$_2$**

For quantification of H$_2$O$_2$, 30 mg samples from 4-week-old plants were completely ground, followed by addition of 200 μL 20 mM K$_2$HPO$_4$ (pH6.5) phosphate buffer. Quantification of H$_2$O$_2$ was performed using a commercial kit (Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer’s recommendation. Detection of superoxide anion was performed by the nitroblue tetrazolium (NBT) staining [104]. Four-week-old seedlings were vacuum infiltrated with 2 ml of 10 mM potassium phosphate phosphate buffer (pH 7.5) containing 10 mM NaN$_3$ and 0.1 % NBT for 30 min, cleared by boiling in 96 % ethanol, remained in 50 % ethanol before taking photos.

**qRT-PCR analysis of gene expression**

Total RNA was extracted using TRIZOL reagent (Invitrogen, Shangha, China) according to the manufacturer’s instructions. First strand cDNA was synthesized from 500 ng of total RNA using SuperScript III Kit (Invitrogen, Shanghai, China). The qPCR reactions contained 12.5 μL SYBR Premix Ex TaqTM (TaKaRa, Dalian, China), 0.1 μg cDNA and 7.5 pmol of each of gene-specific primers in 25 μL reactions. PCR reactions were performed using primers AtBIK1-GUS-1F (5′-ATC AGG GAC GGG GGG AGT TTT CG-3′), a site in bold) and AtBIK1-GUS-1R (5′-GCC ATG ATT GGC ATG AAT AAG-3′), a BamHI site in bold) and cloned into vector pCAMBIA1301. Transformation of wild-type Col-0 plants was performed using the floral dip infiltration method mediated by Agrobacterium tumefaciens strain GV3101. Plants of T2 generations from kanamycin-resistant transformants were used for GUS histochemical staining [105].

**Generation of BIK1pro-GUS transgenic line and GUS staining**

A 2 kb sequence upstream of the BIK1 start codon was PCR amplified using primers AtBIK1-GUS-1F (5′-ATA CTG CAG CTT GTT GAT TGA TTG GAG TTC C-3′, a PstI site in bold) and AtBIK1-GUS-1R (5′-GCC GGA TCC AGA ACT GAA ACA ACC CAT C-3′, a BanHI site in bold) and cloned into vector pCAMBIA1301. Transformation of wild-type Col-0 plants was performed using the floral dip infiltration method mediated by Agrobacterium tumefaciens strain GV3101. Plants of T2 generations from kanamycin-resistant transformants were used for GUS histochemical staining [105].

**Abbreviations**

+Pi, Pi sufficiency; B. cinerea, Botrytis cinerea; BCIP, 5-bromo-4-chloro-3-indolyl phosphate; BIK1, Botrytis-induced kinase1; MPK, mitogen-activated protein kinase; NBT, nitroblue tetrazolium; PAMP, pathogen associated molecular pattern; Pht1, PHOSPHATE TRANSPORTER1; PL, phosphate; −Pi, Pi deficiency; pNPP, p-nitrophenol sodium phosphate; PPO, phosphoenoicpyruvate carboxylase; qRT-PCR, quantitative reverse transcription PCR; ROS, reactive oxygen species; WT, wild type.

**Acknowledgement**

We are grateful to Dr. Tesfaye Mengiste, Purdue University, USA, for providing the Arabidopsis WT and bik1 seeds used in this study.

**Funding**

This work was supported by the National Natural Foundation of Sciences (no. 31272028) and the Ph.D. Program Foundation of Ministry of Education of China (no. 2012011110070).

**Availability of supporting data**

All data supporting the results of this article are included within the article.

**Authors’ contributions**

HZ and FS designed the study and all experiments; HZ, LH and YH conducted all the molecular biology and physiology experiments; HZ and FS analyzed the data; FS drafted the manuscript with HZ. All authors have read and approved the final version of the manuscript.

**Competing interests**

The authors declare that they have no competing interests.

**Consent for publication**

Not applicable.

**Ethics approval and consent to participate**

Not applicable. No human or animals were involved in this study.

**Author details**

1College of Life Science, Taizhou University, Taizhou, Zhejiang 318001, People’s Republic of China. 2National Key Laboratory for Rice Biology, Institute of Biotechnology, Zhejiang University, Hangzhou 310058, People’s Republic of China.
References

1. Raghatha KG. Phosphate transport and signaling. Curr Opin Plant Biol. 2000;3:182–7.
2. Lynch JP, Brown KM. Topsoil foraging—an architectural adaptation of plants to low phosphorus availability. Plant Soil. 2001;237:225–37.
3. Abel S, Ticconi CA, Delatore CA. Phosphate sensing in higher plant. Physiol Plant. 2002;115:1–8.
4. Ticconi CA, Abel S. Short on phosphate: plant surveillance and countermeasures. Trends Plant Sci. 2004;9:548–55.
5. Bucher M, Rauch C, Daram P. Molecular and biochemical mechanisms of phosphorus uptake into plants. J Plant Nutr Soil Sci. 2001;164:209–17.
6. raghatha KG, Karthikeyan AS. Phosphate acquisition. Plant Soil. 2005;274:37–49.
7. williamson LC, Ribrioux SPC, fitter AH, Leyser HMO. Phosphate availability regulates root system architecture in Arabidopsis. Plant Physiol. 2001;126:875–82.
8. Ma Z, Be lenberg DG, Brown KM, Lynch JP. Regulation of root hair density by phosphorus availability in Arabidopsis thaliana. Plant Cell Environ. 2001;24:4459–67.
9. Schmidt W, Schikora A. Different pathways are involved in phosphate and iron stress-induced alterations of root epidermal cell development. Plant Physiol. 2001;125:2078–94.
10. Lopez-Bucio J, Herrández-Abreu E, Sánchez-Calderón OA, Nieto-Jacobo MF, Simpson J, Herrera-Estrella L. Phosphate availability alters architecture and causes changes in hormone sensitivity in the Arabidopsis root system. Plant Physiol. 2002;129:244–56.
11. Lópezbucio J, Cruz-Ramírez A, Herrera-Estrella A. The role of nutrient availability in regulating root architecture. Curr Opin Plant Biol. 2003;6:280–7.
12. Niu YF, Choi RS, Jin GL, Wang H, Tang CX, Zhang YS. Responses of root architecture development to low phosphorus availability: a review. Ann Bot. 2013;112:391–408.
13. Mimura T. Regulation of phosphate transport and homeostasis in plant cells. Int Rev Cytol. 1999;191:149–200.
14. Vance CP, Uhde-Stone C, Allan DL. Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. New Phytol. 2003;157:423–42.
15. Kobyayi K, Masuda T, Takamia K, Ohta H. Membrane lipid alteration during phosphate starvation is regulated by phosphate signaling and auxin/cytokinin cross-talk. Plant J. 2006;47:238–48.
16. Oropesa-Aburto A, cruz-Ramírez A, acevedo-Herrández GJ, Pérez-Torres CA, caballero-Pérez J, Herrera-Estrella L. Functional analysis of the Arabidopsis FLO22 promoter reveals an evolutionarily conserved low-Pi-responsive transcriptional enhancer element. J Exp Bot. 2012;63:2189–202.
17. Rauch C, Bucher M. Molecular mechanisms of phosphate transport in plants. Planta. 2002;216:23–37.
18. López- Arredondo DL, Leyer-González MA, González-Morales SI, López-Bucio J, Herrera-Estrella L. Phosphate nutrition: improving low-phosphate tolerance in crops. Annu Rev Plant Biol. 2014;65:95–123.
19. Franco-Zorrilla JM, González E, Bustos R, Linhares F, Leyva A, Paz-Ares J. The transcriptional control of plant responses to phosphate limitation. J Exp Bot. 2004;55:285–93.
20. Lin WY, Lin SJ, Chiu TJ. Molecular regulators of phosphate homeostasis in plants. J Exp Bot. 2009;60:427–38.
21. Rouached H, Arpaite B, Poirier Y. Regulation of phosphate starvation responses in plants: signaling players and cross-talks. Mol Plant. 2010;3:288–99.
22. Jain A, Nagarajan VK, raghatha KG. Transcriptional regulation of phosphate acquisition by higher plants. Cell Mol Life Sci. 2012;69:3207–24.
23. Zhang Z, Liao H, Lucas JI. Molecular mechanisms underlying phosphate sensing, signaling, and adaptation in plants. J Integr Plant Biol. 2014;56:192–220.
24. Muchhal US, Pardha JM, raghatha KG. Phosphate transporters from the higher plant Arabidopsis thaliana. Proc Natl Acad Sci U S A. 1996;93:10519–23.
25. Daram P, Brunner S, Rauch C, Steiner C, Amrhein N, Bucher M. Pht2;1 encodes a low-affinity phosphate transporter from Arabidopsis. Plant Cell. 1999;11:2153–66.
26. Knappe S, Flügge UI, Fischer K. Analysis of the plastidic phosphate translocator gene family in Arabidopsis and identification of new phosphate translocator-homologous transporters, classified by their putative substrate-binding site. Plant Physiol. 2003;131:1178–90.
27. Guo B, irigoyen S, fowler TB, Versaw WK. Differential expression and phylogenetic analysis suggest specialization of plastid-localized members of the Pht4 phosphate transporter family for photosynthetic and heterotrophic tissues. Plant Signal Behav. 2008;3:784–90.
28. Shin H, Shin HS, Dewbre GR, Harrison MJ. Phosphate transport in Arabidopsis. Pht1:1 and Pht1:4 play a major role in phosphate acquisition from both low- and high-phosphate environments. Plant J. 2004;39:562–9.
29. Nussbaum L, karnio S, Javot H, marin E, pochon N, AYabi A, Nakanishi TM, Thibault MC. Phosphate import in plants: Focus on the PHT1 transporters. Front Plant Sci. 2012;1:283.
30. Remy E, cabrito TR, Batista RA, teixeira MC, Sá-Correia I, duque P. The Pht1:9 and Pht1:8 transporters mediate inorganic phosphate acquisition by the Arabidopsis thaliana root during phosphate starvation. New Phytol. 2012;195:356–71.
31. Cubero B, nakagawa Y, Jiang Xy, miura KI, Li F, raghatha KG, Bressan RA, Hasegawa PM, Pardha JM. The phosphate transporter Pht4;6 is a determinant of salt tolerance that is localized to the Golgi apparatus of Arabidopsis. Mol Plant. 2009;2:535–52.
32. Hassler S, Lemke L, Jung B, Mohlmann T, Krüger F, Schumacher K, Esplen L, Martinia E, Neuhaus HE. Lack of the Golgi phosphate transporter PHT4;6 causes strong developmental defects, constitutively activated disease resistance mechanisms and altered intracellular phosphate compartmentation in Arabidopsis. Plant J. 2012;72:732–44.
33. Liu J, Yang L, luan M, Wang Y, Zhang C, Zhang B, et al. A vacuolar phosphate transporter essential for phosphate homeostasis in Arabidopsis. Proc Natl Acad Sci U S A. 2015;112:E6571–8.
34. Hamburger D, Rezzonico E, MacDonald-Comber Petétot J, Somerville C, Poirier Y. Identification and characterization of the Arabidopsis FYCO1 gene involved in phosphate loading to the xylem. Plant Cell. 2002;14:4889–902.
35. Nagarajan VK, Jain A, Poling MD, Lewis AJ, raghatha KG, Smith AP. Arabidopsis Pht1;5 mobilizes phosphate between source and sink organs and influences the interaction between phosphate homeostasis and ethylene signaling. Plant Physiol. 2011;156:1149–63.
36. Lapis-Gaza HR, Jost R, Finnegan PM. Arabidopsis PHOSPHATE TRANSPORTER1 genes PHT1;8 and PHT1;9 are involved in root-to-shoot translocation of orthophosphate. BMC Plant Biol. 2014;14:334.
37. Rubio V, Linhares F, Solano R, Martín AC, Iglesias J, Leyva, Paz-Ares J. A conserved MYB transcription factor involved in phosphate starvation signaling both in vascular plants and in unicellular algae. Genes Dev. 2001;15:2212–33.
38. Chen ZH, Nimmo GA, Jenkins CJ, Nimmo HG, BHLLH32 modulates several biochemical and morphological processes that respond to Pi starvation in Arabidopsis. Biochem J. 2007;405:191–8.
39. Devaiah BN, Karthikeyan AS, raghatha KG. WRKY75 transcription factor is a modulator of phosphate acquisition and root development in Arabidopsis. Plant Physiol. 2007;143:1789–801.
40. Devaiah BN, Nagarajan VK, raghatha KG. Phosphate homeostasis and root development in Arabidopsis are synchronized by the zinc finger transcription factor ZAT6. Plant Physiol. 2007;145:147–59.
41. Devaiah BN, Madhuvanti R, Karthikeyan AS, raghatha KG. Phosphate starvation responses and gibberellic acid biosynthesis are regulated by the MYB62 transcription factor in Arabidopsis. Mol Plant. 2009;2:423–58.
42. Chen YF, Li LQ, Xu Q, Kong YH, Wang H, Wu WH. The WRKY6 transcription factor modulates PHOSPHATE1 expression in response to low Pi stress in Arabidopsis. Plant Cell. 2009;21:3554–66.
43. Wang H, Xu Q, Kong YH, Chen Y, Duan JY, Wu WH, Chen YF. Arabidopsis WRKY45 transcription factor activates PHOSPHATE TRANSPORTER1 expression in response to phosphate starvation. Plant Physiol. 2014;164:2020–9.
44. Ramaiah M, Jain A, raghatha KG. Ethylene Response Factor070 regulates root development and phosphate starvation-mediated responses. Plant Physiol. 2014;164:1484–98.
45. Su T, Xu Q, Zhang FC, Chen Y, Li LQ, Wu WH, Chen YF. WRKY42 modulates phosphate homeostasis through regulating phosphate translocation and acquisition in Arabidopsis. Plant Physiol. 2015;167:1579–91.
46. Chen CY, Wu K, Schmidt W. The histone deacetylase HDA19 controls root cell elongation and modulates a subset of phosphate starvation responses in Arabidopsis. Sci Rep. 2015;5:15708.
Arabidopsis thaliana

9.

73.
–

1
–

32.
–

15.
–

et al. BMC Plant Biology
–

Arabidopsis
5.
–

61.
Arabidopsis
. Biochem J. 2009;420:57
Affymetrix gene chips determined
–

pho2
75.
99.
–

501.
'

70.
–

high-affinity phosphate
65.
61.

Pseudomonas syringae
23.
–

better safe than sorry. Trends Plant
–

75. Péret B, Clement M, Nussaume L, Desnos T. Root developmental adaptation to phosphate starvation: better safe than sorry. Trends Plant Sci. 2011;16:442–50.

Mittler R, Vanderauwera S, Gollery M, Van Breusegem F. Reactive oxygen gene network of plants. Trends Plant Sci. 2004;9:490–8.

Foyer CH, Noctor G. Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. Plant Cell. 2005;17:1866–75.

Shin H, Shin HS, Chen R, Harrison MJ. Loss of AtH function impacts phosphate distribution between the roots and the shoots during phosphate starvation. Plant J. 2006;45:712–26.

Misson J, Raghothama KG, Jain A, Jouhet J, Block MA, Bligny R, Orpet P, Creff A, Somerville S, Rolland N, et al. A genome-wide transcriptional analysis using Arabidopsis thaliana Affymetrix gene chips determined plant responses to phosphate deprivation. Proc Natl Acad Sci U S A. 2005;102:11934–9.

Lan P, Li W, Schmidt W. Genome-wide co-expression analysis predicts protein kinases as important regulators of phosphate deficiency-induced root hair remodeling in Arabidopsis. BMC Genomics. 2013;14:210.

Gregory A, Hurley BA, Tran HT, Valentine AJ, She YM, Knowles VL, Plaxton WC. In vivo regulatory phosphorylation of the phosphoenolpyruvate carboxylase AtPPC1 in phosphate-starved Arabidopsis thaliana. Biochem. J. 2009;417:57–65.

Sanchez-Calderon L, Lopez-Bucio J, Chacon-Lopez A, Cruz-Ramirez A, Nussaume L. Arabidopsis phospholipid remodeling at the leaf cell surface requires the AtPAP12-AtPAP26 enzyme complex. Plant Cell. 2013;25:3662–76.

Host Microbe. 2010;7:290

Receptor-like cytoplasmic kinases integrate signaling from multiple plant immune receptors and are targeted by a PAMP-triggered immunity. Proc Natl Acad Sci U S A. 2010;107:496–501.

Zhang J, Li W, Xiang T, Liu Z, Laluk K, Ding X, Dietrich RA, Hirt H, Mengiste T. Receptor-like cytoplasmic kinases integrate signaling from multiple plant immune receptors and are targeted by a Pseudomonas syringae effector. Cell Host Microbe. 2010;2:200–31.

Laluk K, Luo H, Chai M, Dheeraw R, Lai Z, Mengiste T. Biochemical and genetic requirements for function of the immune response regulator BORTYRIS-INDUCED KINASE1 in plant growth, ethylene signaling, and PAMP-triggered immunity in Arabidopsis. Plant Cell. 2011;23:2831–49.

Zhou Z, Yang F, Zhan Y, Chen S, Xie Q, Tian X, Zhao JM. BIK1 interacts with PEP17 to mediate ethylene-induced immunity. Proc Natl Acad Sci U S A. 2013;110:20510–5.

Hurley BA, Tran HT, Valentine AJ, She YM, Knowles VL, Plaxton WC. The dual-targeted purple acid phosphatase isozyme AtPAP26 is required for Arabidopsis root acclimation to nutritional phosphorus deprivation. Plant Physiol. 2011;156:1006–14.

McKnight TD, Plaxton WC. The secreted purple acid phosphatase AtPAP12 and AtPAP26 play a pivotal role in extracellular phosphate homeostasis. J Exp Bot. 2007;58:103–13.

Martin AC, del Pozo JC, Iglesias J, Rubio V, Solano R, de la Pena A, Paz-Ares J. Auxin transport is a determinant development program in root of Arabidopsis thaliana. Plant Physiol. 2005;138:2061–74.

Liu Z, Wu Y, Yang F, Zhang Y, Chen S, Xie Q, Tian X, Zhou JM. BIK1 interacts with PEP17 to mediate ethylene-induced immunity. Proc Natl Acad Sci U S A. 2013;110:20510–5.

Lin W, Liu D, Gao X, Jiang J, Su CL, Su CL, Chou TZ. Regulatory network of microRNA399 and PHO2 by systemic signaling. Plant Physiol. 2008;147:732–46.

Hsieh LC, Lin SI, Shih AC, Chen JW, Lin WY, Tseng CY, Wu PC, Chiou TJ. Regulatory network of microRNA399 and PHO2 by systemic signaling. Plant Physiol. 2008;147:732–46.

Murr K, Rus A, Shankhoo A, Yokoi S, Kartikeyan AS, Raghothama KG, Baek D, Koo YD, Jin JB, Bressan RA, Yun DJ, Hasegawa PM. The Arabidopsis SUMO E3 ligase SI21 controls phosphate deficiency responses. Proc Natl Acad Sci U S A. 2005;102:7760–5.

Gray AFM, Hogeweg P, Scheres B. Auxin transport is sufficient to generate a maximum and gradient guiding root growth. Nature. 2007;449:1008–13.

Martin AC, del Pozo JC, Iglesias J, Rubio V, Solano R, de la Pena A, Paz-Ares J. Influence of cytokinins on the expression of phosphate starvation responsive genes in Arabidopsis. Plant J. 2000;24:559–70.

Nacry P, Canivenc G, Muller B, Azmi A, Van Onckelen H, Rossignol M, Doumas P. A role for auxin redistribution in the responses of the root system architecture to phosphate starvation in Arabidopsis. Plant Cell. 2005;17:2061–74.

Lopez-Bucio J, Hernandez-Arbus E, Sanchez-Calderon L, Perez-Torres A, Rampey RA, Bartel B, Herrera-Estrella L. An auxin transport independent pathway is involved in phosphate stress-induced root architectural alterations in Arabidopsis. Identification of BIG as a mediator of auxin in pericycle cell activation. Plant Cell. 2005;17:6761–81.

Nacy P, Canivenc G, Muller B, Azmi A, Van Onckelen H, Rossignol M, Doumas P. A role for auxin redistribution in the responses of the root system architecture to phosphate starvation in Arabidopsis. Plant Cell. 2005;17:2061–74.

Gineintein VA, Xu J, Marée AFM, Hogeweg P, Scheres B. Auxin transport is sufficient to generate a maximum and gradient guiding root growth. Nature. 2007;449:1008–13.

Martin AC, del Pozo JC, Iglesias J, Rubio V, Solano R, de la Pena A, Paz-Ares J. Influence of cytokinins on the expression of phosphate starvation responsive genes in Arabidopsis. Plant J. 2000;24:559–70.

Wang X, Yi K, Tao Y, Wang F, Wu Z, Jiang D, Chen X, Zhou L, Wu P. Cytokinin represses phosphate-starvation response through increasing of intracellular phosphate level. Plant Cell Environ. 2006;29:1924–35.

Ma Z, Basak TI, Brown KM, Lynch JP. Regulation of root elongation under phosphate stress involves changes in ethylene responsiveness. Plant Physiol. 2005;131:1381–90.

Zhang Y, Lynch JP, Brown K. Ethylene and phosphate availability have interacting yet distinct effects on root hair development. J Exp Bot. 2003;54:2351–62.

Nagarajan VK, Smith AP. Ethylene’s role in phosphate starvation signaling: more than just a root growth regulator. Plant Cell Physiol. 2012;53:727–36.
purple acid phosphatase isozymes secreted by phosphate-starved Arabidopsis thaliana. Plant Cell Environ. 2010;33:1789–803.

89. Torres MA. ROS in biotic interactions. Physiol Plant. 2010;138:414–29.

90. Suzuki N, Kousevitzy S, Mittler R, Miller G. ROS and redox signalling in the response of plants to abiotic stress. Plant Cell Environ. 2012;35:259–70.

91. Baxter A, Mittler R, Suzuki N. ROS as key players in plant stress signalling. J Exp Bot. 2013;65:1229–40.

92. Shin R, Berg RH, Schachtman DP. Reactive oxygen species and root hairs in Arabidopsis root response to nitrogen, phosphorus and potassium deficiency. Plant Cell Physiol. 2002;46:1350–7.

93. Foreman J, Demidchik V, Bothwell JH, Mylona P, Miedema H, Torres MA, Linstead P, Costa S, Brownlee C, Jones JD, Davies IM, Dolan L. Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. Nature. 2003;422:442–6.

94. Rentel MC, Lecourieux D, Ouaked F, Usher SL, Petersen L, Okamoto H, Knight H, Peck SC, Grierson CS, Hirt H, Knight MR. OX1 kinase is necessary for oxidative burst-mediated signalling in Arabidopsis. Nature. 2004;427:858–61.

95. Tyburski J, Dunajskja K, Tretyn A. Reactive oxygen species localization in roots of Arabidopsis thaliana seedlings under phosphate deficiency. Plant Growth Reg. 2009;59:27–36.

96. Chacón-López A, Ibarra-Laclette E, Sánchez-Calderón L, Gutiérrez-Alanis D, Herrera-Estrella L. Global expression pattern comparison between low phosphorus insensitive 4 and WT Arabidopsis reveals an important role of reactive oxygen species and jasmonic acid in the root tip response to phosphate starvation. Plant Signal Behav. 2011;6:382–92.

97. Kadota Y, Sklenar J, Derbyshire P, Stansfeld L, Asai S, Ntoukakis V, Jones JD, Shirasu K, Menke F, Jones A, Zipfel C. Direct regulation of the NADPH oxidase RBOH:D by the PRR-associated kinase BIK1 during plant immunity. Mol Cell. 2014;54:43–55.

98. Li L, Li M, Yu L, Zhou Z, Liang X, Liu Z, Jones JD, Shirasu K, Menke F, Jones A, Zipfel C. The FLS2-associated kinase BIK1 directly phosphorylates the NADPH oxidase RbohD to control plant immunity. Cell Host Microbe. 2014;15:329–38.

99. Karthikeyan AS, Varadarajan DK, Mukatira UT, D’Urzo MP, Darnz B, Raghothama KG. Regulated expression of Arabidopsis phosphate transporters. Plant Physiol. 2002;130:221–33.

100. Lange H, Shropshire W, Mohr H. An analysis of phytochrome-mediated anthocyanin synthesis. Plant Physiol. 1971;47:649–55.

101. Richardson AE, Hadobas PA, Hayes JE. Extracellular secretion of Aspergillus phytase from Arabidopsis roots enables plants to obtain phosphorus from phytate. Plant J. 2001;25:641–9.

102. Tomchuk JL, Trull MC, Deikman J, Lynch JP, Guilfoon MJ. Phosphatase under-producer mutants have altered phosphorus relations. Plant Physiol. 2004;135:334–45.

103. Trull MC, Deikman J. An Arabidopsis mutant missing one acid phosphatase isoform. Planta. 1998;206:644–50.

104. Doke N. Involvement of superoxide anion generation in the hypersensitive response of potato tuber tissue to infection with an incompatible race of Phytophthora infestans and to the hyphal wall components. Physiol Plant Pathol. 1983;23:345–57.

105. Jefferson RA, Kavanagh TA, Bevan MW. GUS fusions: beta-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J. 1987;6:3901–7.

Submit your next manuscript to BioMed Central and we will help you at every step:

- We accept pre-submission inquiries
- Our selector tool helps you to find the most relevant journal
- We provide round the clock customer support
- Convenient online submission
- Thorough peer review
- Inclusion in PubMed and all major indexing services
- Maximum visibility for your research

Submit your manuscript at www.biomedcentral.com/submit