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# An ABC transporter complex encoded by *Aluminum Sensitive 3* and *NAP3* is required for phosphate deficiency responses in *Arabidopsis*

Rania Belal [a, b], Renjie Tang [a], Yangping Li [a], Yasser Mabrouk [b], Effat Badr [b], Sheng Luan [a, *]

[a] Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720, USA
[b] Department of Genetics, Faculty of Agriculture, University of Alexandria, Alexandria 21545, Egypt

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ABSTRACT

Phosphate is essential for cell metabolism in all organisms. As it is often limiting in the soil, plants have evolved various mechanisms to cope with low-phosphate conditions. Here, we report that *Aluminum Sensitive 3* and *NAP3*, two genes previously identified to function in aluminum tolerance, play a critical role in plant response to phosphate deficiency. Two T-DNA insertional alleles of *ALS3* gene in *Arabidopsis* showed hypersensitive responses to phosphate limiting conditions. Compared to the wild type, *als3* mutant plants exhibited more severe root growth inhibition and developed more root hairs under phosphate starvation. Interestingly, these phenotypic changes occurred only when the low-phosphate medium is supplemented with sucrose, suggesting that *ALS3* regulates low-phosphate response in a sugar-dependent manner. Furthermore, *NAP3*, a gene encoding the nucleotide binding domain protein that physically interacts with *ALS3*, was implicated in the same pathway in response to low-P. The *nap3* mutant showed the same phenotype as the *als3* mutant when grown on phosphate depletion medium. We conclude that *ALS3* and *NAP3* protein form an ABC transporter complex that is required for sugar-dependent response to phosphate deficiency.



## 1. Introduction

Phosphorous (P) is one of the mineral nutrients essential for plant growth, development, and reproduction. Not only is it a major component of fundamental macromolecules, such as nucleic acids and phospholipids, but it also plays an important role in energy transfer as well as the regulation of enzyme reactions and metabolic pathways [1,2]. Most of the P in the soil is converted to organic compounds by microorganisms or turns insoluble by interacting with cations [3–5], becoming unavailable for plant uptake. To overcome problems with P availability, plants have evolved a series of metabolic and developmental strategies to maintain P homeostasis. These responses include conservation and remobilization of internal Pi and enhanced acquisition of external P which involve rapid and distinct changes in gene expression [4,6]. Remodeling of root system architecture, increased root hair formation and association with symbiotic mycorrhizal fungi to accelerate soil exploration are typical developmental responses to low P [7–10].

Sugars in plants, derived from photosynthesis, act as substrates for energy metabolism and biosynthesis of complex carbohydrates, and provide sink tissues with the necessary resources to grow and develop. In addition, sugars can act as signals that regulate plant growth and development in response to biotic and abiotic stresses [11]. Experiments have suggested that sugar signaling may also be involved in plant responses to P starvation. Stem girdling of white lupin (*Lupinus albus*) to block the movement of sugars from shoots to roots severely affected the expression of phosphate starvation inducible genes in P-starved roots [12]. The *Arabidopsis pho3* mutant with a defective copy of the sucrose transporter 2 (*SUC2*) gene has substantially reduced transport of sucrose from shoots to roots, leading to an increase in expression of an array of Pi-starvation responsive genes [13–15].

Aluminum toxicity and low-phosphate availability usually co-exist in acid soil because of the formation of aluminum phosphate and this combination severely limits crop yield [16]. In soybean, it was found that adding P to acid soils could enhance Al tolerance in the P-efficient genotype *HN89*. *HN89* plants released more malate under conditions mimicking acid soils suggesting that root malate exudation may be critical for soybean adaptation to both Al toxicity and P-deficiency. Malate release is regulated by three factors including pH, Al, and P status through the expression of *GmALMT1* gene that encodes a malate transporter in plants [17]. In addition, two P-efficient genotypes appeared to be more Al tolerant than the

* Corresponding author.
*E-mail address:* sluan@berkeley.edu (S. Luan).

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two P-inefficient genotypes under high-P conditions [18]. Analysis of root exudates indicated that Al toxicity induced citrate exudation, P-deficiency triggered oxalate exudation, and malate release was induced by both treatments, indicating that P efficiency and Al tolerance are inter-related in soybean and likely in other plants. Phosphorus-efficient genotypes may be able to enhance Al tolerance not only through direct Al–P interactions but also through indirect interactions associated with stimulated exudation of different Al-chelating organic acids in specific roots and root regions [19].

To address Al–P interaction at the molecular level and identify components that may function in such interaction, we screened some of the previously described mutants altered in Al tolerance under low-P conditions. We report here that the Aluminum Sensitive 3 and its interacting protein NAP3 function in low-P response in a sugar-dependent fashion.

## 2. Materials and methods

### 2.1. Plant material and growth condition

*Arabidopsis thaliana* ecotype Col-0 was used in this study. T-DNA insertion mutants of two lines *als3a* (SALK_011435) and *als3b* (SALK_004094) were obtained from *Arabidopsis* Biological Resource Center (http://abrc.osu.edu/). The homozygous *als3a* and *als3b* mutants were identified using gene specific primers in combination with T-DNA left border primers.

***als3aF***5′AGACGACGACAAAACAAAAAAGT3′,***als3aR***5′ATTGGGATCATATACCTCGGAGT3′,***als3bF***5′TTGCGTATCTCTTCATGGTTAGT3′,***als3bR***5′CCCATTATCATACCAGTCATTGC 3′, ***LBa1*** 5′TGGTTCACGTAGTGGGCCATCG3′. Wild-type (Col-0) and mutant *Arabidopsis* plants were grown in soil at 22 °C under 12-h-light/12-h-dark cycles in the green house. For all germination assay, unless otherwise mentioned, wild-type and mutant seeds were sterilized with 0.5% sodium hypochlorite for 5 min, washed four times with sterilized water, and sown on 1/6-strength MS medium with or without phosphate (containing 1% sucrose, pH = 5.8, solidified with 1% agarose). The plates were placed at 4 °C for 2 days, and then the seeds were germinated vertically at 24 °C under normal conditions. Aluminum medium was prepared as previously described [20].

### 2.2. RT-PCR and qRT-PCR analyses

Total RNA was isolated from *Arabidopsis* seedlings using TRIzol Reagent (Invitrogen). RNA samples were obtained from 7-days old

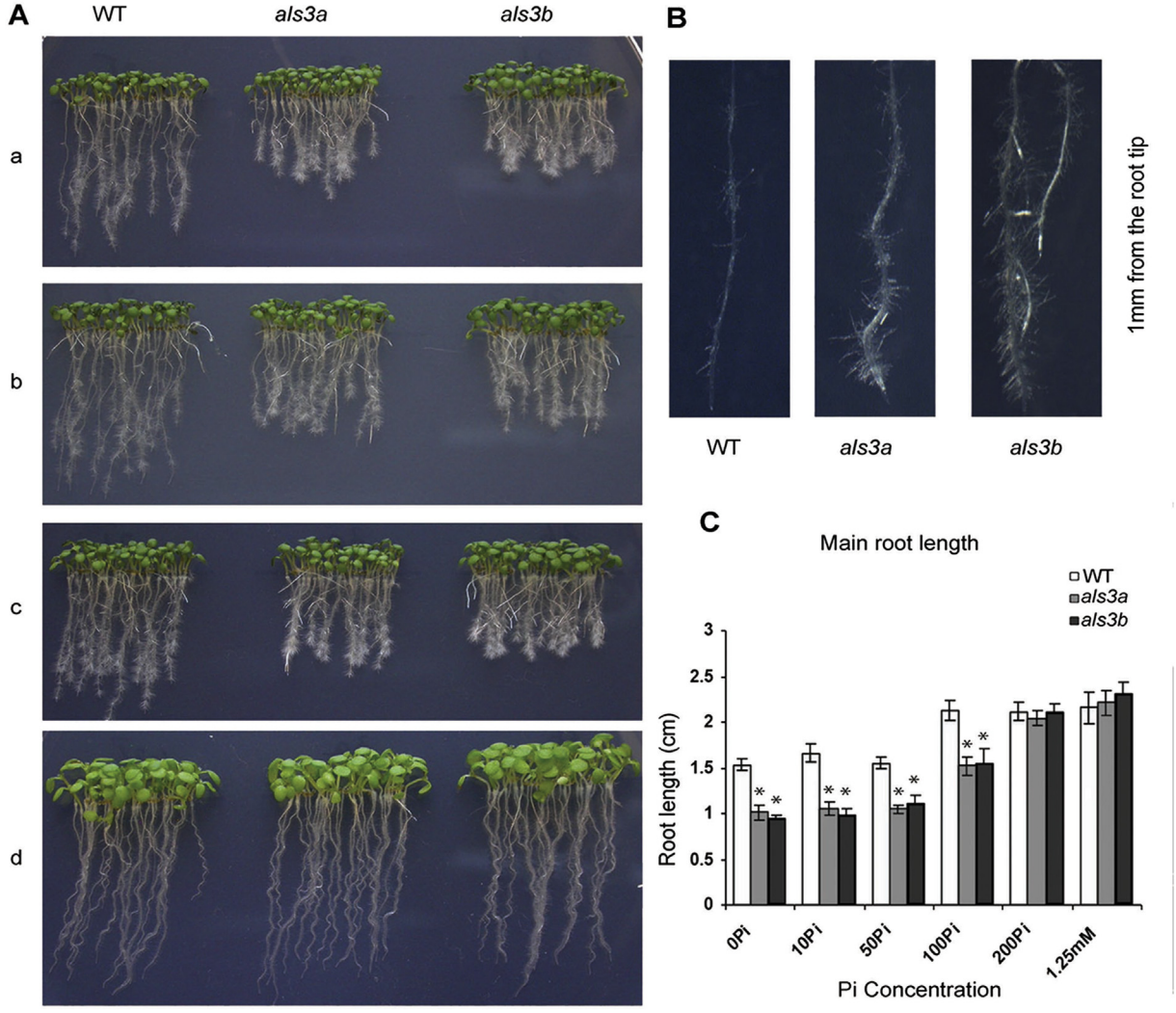


**Fig. 1.** Phenotyping of *als3* mutants. (A)7days old seedlings grown on 1/6 Murshige and Skoog medium (MS) lacking phosphate and supplemented with different concentrations of Pi. (a) 1/6 MS-Pi, (b) 10 μM Pi (c) 50 μM Pi (d) 1.25 mM Pi. (B) Microscopic pictures for about 1 mm from the root tip of WT and mutant lines on −Pi medium showing the density of root hairs in mutant in compare to the wild type. (C) Histogram showing the primary root length of WT and *als3* on different Pi concentrations. Using Student (t-test), significant difference appears between WT and *als3* $P < 0.05$, error bar present ± S.E.

seedlings grown on 1/6 MS medium with or without phosphate. 2 μg of total RNA was subjected to reverse transcription reaction at 42 °C for 1 h. The resulting cDNA was used for PCR amplification with the gene specific primers*ALS3PF*5′CCGGTTCTGGGTTTCATTGT TTA3′,*ALS3PR*5′GTCTCTGTTTCTCTCTGTCTCTCG3′. qRT-PCR analysis was performed on the RotorGene 3000 system (Corbett Research) with the SYBR Green real time PCR Master Mix (TOYOBO) to monitor double-stranded DNA products. Data analysis was performed with Rotor-Gene software version 6.0 and relative amounts of mRNA were calculated based on the comparative threshold cycle method. The relative expression of *ALS3* the housekeeping gene *ACTIN2*.

## 3. Results

### 3.1. The als3 mutants are hypersensitive to phosphate deficiency

We grew the wild-type plants and several mutants obtained from *Arabidopsis* Biological Resource Center (http://abrc.osu.edu/) that are defective in aluminum tolerance, including *als3*, *als4*, *als5* (*Aluminum Sensitive 3*, *4*, *5*), and *almt1* (*Aluminum activated Malate Transporter 1*) on both normal MS and P-depleted MS medium. As compared to the WT, only *als3* showed altered phenotype associated with P-deficiency (Fig. S1). The *als3* plants displayed significantly shorter primary roots and higher root hair density,

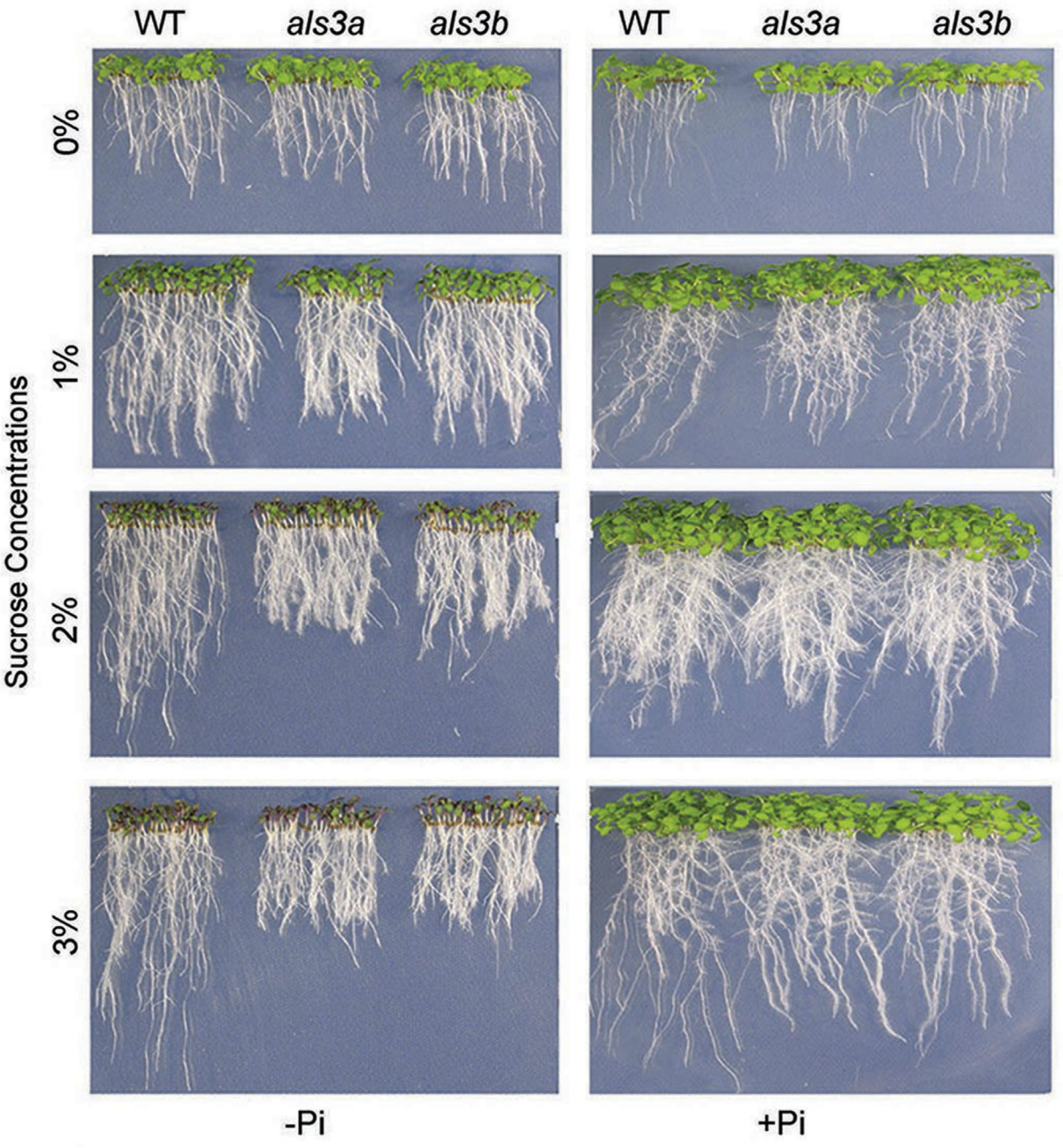


**Fig. 2.** Effect of Sugar and phosphate on plant growth Root Phenotype of 7 days old WT and mutants plants grown on different concentrations of phosphate and sugar.

symptoms associated with more severe Pi deficiency. Although *als4* plants were also stunted, they showed the same phenotype on either −Pi medium or normal MS medium (+Pi), suggesting that the defect in *als4* growth is not related to Pi depletion conditions.

To characterize the function of *ALS3* gene in low-P response, we isolated two more T-DNA insertional alleles for further analysis. The *als3a* (SALK_011435) contained a T-DNA insertion in the first exon, and *als3b* (SALK_004094) carried a T-DNA insertion in the third exon of the *ALS3* gene. The homozygous *als3a* or *als3b* mutants were isolated by PCR-based screening using gene specific primers in combination with T-DNA left border primers. Consistent with the original observation that the EMS mutant of *ALS3* was more sensitive to aluminum than the WT the newly isolated two T-DNA mutants also showed hypersensitivity to high aluminum concentrations (Fig. S2).

Remodeling of root system architecture (RSA) is a typical response to Pi insufficient conditions. This includes inhibition of primary root growth, increase in the length and density of lateral roots and root hairs [21,22]. Compared to the wild type, both *als3a* and *als3b* exhibited more severe reduction in primary root length as Pi concentration in the medium decreased. More specifically, primary roots of mutant plants were about 30% shorter than those in the wild type when grown on 1/6 MS media containing 0, 10, 50 and 100 μM Pi. When Pi concentration reached 200 μM in the medium, the mutants showed no difference from the WT (Fig. 1A and C). In addition, the two mutants had more and longer root hairs under low-Pi conditions comparing to the wild type (Fig. 1B). We also compared the growth of the mutants and WT on the low-Pi media after transferring seedlings grown on normal medium. Seeds were germinated on 1/2 MS for 7 days before transferred to the 1/6 MS medium lacking Pi and grown for another 7 days. The same differences between mutants and the wild type had been observed as in the germination assay (Fig. S3).

### 3.2. Sucrose levels in the medium are critical for the function of ALS3 in Pi deficiency response

In plants, sucrose is synthesized in source tissues and transported to sink tissues, where it is utilized or stored [23]. It has been shown that sucrose is both a metabolite and a signaling molecule in plants [24,25], and under phosphate starvation, plants accumulate sugar and starch in their leaves. Increased loading of sucrose to the phloem under Pi starvation primarily functions to relocate carbon resource to the roots to promote a larger root system to adapt to the low-P conditions [26].

In order to investigate a possible role of sugar in *ALS3*-mediated low-Pi response, we grew WT and *als3* mutants on media with or without phosphate supplemented with different concentrations of sucrose. After 7 days of growth, *als3* mutants showed a clear hypersensitive response to low-Pi in the presence of 2% or 3% sucrose (Fig. 2). Strikingly, there was no discernable difference between the mutants and WT on medium without sucrose despite lacking Pi. In media lacking phosphate and supplemented with 2 or 3% sucrose, growth of the primary roots was strikingly reduced in the mutants in comparison to the wild type. On medium lacking Pi supplemented with 1% sucrose, the difference was observed but not as striking. The phenotypic changes were not limited to roots but the shoots appeared different as well when sucrose concentration increased to 2–3%. The leaves of mutant plants became smaller under high sucrose concentration comparing to the wild type (Fig. 2). We also measured the expression of *ALS3* using RT-PCR to examine the effect of different combinations of phosphate and sugar on *ALS3* gene expression. Results showed that the expression of *ALS3* gene in media without sucrose was similar under sufficient or insufficient phosphate conditions. The expression of *AlS3* was increased when both sucrose and Pi were added to the medium (+Pi + Suc). Interestingly, the expression level of *ALS3* under the phosphate starvation condition in the presence of sucrose was the highest (−Pi + Suc). This result suggests that *ALS3* gene expression is particularly needed under low-Pi conditions when sugar supply is normal, consistent with the finding that the *als3* mutant is specifically defective under the same conditions (Fig. 3).

### 3.3. NAP3 gene is required for sugar-dependent low-P response

*NAP3* gene encodes a nucleotide binding domain of an ABC bacterial transporter [27], while *ALS3* was identified as the transmembrane domain of a bacterial-type ATP binding cassette (ABC) transporter [20]. It was suggested that *ALS3* and *NAP3* interact to form a bacterial-type ABC transporter that has a role in aluminum tolerance in rice [28]. With the finding that *ALS3* is involved in low-P response, we speculated that *NAP3* may be a partner of *ALS3* in the same process. We thus examined *nap3* knockout mutant under phosphate starvation conditions, and indeed it showed the same phenotype as *als3* mutants (Fig. 4A and B). We examined *NAP3* gene expression under the combinations of phosphate and sugar concentrations used previously with *als3* mutants. The results showed that the transcript level of *NAP3* was, like that of *ALS3*, induced by the combination of low-Pi plus sugar (Fig. 4C). Taken together, these findings support the hypothesis that *NAP3* interacts with *ALS3* to form an ABC transporter that functions in both aluminum tolerance [27,28]and in low-P response.

## 4. Discussion

Phosphorous is an essential macronutrient for all organisms. Its availability is a major factor limiting growth, development, and productivity of plants. Aluminum and phosphorus coexist in acid soil and recent studies suggest plant responses to aluminum and phosphate may be physiologically connected. For example, Pi

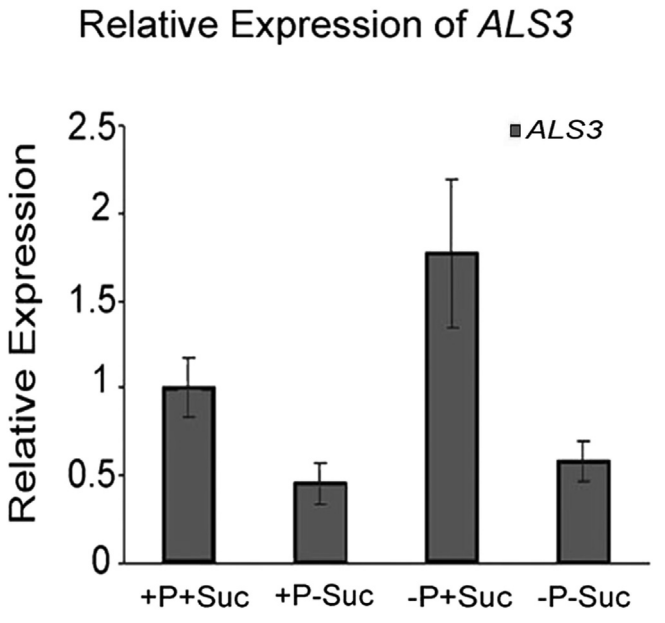


**Fig. 3.** Relative gene expression of *ALS3*. Relative gene expression of *ALS3* in different combinations of Phosphate and sugar concentrations. For each sample total RNA was extracted from 7 days old seedling and RNA was transcribed to C-DNA. *ALS3* was normalized using the housekeeping gene *Actin 2*. Error bar present ± S.E.

availability could ameliorate Al toxicity in sorghum by improving root development and nutrition uptake [29]. The immobilization of Al by Pi in the root cell wall is another potential mechanism for Al tolerance in buckwheat [30], barley [31], and maize [32]. Moreover, organic acid exudation has been suggested to be involved in the P-dependent Al tolerance in plants [33]. In this study, we identified two previously known aluminum tolerance genes to be involved in low-P response, making a connection at the molecular level.

*ALS3* encodes trans-membrane domain (TMD) of an ABC transporter characterized to be important for aluminum tolerance in *Arabidopsis*. The *Arabidopsis* mutant *als3* exhibited Al hypersensitivity in root growth inhibition assay [20]. In addition to *ALS3*, *NAP3* has been shown to physically interact with *ALS3* and, like *ALS3*, play a role in Al tolerance in rice [27,28]. Although *ALS3-NAP3* may form a bacterial-type ABC transporter critical for Al tolerance, its substrate does not include aluminum, instead, some of the glycosides may serve as substrates [27]. The mechanism of *ALS3-NAP3* action in Al tolerance remains unknown.

In pursuing a connection between Al tolerance and low-Pi response, we show here that *als3* and *nap3* mutants displayed typical features found in many other low-Pi response mutants, including those required for the modification of RSA (Root System Architecture), gene expression modulation [34,8,13,35]. In particular, root system in the *als3* mutant showed exaggerated reconstruction response by severely attenuating the primary root growth and increasing lateral roots and root hairs under phosphate starvation. These phenotypic changes in the mutant required high levels of sucrose in the medium, consistent with the finding that low-phosphate response in the roots appears to depend on sugar status [36,37,25,26,38,39]. Mutation in *PHO3*, a gene encoding a sucrose transporter involved in the phloem loading, disrupted low-P response [14], supporting the hypothesis that sugar status in roots is critical for root development under P-starvation [39]. In this study, by examining the effect of sucrose on the expression of *ALS3* and *NAP3* genes, we found that both genes were induced under low-P condition, but only in the presence of sugar in the medium. This sugar-dependent expression pattern is consistent with their function in sugar-dependent manner.

Although *ALS3-NAP3* may function as a transporter [27], it remains difficult to conclude how this transporter may work in low-P response. We speculated that *ALS3-NAP3* complex may function in Pi transport between shoots and roots thus affecting Pi status. However, Pi content in the *als3* and *nap3* mutants did not seem to be significantly different from the WT plants. Based on an earlier study [27], *ALS3-NAP3* has ADP-G transport activity. It is possible that such activity can extend to sugar transport, such as transport of sucrose. This hypothesis remains to be tested by further analysis.

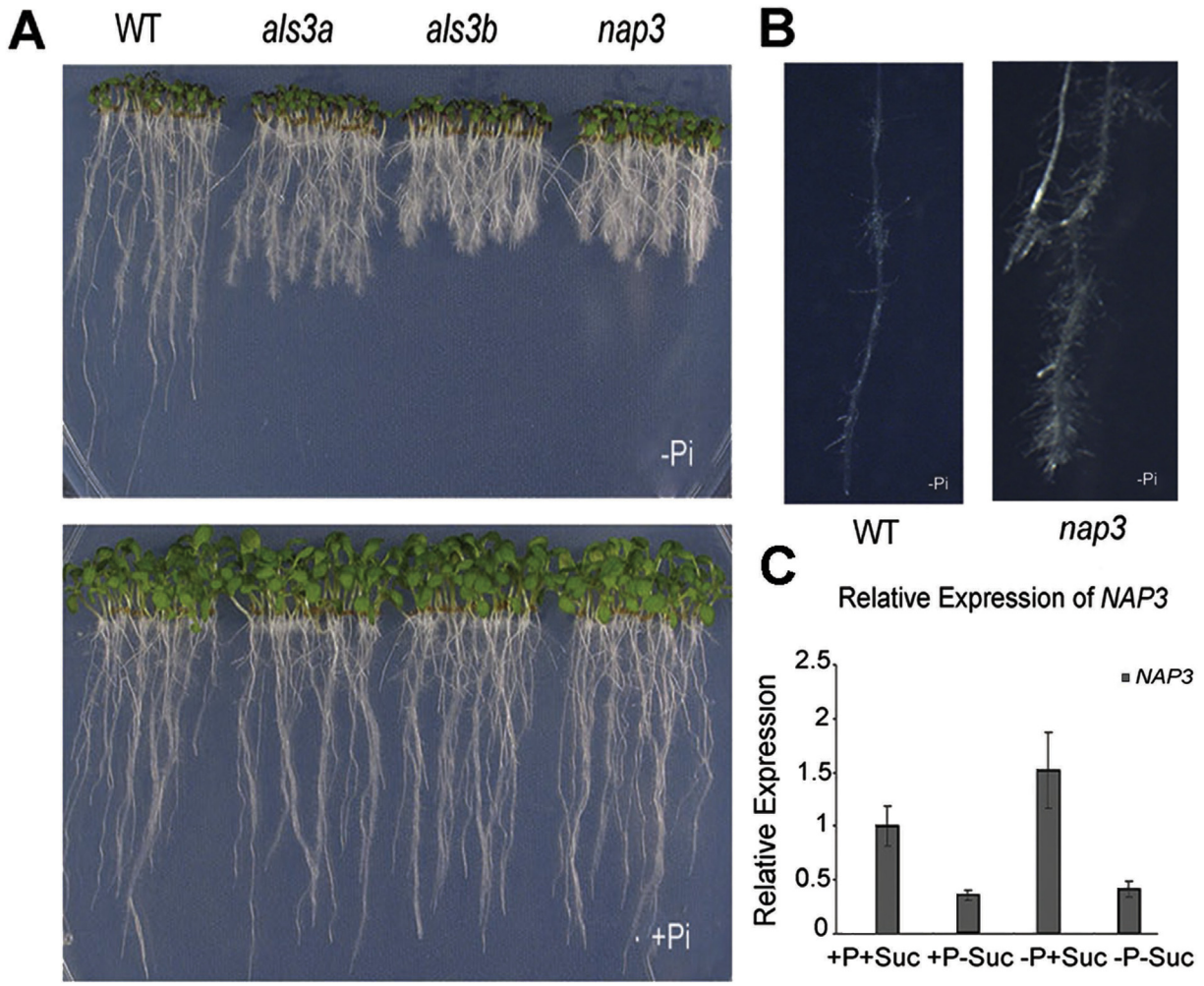


**Fig. 4.** *nap3* analysis (A) Phenotyping of *nap3* mutant, 7days old seedlings grown on 1/6 Murshige and skoog (MS) medium with or without phosphate. (B) Microscopic picture for about 1 mm from the root tip of *nap3* mutant on -Pi media showing the density of root hair. (C) Gene expression analysis of 7 days old *nap3* seedlings grown on different combinations of phosphate and sucrose. *Nap3* was normalized using the housekeeping gene *Actin 2*. Error bar present ± S.E.

## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.05.009.

## Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.05.009.

## References


[1] R.L. Bieleski, I.B. Ferguson, Physiology and metabolism of phosphate and its compound, in: A. Lauchi, R.L. Bieleski (Eds.), Encyclopedia of Plant Physiology, Inorganic Plant Nutrient Springer-Verlag, Berlin, 1983, pp. 422–429.

[2] M.E. Theodorou, W.C. Plaxton, Metabolic adaptations of plant respiration to nutritional phosphate deprivation, Plant Physiol. 101 (1993) 339–344.

[3] H. Marschner, Mineral Nutrition of Higher Plants, Academic, London, 1995.

[4] K.G. Raghothama, Phosphate acquisition, Annu. Rev. Plant Physiol. Plant Mol. Biol. 50 (1999) 665–693.

[5] H. Tiessen, Phosphorus in the global environment, in: P.J. White, J.P. Hammond (Eds.), The Encyclopedia of Plant Phosphorus Interactions, vol. 7, 2008, pp. 1–7.

[6] Y. Poirier, M. Bucher, Phosphate transport and homeostasis in Arabidopsis, in: The *Arabidopsis* Book, 2002.

[7] M.j. Harrison, Molecular and cellular aspects of arbuscular mycorrhizal symbiosis, Annu. Rev. Plant Physiol. Plant Mol. Biol. 50 (1999) 219–243.

[8] j. Lopez-Bucio, A. Cruzy Ramirez, L. herrera-Estrella, The role of nutrient availability in regulating root architecture, Curr. Opin. Plant Biol. 6 (2003) 280–287.

[9] Z. Ma, D.C. Bielenberg, K.M. Brown, J.P. Lynch, Regulation of root hair density by phosphorous availability in *Arabidopsis Thaliana*, Plant Cell Environ. 24 (2001) 459–467.

[10] L.c. Williamson, S.P.C.P. Ribrioux, A.H. Fitter, H.M. ottoline Leyser, Phosphate availability regulates root system architecture in *Arabidopsis*, Plant Physiol. 126 (2001) 875–890.

[11] J.P. Hammond, P.J. White, Sucrose transport in the phloem: integrating root responses to phosphorus starvation, J. Exp. Bot. 59 (2007) 93–109.

[12] J.Q. Liu, D.A. Samac, B. Bucciarelli, D.L. Allan, C.P. Vance, Signaling of phosphorus deficiency-induced gene expression in white lupin requires sugar and phloem transport, Plant J. 41 (2005) 257–268.

[13] O.V. Zakhleniuk, C.A. Raines, J.C. Lloyd, pho3: a phosphorus deficient mutant of *Arabidopsis thaliana (L.) Heynh*, Planta 212 (2001) 529–534.

[14] J.C. Lloyd, O.V. Zakhleniuk, Responses of primary and secondary metabolism to sugar accumulation revealed by microarray expression analysis of the *Arabidopsis* mutant, *pho3*, J. Exp. Bot. 55 (400) (2004) 1221–1230.

[15] M. Lei, Y. Liu, B. Zhang, Y. Zhao, X. Wang, Y. Zhou, G. Kashchandra Raghothama, D. Liu, Genetic and gemonic evidence that sucrose is a global regulator of plant responses to phosphate starvation in Arabidopsis, Plant Physiol. 156 (2011) 1116–1130.

[16] L.V. Kochian, O.A. Hoekenga, M.A. Pĩneros, How do plants tolerate acid soil? mechanism of aluminum tolerance and phosphorus, Annu. Rev. Plant Biol. 55 (2004) 459–493.

[17] C. Liang, M.A. Piñeros, j. Tian, Z. Yao, L. Sun, j Liu, J. Shaff, A. Coluccio, L.V. Kochian, H. Liao, Low pH, aluminum, and phosphorus coordinately regulate malate exudation through *GmALMT1* to improve soybean adaptation to acid soils, Plant Physiol. 161 (2013) 1347–1361.

[18] D. Dong, X. Penga, X. Yan, Organic acid exudation induced by phosphorus deficiency and/or aluminum toxicity in two contrasting soybean genotypes, Physiol. Plant. 1221 (2004) 90–199.

[19] H. Liao, H. Wan, j. Shaff, X. Wang, X. Yan, L.V. Kochian, Phosphorus and aluminum interactions in soybean in relation to aluminum tolerance, exudation of specific organic acids from different regions of the intact root system, Plant Physiol. 141 (2006) 674–684.

[20] P.B. Larsen, M.J.B. Geisler, C.A. Jones, K.M. Williams, J.D. Cancel, *ALS3* encodes a phloem-localized ABC transporter-like protein that is required for aluminum tolerance in *Arabidopsis*, Plant J. 413 (2005) 53–363.

[21] K.S. Osmont, R. Sibout, C.S. Hardtke, Hidden branches: developments in root system architecture, Plant Biol. 58 (2007) 93–113.

[22] T. Desnos, Root branching responses to phosphate and nitrate, Curr. Opin. Plant Biol. 11 (2008) 82–87.

[23] J. Wind, S. Smeekens, J. Hanson, Sucrose: metabolite and signaling molecule, Phytochemistry 71 (2010) 1610–1614.

[24] L. Barker, C. Kuhn, A. Weise, A. Schulz, C. Gebhardt, B. Hirner, H. Hellmanna, W. Schulzea, J.M. Warda, W.B. Frommer, SUT2, a putative sucrose sensor in sieve elements, Plant Cell 12 (2000) 1153–1164.

[25] A.S. Karthikeyan, D.K. Varadarajan, A. Jain, M.A. Held, N.C. Carpita, K.G. Raghothama, Phosphate starvation responses are mediated by sugar signaling in *Arabidopsis*, Planta 225 (2007) 907–918.

[26] C. Hermans, J.P. Hammond, P.J. White, N. Verbruggen, How do plants respond to nutrient shortage by biomass allocation? Trends Plant S 11 (2006) 610–617.

[27] C.F. Huang, N. Yamaji, N. Mitani, M. Yano, Y. Nagamura, J.F. Ma, A Bacterial-Type ABC transporter is involved in aluminum tolerance in Rice, Plant Cell 21 (2009) 655–667.

[28] C.F. Huang, N. Yamaji, J.F. Ma, Knockout of a Bacterial-Type ATP-Binding cassette transporter gene, *AtSTAR1*, results in increased aluminum sensitivity in Arabidopsis, Plant Physiol. 53 (2010) 1669–1677.

[29] K. Tan, W.G. Keltjens, Interaction between aluminium and phosphorus in sorghum plants, studies with the aluminium sensitive sorghum genotype *TAM428*, Plant Soil 124 (1990) 15–23.

[30] S.J. Zheng, J.L. Yang, Y.F. He, X.H. Yu, L. Zhang, J.F. You, R.F. Shen, H. Matsumoto, Immobilization of aluminum with phosphorus in roots is associated with high aluminum resistance in buckwheat, Plant Physiol. 138 (2005) 297–303.

[31] L.H. McCormick, F.Y. Borden, Phosphate fixation by aluminum in plant roots, Soil Sci. Soc. Am. J 36 (1972) 779–802.

[32] A. Gaume, F. Machler, E. Frossard, Aluminum resistance in two cultivars of *Zea mays* root exudation of organic acid and influence of phosphorus nutrition, Plant Soil 234 (2001) 73–81.

[33] A. Ligaba, H. Shen, K. Shibata, Y. Yamamoto, S. Tanakamaru, H. Matsumoto, The role of phosphorus in aluminium-induced citrate and malate exudation from rape (*Brassica napus*), Physiol. Plant. 120 (2004) 575–584.

[34] C. Ticconi, S. Abel, Short on phosphate: plant surveillance and countermeasures, Trends Plant S 9 (2004) 548–555.

[35] D.L. Chen, C.A. De latorre, A. Bakker, S. Abel, Conditional identification of phosphate starvation -response mutants in *Arabidopsis thaliana*, Planta 211 (2000) 13–22.

[36] P.J. White, M.R. Broadley, D.J. Greenwood, J.P. Hammond, Genetic modifications to improve phosphorus acquisition by roots, in: Proceedings of the International Fertiliser Society, York, UK, 2005.

[37] A. Amtmann, J.P. Hammond, P. Armengaud, P.J. White, Nutrient sensing and signalling in plants: potassium and phosphorus, Adv. Bot. Res. 43 (2006) 209–256.

[38] R. Muller, M. Morant, H. Jarmer, T.H. Nilsson, Genome-wide analysis of the *Arabidopsis* leaf transcriptome reveals interaction of phosphate and sugar metabolism, Plant Physiol. 143 (2007) 156–171.

[39] A. Jain, M.D. Poling, A.S. Karthikeyan, J.J. Blakeslee, W.A. Peer, B. Titapiwatanakun, A.S. Murphy, K.G. Raghothama, Differential effects of sucrose and auxin on localized phosphate deficiency-induced modulation of different traits of root system architecture in Arabidopsis, Plant Physiol. 144 (2007) 232–247.