Characterization of the rice NLA family reveals a key role for OsNLA1 in phosphate homeostasis

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Abstract

Background: Phosphate (Pi), an essential mineral nutrient for plant development and reproduction, is one of the main components of fertilizers in modern agriculture. Previous research demonstrated that AtNLA1 mediates ubiquitination of Pi transporters in the plasma membrane and triggers their endocytosis and degradation in Arabidopsis. In this study, we researched the function of NLA homologous proteins in Pi homeostasis in rice.

Findings: Two OsNLA homologs from rice (Oryza sativa L.) were identified by bioinformatics and phylogenetic analysis and designated OsNLA1 and OsNLA2. The OsNLA1 clustered with Arabidopsis AtNLA1, was expressed higher than OsNLA2 and was transcriptionally repressed under Pi-deficient condition. Loss-of-function of OsNLA1 caused P overaccumulation and growth inhibitions in both root and shoot under Pi-sufficient condition. Furthermore, mutation of OsNLA1 affected expression of Pi transporters and root hair development under Pi-sufficient and/or Pi-deficient conditions.

Conclusions: OsNLA1 plays a key role in maintaining phosphate homeostasis in rice.

Keywords: Rice, Phosphate, OsNLA1, Pi-homeostasis

Findings

Phosphorus (P) is a mineral nutrient essential for plant development and reproduction, and is integral to several macromolecules such as phospholipids and nucleic acids. Despite the indispensable role of P for plants, levels of phosphate (orthophosphate; Pi), the only form of P that can be taken up by plants, are commonly limited because of chemical fixation and microbial activity (Raghothama, 1999). To cope with suboptimal Pi conditions, plants have developed a series of adaptive responses, such as induction of Pi transporters and modification of root system architecture (Raghothama, 1999; Lin et al., 2009; Wu et al., 2013). Plant uptake of Pi is largely mediated by plasma membrane -localized Pi transporters belonging to the PHOSPHATE TRANSPORTER1 (PT) symporter family. Thirteen PT genes have been identified in rice (Oryza sativa) and nine in Arabidopsis thaliana (Goff et al., 2002; Karthikeyan et al., 2002). OsPTs differ in tissue expression patterns and affinities for Pi, resulting in diverse functions in plants. For instance, the high-affinity Pi transporter OsPT8 is universally expressed in rice, and is responsible for half of its Pi uptake (Chen et al., 2011; Jia et al., 2011). Although most OsPTs in rice are induced at the transcriptional level by Pi starvation or mycorrhizal symbiosis (Yang et al., 2012; Secco et al., 2013), post-transcriptional regulating of OsPT family proteins is also important to their activities (Gonzalez et al., 2005; Bayle et al., 2011; Chen et al., 2011; Chen et al., 2015). NITROGEN LIMITATION ADAPTATION (NLA), designated AtNLA1 in this study, was first identified as a positive regulator for the adaptability of Arabidopsis to nitrogen limitation (Peng et al., 2007), and later analysis of Pi concentration revealed that the early senescence phenotype of atnla mutant plants was due to Pi toxicity (Kant et al., 2011). In Arabidopsis, AtNLA1 can interact with AtPTs members via its SPX domain, and mediate ubiquitination of AtPTs in the plasma membrane and trigger their endocytosis and degradation (Lin et al., 2013; Park et al., 2014). Recently, two research groups separately reported roles of OsNLA1 in maintaining Pi homeostasis in rice (Yue et al.,...
AtNLA1 plays a major role in regulating Pi homeostasis, as OsNLA1 remained at relatively constant levels (Fig. 1e). The OsNLA2 et al., 2013); however, the expression level of OsNLA2 in rice plants were compared, and found that they were approximately equal (95.5% for OsNLA1 and 94.3% for OsNLA2) (Additional file 2: Figure S1 and Additional file 3: Table S1). Then, the transcript level of OsNLA1 and OsNLA2 in plants were compared, and found that the transcript level of OsNLA1 was higher than that of OsNLA2 in all tissues tested, with about 1.5-fold in shoot base, 4.5-fold in root and 80-fold in leaf sheath (Fig. 1b and c). Previous transcriptome analysis also shown that OsNLA1 abounance was higher than OsNLA2 in both root and shoot (Secco et al., 2013). Furthermore, the OsNLA1 transcript was differentially regulated by Pi availability, with higher expression in Pi-sufficient and lower expression in Pi-deficient conditions (Fig. 1d), as AtNLA1 in Arabidopsis (Lin et al., 2013); however, the OsNLA2 transcripts remained at relatively constant levels (Fig. 1e). The transcriptional change of OsNLA1 in response to Pi supply was also identified by RNA sequencing (Secco et al., 2013). Based on phylogenetic relationships and expression levels and responses to Pi starvation of the NLA family in rice, we suggest that OsNLA1 might plays a major role in regulating Pi homeostasis, as does AtNLA1 in Arabidopsis.

To characterize the functions of the NLA gene family in rice, we searched for publicly available mutants in different rice genomic resources. One T-DNA null mutant in OsNLA1 gene (PFG_1B-12,301) was obtained from RISD DB (Rice T-DNA Insertion Sequence Database) (Fig. 2a and b). After growth for 30 d under Pi-sufficient condition (300 μM Pi; +P), the shoots and roots of osnla1 were inhibited compared with wild-type (WT) plants (Fig. 2c and d). In addition, osnla1 displayed leaf tip necrosis on old leaves, which was a typical Pi toxicity symptom in rice (Fig. 2c, Additional file 2: Figure S1). This symptom in osnla1 was not observed when grown in Pi-deficient condition (10 μM Pi; -P). Moreover, the inhibited root phenotype of Osnla1 was reversed when grown in Pi-deficient condition (Fig. 2c and d). Total P concentrations in all tissues of osnla1 were higher than of WT, with 1.24-fold in roots and 1.46-fold in leaves under Pi-sufficient condition (Fig. 2e). This indicating that OsNLA1 played a key role in Pi uptake in rice, as previously reported (Yue et al., 2017; Zhong et al., 2017). However, under Pi-deficient condition, total P concentrations in old leaves (leaves 2 and 3) of osnla1 were decreased by 17–25%, while total P concentrations in youngest leaves (leaves 7) of osnla1 were increased by 21% compared with WT. The total P distribution rate in WT plants grown in Pi-deficient condition was 1.68-fold higher than that in plants grown in Pi-sufficient condition. However, the rate in osnla1 mutants grown in Pi-deficient condition was 3.19-fold higher than that in plants grown in Pi-sufficient condition (Fig. 2f). This significant increased total P distribution rate under Pi limiting condition sustained the newly leaves growth (Fig. 2c and d). Pi is the major form of P transported within the plants, and old leaves Pi pool would be the source of Pi in regard to young leaves under Pi-deficient condition, resulting in higher P concentration in young leaves (Li et al., 2015, b). Thus, these results indicated that OsNLA1 was also involved in Pi remobilization besides Pi uptake. This was expected because OsPT1 and OsPT8 also function in the redistribution of Pi from source to sink organs (Sun et al., 2012; Li et al., 2015, b). In a recent study, AtNLA1 was also involved in mediating degradation of NRT1.7 and further remobilizing nitrate from source to sink in Arabidopsis (Liu et al., 2016). Whether OsNLA1 also functions in nitrate remobilization need further studies.

As plants may modify root system architecture when growth in suboptimal Pi condition, we analyzed root hairs when osnla1 and WT were grown in Pi-sufficient and -deficient conditions. After Pi-deficient growth for 10 d, WT developed many root hairs (Fig. 3a and b), as previously reported (Zhou et al., 2008; Sun et al., 2012). However, the length of root hairs on osnla1 mutant was 1.5-fold those of WT plants grown in Pi-deficient condition. Furthermore, osnla1 mutant also had increased root hairs length under Pi-sufficient condition compared with WT plants. Since OsNLA1 could mediate
the degradation of OsPT2 and OsPT8 (Yue et al., 2017), inhibition of root growth and induce of root hair in osnla1 was expected because OsPTs were involved in regulating root growth and root hair development (Jia et al., 2011; Sun et al., 2012).

Since changing the expression of OsPT4 or OsPT8 affects the expression of other Pi transporters in rice (Jia et al., 2011; Sun et al., 2012; Li et al., 2015, b) and protein levels of OsPT2 and OsPT8 accumulated in osnla1 mutants (Yue et al., 2017), we then analyzed the transcriptional levels of OsPTs following WT and osnla1 mutant growth in Pi-sufficient and -deficient conditions for 10d. In the shoot of osnla1 mutant, transcripts of most of Pi transporters were induced (Fig. 3c). Compared with the WT, expressions of OsPT6 and OsPT8 were greatly induced under both Pi-sufficient and -deficient conditions. Although OsPT2, OsPT4 and OsPT10 were also up-regulated under Pi-sufficient condition, their transcript levels did not change under Pi-deficient condition. Expression of OsPT1 was induced only when osnla1 mutant was
grown under Pi-deficient condition. However, contrary to our finding, Yue et al. (2017) found that OsPT2 and OsPT8 were unchanged in leaf under Pi-sufficient conditions. This might be resulted from transcriptional levels of OsNLA1 and Pi transporters differed in various tissues (Fig. 1b; Remy et al., 2012). Unlike Pi transporters induced in the shoot, transcripts of Pi transporters were differentially regulated under Pi-sufficient and -deficient conditions in root of osnla1 mutant (Fig. 3c). The transcriptional levels of OsPT1 and OsPT4 were induced under Pi-sufficient condition, but unchanged under Pi-deficient condition. In contrast, OsPT6, OsPT8 and OsPT10 were downregulated under Pi-deficient condition, but unchanged under Pi-sufficient condition. The increased or repressed expression of these Pi transporters was caused, at least in part, by accumulated protein level of Pi transporters in osnla1 mutant, because changing the expression of OsPT4 or OsPT8 affects the expression of Pi transporters in rice (Jia et al., 2011; Zhang et al., 2015). Moreover, induced expression of OsPT1 and OsPT8 in shoot of osnla1 mutant under Pi-deficient condition would further remobilize Pi from old to young leaves (Sun et al., 2012; Li et al., 2015b).
Since OsNLA1 mediates degradation of OsPTs and plays a key role in maintaining Pi homeostasis in rice. In this research, we identified OsNLA1 could regulate root system architecture, Pi transporters at the transcriptional levels and Pi redistribution from source to sink organs. These results presented here will provide a novel insight into the function of OsNLA1 in rice.

Additional files

**Additional file 1:** Materials and methods. (DOCX 19 kb)
**Additional file 2:** Figure S1. Calculation of PCR efficiencies. Figure S2. Leaf blades of 30-d-old WT and osnla1 grown under Pi-sufficient (300 μM; +P) and Pi-deficient (10 μM; -P) conditions. (PPTX 304 kb)
**Additional file 3:** Table S1. Primers used in this study. (DOCX 14 kb)

**Abbreviations**
NLA: Nitrogen Limitation Adaptation; Pi: Phosphate; PT: Phosphate transporter; RISD DB: Rice T-DNA Insertion Sequence Database; WT: Wild type

**Acknowledgements**
This work was supported by the National Natural Science Foundation of China (31701984), Foundation of Sichuan University (2017SCU12007) and National Key Research and Development Program of China (2016YFD0100700)

**Authors’ Contributions**
HHL, CZM and JY designed the experiments. JY developed relevant research materials and performed the experiments together with LW. HHL, CZM and JY wrote the manuscript. All authors read and approved the final manuscript.

**Competing Interests**
The authors declare that they have no competing interests.
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Received: 15 July 2017 Accepted: 12 December 2017
Published online: 28 December 2017

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