Plant growth promotion under phosphate deficiency and improved phosphate acquisition by new fungal strain, Penicillium olsonii TLL1

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March 29, 2023

Abstract

Microbiomes in soil ecosystem play a significant role in solubilizing insoluble inorganic and organic phosphate sources with low availability and mobility in the soil. They transfer the phosphate ion to plants, thereby promoting plant growth. In this study, we isolated an unidentified fungal strain, POT1 (*Penicillium olsonii* TLL1), and confirmed its ability to promote root growth, especially under phosphate deficiency as well as solubilizing activity for insoluble phosphates such as $AlPO_4$, $FePO_4{\cdot}4H_2O$, $Ca_3(PO_4)_2$ and hydroxy apatite. Indeed, in vermiculite containing low and insoluble phosphate, POT1 promoted the growth of Arabidopsis and leafy vegetable. We also tested the growth of crops in Singapore local soil containing highly insoluble phosphate and confirmed an improved crop growth for Bok Choy and Rice with POT1. Furthermore, we demonstrated that plant growth promotion and phosphate solubilizing activity of POT1 were more effective than those of four different *Penicillium* strains such as *P. bilaiae*, *P. chrysogenum*, *P. janthinellum* and *P. simplicissimum* under phosphate limiting conditions. Our findings uncover a new fungal strain and provide a better understanding of symbiotic plant–fungal interactions and suggest the potential use of POT1 as a biofertilizer to improve phosphate uptake and use efficiency in phosphate-limiting conditions.

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Abstract

Microbiomes in soil ecosystem play a significant role in solubilizing insoluble inorganic and organic phosphate sources with low availability and mobility in the soil. They transfer the phosphate ion to plants, thereby promoting plant growth. In this study, we isolated an unidentified fungal strain, POT1 (*Penicillium olsonii* TLL1), and confirmed its ability to promote root growth, especially under phosphate deficiency as well as

solubilizing activity for insoluble phosphates such as $AlPO_4$, $FePO_4{\cdot}4H_2O$, $Ca_3(PO_4)_2$ and hydroxy apatite. Indeed, in vermiculite containing low and insoluble phosphate, POT1 promoted the growth of Arabidopsis and leafy vegetable. We also tested the growth of crops in Singapore local soil containing highly insoluble phosphate and confirmed an improved crop growth for Bok Choy and Rice with POT1. Furthermore, we demonstrated that plant growth promotion and phosphate solubilizing activity of POT1 were more effective than those of four different *Penicillium* strains such as *P. bilaiae* , *P. chrysogenum* , *P. janthinellum* and*P. simplicissimum* under phosphate limiting conditions. Our findings uncover a new fungal strain and provide a better understanding of symbiotic plant–fungal interactions and suggest the potential use of POT1 as a biofertilizer to improve phosphate uptake and use efficiency in phosphate-limiting conditions.

Keywords:

Biofertilizer, *Penicillium olsonii* TLL1, Phosphate deficiency stress, Phosphate solubilizing fungus, Plant growth promotion

1. Introduction

The availability of macro- and micronutrients plays a pivotal role in growth and development of plants. Among the macronutrients, phosphorus is an essential element involved in metabolic processes and signal transduction pathways in plants (Nesme et al., 2018). Phosphate limitation is a major nutritional constraint as it affects plant growth development and compromises optimal yields. Phosphate deficiency in agriculture has often been addressed by applying phosphate fertilizers, but the phosphate use efficiency in plants is only 5-25% due to the rapid fixation into insoluble forms (Schnug and Haneklaus, 2016). Moreover, the over-application led to loss of soil fertility and associated environmental hazards (Sharma et al., 2013).

Plants respond to phosphate limitation through various physiological, morphological and biochemical strategies (Raghothama, 1999). One of the acclimatization responses is the root morphology change to acquire available phosphate. The plant root morphology is remodeled by inhibiting primary root elongation and enhancing the formation of lateral roots and root hairs, which increases the effective surface area (Bates and Lynch, 1996; Lynch and Brown, 2001).

In Arabidopsis, the transcription factor STOP1 (SENSITIVE TO PROTON RHIZOTOXICITY 1) regulates citrate and malate exudation by activating the expression of its downstream genes, *MATE and ALMT1* efflux transporters, respectively, which were important for Al tolerance (Sawaki et al., 2009). In addition, RAE1 functions as an E3 ligase ubiquitinating *STOP1* and controls the expression of STOP1-regulated *AtALMT1* in Al resistance and low Pi response (Kobayashi et al., 2007; Zhang et al., 2019). The inhibition of primary root elongation under phosphate stress is often linked to iron accumulation in the root apical meristem (Ward et al., 2008) which is mediated by two genes in Arabidopsis, *LPR1* (Low Phosphate Root 1) encoding multicopper oxidase with ferroxidase activity and *PDR2*(Phosphate Deficiency Response 2) encoding single P-type ATPase (Svistoonoff et al., 2007; Ticconi et al., 2004). The interaction of LPR1-PDR2 result in accumulation of iron in the root apical meristem (RAM) and elongation zone (EZ) leading to callose deposition at the cell walls of RAM and EZ. Callose deposition impairs the expression of the transcription factor, *SHR* (Short Root), which is required for root patterning (Petricka et al., 2012). This results in blocked cell-to-cell communication and reduced RAM activity (Muller et al., 2015). Phosphate-deficiency stress also promotes the accumulation of Reactive Oxygen Species (ROS) and peroxidase in the cell walls of the root apical meristem (RAM) and elongation zone (EZ) in Arabidopsis plants. Peroxidase activity crosslinks cell wall components which lead to cell wall stiffening eventually reducing root cell expansion (Balzergue et al., 2017). Phosphate deprivation leads to higher levels of nitric oxide (NO) in the roots (Royo et al., 2015; Wang et al., 2010). NO acts as a signal molecule that mediates multiple responses, including reduction of primary roots (Wu et al., 2014), development of lateral roots (Correa-Aragunde et al., 2004), growth of root hair (Lombardo et al., 2006), and reutilization of phosphate in cell wall (Zhu et al., 2017) under phosphate deficiency in plants.

Phosphate-solubilizing microorganisms (PSM) help in phosphate assimilation from both organic and inorganic forms of phosphates in soil, making it available for plant uptake. Soluble phosphate acquisition by

PSM is attributed to several mechanisms such as chelation and ion exchange processes, production of organic acids, secretion of microbial-derived enzymes such as acid phosphatases, phytases, and production of phytohormones (Bergkemper et al., 2016). Although PSM includes both fungi and bacteria, the phosphate-solubilizing activity of fungi are comparatively higher than that of bacteria (Khan et al., 2010). Mycorrhizal associations with the plant roots are the best characterized beneficial interactions which help to mobilize soil nutrients and improve plant growth. However, endophytic fungi are the major contributors of phosphate solubilization in Brassicaceae family as mycorrhizal associations are rare (Bonfante and Genre, 2010). Most of the phosphate-solubilizing fungi (PSF) have been reported in the genera*Penicillium* (Li et al., 2016), *Aspergillus* (Yin et al., 2017) and *Trichoderma* (Lei and Zhang, 2015) and have been utilized as microbial inoculants in a variety of crops for improved phosphate uptake, and thereby increased biomass and yield. For example,*Penicillium oxalicum* and *Aspergillus niger* increase the bioavailability of phosphate and promote maize growth in calcareous soil (Yin et al., 2015). *Penicillium guanacastense* was reported as a potent biological fertilizer as it enhances the growth of *Pinus massoniana* under phosphate-limiting conditions (Qiao et al., 2019). Efficient PSF is a promising eco-friendly strategy to improve phosphate uptake and promote plant growth in modern agriculture. Nevertheless, it remains unclear how symbiotic fungi control the growth condition and regulate gene expression of their host plants under phosphate limitation.

In this study, we identify a new fungal strain, *Penicillium olsonii* TLL1 (POT1) and investigate gene regulation for root growth, especially under limited phosphate availability such as phosphate deficiency and insoluble phosphate conditions in soil, testing its phosphate solubilizing capability by fungus-plant interaction. Furthermore, we show that POT1 as a biofertilizer functions in mono/dicot plants and propose that it can be applied effectively in phosphate-limiting soils in both upland and paddy environments.

2. Materials and methods

2.1. PSF strain and plant materials

PSF was isolated from indoor dust samples and monocultured on Pikovskaya agar (Pikovskaya, 1948) containing 0.5% tricalcium phosphate (CaP), pH 6.8-7.2, by incubating at 250C for 7 days.

For seedling growth, *Arabidopsis* WT (Col-0), Bok Choy (*Brassica rapa subsp . parachinensis*) and Rice (Temasek Rice) seeds were germinated on media containing Murashige and Skoog salts, 0.25 mM MES and 10 g/L sucrose and 0.8% agar.

2.2. Identification and Genotyping of PSF strain

Total genomic DNA was extracted from the PSF strain using the CTAB method (Edel et al., 2001). One hundred nanograms of genomic DNA of the PSF strain were used to amplify five genetic loci such as Internal transcribed spacer (*ITS*), Translation elongation factor-1-α (*TEF1* α), Small Ribosomal subunit (*SSU*), Large ribosomal subunit (*LSU*), and Mini-chromosome maintenance protein (*MCM7*) using Hi-fidelity Phusion DNA polymerase. The genotyping was performed with primers in Table S1. The PCR reaction profile consisted of initial denaturation at 980C for 3 minutes, followed by 35 cycles of 980C for 10 seconds, 55-680C for 30 seconds, and 720C for 1 minute and final extension of 720C for 5 minutes. The sequences of PCR amplicons were determined using Sanger's Sequencing, and the contigs were assembled using Bioedit tool and were analyzed by BLAST searches at NCBI.

A phylogenetic tree was constructed using MEGA v. 7.0 separately for each genetic locus using Maximum-likelihood (ML) algorithm using Tamura Nei model with 1000 bootstrap replications. The reference sequences of the genes from the genus *Penicillium* were also used for alignment. The final tree was presented to show bootstrap cutoff level above 65% (Visagie et al., 2014).

2.3. Colony morphology and microscopy analysis

Colony morphology of POT1 was studied by culturing on PDA and MEA plates at 28 ± 2°C for 7 days. The colonization of POT1 was analyzed under full P conditions using co-staining with Wheat Germ Agglutinin-Alexa Fluor 488 conjugate (WGA-AF488) and propidium iodide (PI) as per the protocol mentioned in

Redkar et al., (2018). The confocal analysis was performed with TCS SP8 confocal laser scanning microscope. The excitation wavelength and detection wavelength for WGA-AF488 were 488 nm and 500-540 nm, respectively. For PI staining, the excitation wavelength and detection wavelength were 561 nm and 580-630 nm, respectively.

2.4. Bioassay for assessment of plant growth promoting potential

In vitro growth test of Arabidopsis ecotype, Col-0 was assessed in modified Hoagland's solution under full P, low P and -P conditions with 0.50 mM, 0.01 mM and 0 mM of KH_2PO_4(pH 5.6), respectively (Millner and Kitt, 1992). Four-day-old Arabidopsis seedlings were transferred to the test media precultured with POT1 inoculum. The root length of the seedlings was estimated after 10 days using Image J software (https://imagej.nih.gov/ij/download/). Other growth parameters such as shoot and root fresh weight were also determined.

The total phosphorus content in the shoot was measured by digesting 45 mg of the leaf or root tissue in 1 mL of 3:1 HNO_3/HCl in a microwave oven at 240°C for 15 minutes, and after digestion, it was diluted by ten times with water. The phosphorus content was determined using inductively coupled plasma-atomic emission spectroscopy (ICP-OES).

2.5. Quantitative RT-PCR

Total RNA was isolated from shoots and roots of *A. thaliana*plants grown in full P and low P conditions with and without POT1 using FavorPrep^{TM} plant total RNA minikit (Favorgen). Reverse transcription was performed with 1 µg total RNA using the iscript cDNA synthesis kit (Bio-Rad). Quantitative RT-PCR was performed on CFX96 real time system (Bio-Rad) using SsoAdvanced Universal SYBR Green Supermix (Bio-Rad) following manufacturer's instructions. The expression of *RAE1, STOP1, ALMT1* , *MATE1* were analyzed with primers in Table S2. Actin was used as the reference gene, and the gene expression level was analyzed by Genex Program (BioRad). The relative expression levels of each gene were normalized to their expression levels under full P conditions without POT1.

2.6. Determination of STOP1 protein levels in plants after POT1 inoculation

Full length CDS of *STOP1* gene was amplified from Arabidopsis cDNA using STOP1F: CACCATGGAAACTGAAGACGATTTGTGCAA and STOP1R: GAGACTAGTATCTGAAACAGACTCACCAAC and cloned in pENTR/D vector (Invitrogen) and then transferred to pBCo-DC-3HA plant expression vector by Gateway cloning (Invitrogen) using LR clonase enzyme II (Invitrogen) to generate 35S: STOP1-3HA construct. The construct was then introduced into the Arabidopsis ecotype Columbia-0 (Col-0) through*Agrobacterium* -mediated floral dipping (Zhang et al., 2006). To detect STOP1 accumulation in plants, transgenic Arabidopsis seedlings harbouring 35S:STOP1-3HA transgenes were grown in full P and -P conditions with and without POT1 at pH 5.6 for 10 days. The plants (100 mg) were flash frozen in liquid nitrogen and homogenized with 500 µL IP lysis buffer containing 1X complete protease inhibitor tablets (Roche). The total protein contents were separated on a 10% SDS-PAGE and STOP1-3HA proteins were analyzed by standard immunoblot using anti-HA antibody. Protein bands on membrane were captured using ChemiDoc Touch Imaging system (Bio-Rad, USA).

2.7. Determination of callose and NO

The roots excised from Arabidopsis plants grown under full P and low P conditions for 10 days were incubated for 2 hours in 150 mM K_2HPO_4 and 0.01% aniline blue under dark (Schenk and Schikora, 2015). Callose depositions were analyzed in a fluorescent microscope using DAPI filter [excitation 370 nm and emission 509 nm, Olympus FV 3000 (20X objective)].

For NO determination, the roots were treated with 10 µM DAF-FM DA (3-amino, 4-aminomethyl-2', 7 '-Difluorescein diacetate) for 15 minutes, and samples were washed with HEPES buffer (pH 4.0) for 10 minutes (Lombardo and Lamattina, 2012). The fluorescence was observed with a fluorescence microscope [excitation 495 nm and emission 515 nm, Olympus FV 3000 (20X objective)].

2. 8. Analysis of insoluble phosphate-solubilizing activity of POT1

POT1 was cultured on Pikovskaya agar and NBRIP containing tricalcium phosphate (CaP), pH 6.8-7.2. The colony diameter (D) and circular band of dissolved phosphorus (d) were measured, and the ratio d/D was calculated to find the phosphate-solubilizing rate.

For determination of soluble P concentration in broth, POT1 was cultured on Prune agar medium (40 mL/L prune juice, 1 g/L yeast extract, 2.5 g/L lactose, 2.5 g/L sucrose, 20 g/L agar, pH 6.5) for two days in dark and three days in light for sporulation. The conidial spore suspension (10^8 spores/mL) was inoculated into 100 mL of modified Pikovskaya broth containing 1g/L of sparingly soluble inorganic phosphates such as tricalcium phosphate (pH 7.0), iron phosphate (pH 2.5), aluminium phosphate (pH 4.0) and hydroxyapatite (pH 7.0) and incubated at 250C, 120 rpm for 14 days. Uninoculated media with different P sources were also kept as controls. The cultures were centrifuged at 5000 rpm for 15 minutes, and the supernatant was filtered with 0.22 μm syringe filter. The soluble phosphorus content was determined using phosphomolybdenum spectrophotometry (Ames, 1966).

2.9. Analysis of organic acids secreted by POT1

POT1 cultures grown under full P and low P were filtered using 0.22 μm filter and 10 μL of the samples were analyzed on a Liquid Chromatography-Q Exactive Orbitrap Mass Spectrometer system (Thermo Fisher Scientific, Waltham, US). The temperature in the multi-sampler was set at 4°C. LC-separation was performed on an Accucore C18 column (2.1 mm × 30 mm, 2.6 μm) at 22°C under a flow rate of 300 μL/min. The mobile phases for the reversed-phase (RP)-LC were solution A, 0.2% v/v formic acid in water, and solution B, 0.2% formic acid in 100% methanol. The MS was run in the negative mode for parallel reaction monitoring (PRM) using the ions listed in Table S3. Calibration stock solutions were prepared and stored at -80°C, and in each batch, 6-point calibration curves were made with freshly prepared dilutions.

2.10. Analysis of plant growth by POT1 under insoluble phosphate conditions

For the soil test, vermiculite was used as nutrient-free soil for studying plant growth under insoluble phosphate conditions. It was washed three times with deionized water to remove any soluble P and air-dried at room temperature. To test plant growth by P solubilizing activity, POT1 mycelia (0.5 g) were drenched in vermiculite for 7 days prior to the transfer of the seedlings. 7-day-old seedlings of Arabidopsis and Bok Choy were transferred to the vermiculite and subjected to different phosphate sources.

The plants were irrigated weekly twice with Hoagland's that contained full P (0.5 mM KH_2PO_4), low P (0.01 mM KH_2PO_4), -P (0 mM KH_2PO_4), tricalcium phosphate (0.5 mM CaP), aluminium phosphate (0.5 mM AlP), iron phosphate (0.5 mM FeP), and hydroxy apatite (0.5 mM HA), and were grown at 220C (Arabidopsis) and 250C (Bok Choy) under 75% relative humidity. Growth index parameters such as shoot fresh weight, leaf area index, number of leaves and shoot P content were analyzed.

The anthocyanin content in leaves of Arabidopsis plants was determined following the protocol by Laby et al., (2000). The leaves of 14-day old plants co-cultivated with and without POT1 were divided into three groups (leaves 1-4, 5-8, 9-12). The leaves were extracted with 99:1 methanol:HCl (v/v) overnight at 4°C. The anthocyanin contents were determined by acquiring OD at 530 and 657 nm and were calculated using the formula: OD530-(0.25 × OD657) × extraction volume (mL) × 1/ weight of the tissue.

2.11. Effect of POT1 under local soil conditions

The growth test of Bok Choy and Temasek Rice was conducted in soil collected from Lim Chu Kang, Singapore (N 1° 26' 7.3284", E 103° 42' 48.456"). Prior to that, a soil nutrient analysis was conducted to determine the available nutrients in the Lim Chu Kang soil and commercial soil used for the cultivation of Bok Choy and Rice. 1 kg of commercial soil and local soil collected from Lim Chu Kang, Singapore were analyzed for nitrogen (Kjeldahl, 1883), phosphorus, potassium, calcium, magnesium, sodium, copper, manganese, zinc and boron using Mehlich 3 extraction (Mehlich, 1984) followed by ICP-OES analysis at

National Parks Board, Singapore. 10 g of soil was mixed with 20 mL of sterile distilled water on a magnetic stirrer for 1 hour, and the pH of the soil mixture was recorded using pH meter.

For growth test, POT1 mycelia (0.5 g per pot) and heat-killed POT1 were drenched in Lim Chu Kang soil seven days before transferring the Bok Choy and Rice seedlings. The plants were grown under greenhouse conditions and the growth index parameters were determined.

2.12. Comparison of POT1 with other *Penicillium* species

The insoluble phosphate solubilizing activity of POT1 was compared with that of other *Penicillium* strains such as *P. bilaiae* (ATCC 20851), *P. chrysogenum* (NBRC 4626), *P. janthinellum* (NBRC 31133) and *P. simplicissimum* (NBRC 106922) in CaP amended modified Pikovskaya broth. The phosphate solubilizing rate was calculated using the following formula: [(soluble phosphorus content of inoculation group-soluble phosphorus content of control group)/inorganic phosphorus content of each experimental group × 100] described by Qiao et al., (2019). *In vitro* growth test was performed to compare the effect of POT1 with other *Penicillium*strains under -P conditions.

In addition, we conducted experiments in vermiculite soil medium to compare the CaP solubilization ability of POT1 with other*Penicillium* strains. The fungal mycelia (0.5 g) were drenched in vermiculite for 7 days prior to the transfer of the Arabidopsis seedlings, and the plants were irrigated weekly twice with Hoagland's solution that contained tricalcium phosphate as the sole source of phosphorus. The plants were grown at 220C and the growth index parameters were determined.

3. Results

3.1. Identification and genotyping of POT1

The phylogenetic relationships between POT1 and the representative fungal strains in maximum-likelihood trees were constructed using five barcode markers such as *ITS* , *TEΦ1a, ΣΣϒ, ΛΣϒ,* and*MCM7* . The *ITS* sequence of POT1 clustered with two other strains of *P. olsonii* (boot strap=82) (Fig. 1A and S1). The*TEΦ1a* sequences of POT1 clustered with five other strains of*P. olsonii* with a bootstrap value of 87 (Fig. S2). The *SSU*and *LSU* sequences of *P. olsonii* were also grouped with other strains of *P. olsonii* with bootstrap values of 97 and 99, respectively (Fig. S3 and S4). The same or closely related*Penicillium* species were clustered as a clade on the resulting phylogenetic tree, while the out-group cluster of *Aspergillus sp* . formed non-similarity clusters. Except for *MCM7* (Fig. S5), all the sequences of the DNA barcode markers of POT1 strain clustered with*P. olsonii* sequences and the strain was ascertained as *P. olsonii* .

The colony morphology of POT1 was recorded after 7 days of growth on MEA and PDA (Fig. 1B). When we stained the Arabidopsis roots co-cultivated with POT1 using WGA-AF488 and PI, the POT1 fungal hyphae appeared green and the plant root cell walls appeared red (Fig. S6). The co-staining revealed that POT1 is colonizing on the surface of the roots without any penetration into the root cortical cells.

3.2. Plant root growth promotion by POT1 under P-sufficient and deficient conditions

We conducted experiments to assess the growth promotion of Arabidopsis mediated by *P. olsonii* TLL1 (POT1) under both P-sufficient and P-deficient media conditions (Fig. S7). Under P-sufficient condition, Arabidopsis plants inoculated with POT1 exhibited 1.2-fold longer primary roots (Fig. 1C, 1D), but there was no significant increase in shoot fresh weight, root fresh weight, or shoot P content compared to uninoculated plants (Fig. 1E, 1F, 1G). Under P-deficient condition, POT1-inoculated plants showed 2-fold longer primary root length (Fig. 1H, 1I) and their shoot and root fresh weight were significantly increased by 1.5- and 2-fold (Fig. 1J, 1K), respectively, compared with uninoculated plants (Table S4). Additionally, we tested the growth Arabidopsis by POT1 inoculation under no P condition (Fig. S8A). Despite the absence of a phosphate source in the medium, the co-cultivated plants with POT1 showed 2-fold longer primary roots and 2-fold increased root fresh weight, whereas there was no difference in shoot fresh weight between inoculated and non-inoculated plants (Fig. S8B, S8C, S8D and Table S5). The shoot phosphate content in plants

inoculated with POT1 under no P conditions increased 1.5-fold compared to uninoculated plants (Fig. S7E).

3.3. Altered ALMT1 expression by POT1

To identify the role of *RAE1* , *STOP1* , *ALMT1* , and*MATE1* in promoting Arabidopsis growth by POT1, we quantified their relative expression in shoots and roots using qPCR (Fig. 2 and Table S6). Our results showed that the expression level of *RAE1, STOP1 and MATE1* showed no significant changes in response to phosphate sufficiency or deficiency in either shoots or roots (Fig. 2A, 2B, 2D, 2E, 2F, 2H). However, the expression of *ALMT1* was increased under phosphate deficient conditions in root. Consistent with the root phenotype in Arabidopsis, *ALMT1* expression was decreased upon POT1 inoculation (Fig. 2C). Under phosphate sufficient condition,*ALMT1* expression was significantly decreased in the shoots upon POT1 inoculation (Fig. 2G).

3.4. STOP1 protein was unstable when co-cultivated with POT1

The interaction between POT1 and STOP1 protein under full P and low P conditions was demonstrated in transgenic Arabidopsis plant. The STOP1 protein was found to be degraded by POT1 under both phosphate sufficient and deficient conditions (Fig. 3A). Under phosphate-deficient condition, POT1 induced a greater decrease in STOP1 protein levels under phosphate-deficient conditions compared to phosphate-sufficient conditions.

3.5. Inhibition of callose deposition and NO accumulation by POT1

To confirm that POT1 inhibits negative components of root growth, we investigated its effect on callose deposition and Nitric Oxide (NO) accumulation under phosphate sufficient and deficient conditions. Under phosphate deficiency, POT1-inoculated plants showed decreased callose deposition in the root apical meristem compared to the massive callose formation observed in uninoculated plants (Fig. 3B). However, under phosphate-sufficient conditions, no difference in callose deposition was observed in plants with/without POT1. The capability of POT1 to inhibit endogenous NO accumulation in roots was tested using the fluorescent probe DAF-FM DA. Green fluorescence was clearly observed under phosphate deficient condition, indicating phosphate-dependent NO production. In contrast, after inoculating POT1, the NO level in root was decreased, suggesting the involvement of POT1 in root development under phosphate deficiency (Fig. 3C).

3.6. Quantitative assay for phosphate-solubilizing activity of POT1

When POT1 was grown on PVK and NBRIP media amended with $Ca_3(PO_4)^2$ for 7 days at 25°C, POT1 generated visible halo zones of dissolved phosphorus, indicating recalcitrant phosphate solubilization (Fig. 4A). When cultured on Pikovskaya and NBRIP media, POT1 showed a phosphate solubility index (d/D ratio) of 1.161 and 1.212, respectively (Fig. 4B and Table S7).

The POT1 was found to solubilize tricalcium phosphate [$Ca_3(PO_4)^2$], hydroxyapatite [$Ca_{10}(PO_4)^6(OH)^2$], aluminium phosphate ($AlPO_4$), and iron phosphate ($FePO_4.4H_2O$), accompanied by a decrease in the pH of the supernatants in modified PVK media (Table S8). POT1 had the highest phosphate-solubilizing activity in media containing $Ca_3(PO_4)^2$, followed by hydroxyapatite, aluminium phosphate, and iron phosphate (Fig. 4C). The soluble phosphorus concentration reached 2.95 mg/mL in CaP and 1.574 mg/mL in hydroxyapatite. POT1 could also solubilize $AlPO_4$ and $FePO_4$ to release soluble P at 0.924 mg/mL and 0.436 mg/mL, respectively, after 14 DPI (Fig. 4D). The phosphate-solubilizing rate was 57% for CaP, 31% for hydroxyapatite, 20% for AlP and 3% for FeP (Table S8).

3.7. Secretion of organic acids by POT1

The organic acids released by POT1 were measured under both full P and low P conditions. Secretion of gluconic acid (238 mg/L) was higher under low P conditions, whereas secretion of citric acid (212 mg/L) was higher under full P conditions (Fig. S9). Malic acid concentration in low P (3 mg/L) was reduced by

45-fold when compared to full P conditions (136 mg/L). Similarly, succinic acid secreted by POT1 into low P culture filtrate was reduced 25-fold.

3.8. Growth promotion in Arabidopsis and leafy vegetable by POT1 under insoluble phosphate conditions

To investigate the growth promotion and phosphate-solubilizing capability of POT1 on Arabidopsis growth under P sufficient and deficient conditions including insoluble P sources, we co-cultivated Arabidopsis plants in vermiculite, which is a nutrient-free soil (Fig. 5 and Table S9). Arabidopsis plants grown in low P and insoluble P sources such as tricalcium phosphate (CaP), aluminium phosphate (AlP), iron phosphate (FeP) and hydroxyapatite (HA) conditions are stunted without POT1 inoculation (Fig. 6A). Consistent with Arabidopsis growth condition, shoot fresh weight, leaf index, number of leaves and shoot phosphate content were higher in POT1 inoculated plants compared to non-inoculated plants (Fig. 5B, 5C, 5D, 5E and S10). The anthocyanin accumulation, which is the typical phenotype of plants grown under phosphate deficiency (Jiang et al., 2007), was lower in POT1 inoculated plants than that of non-inoculated plants (Fig. S11, Table S10). Under no P condition in vermiculite soil, shoot fresh weight, number of leaves, leaf area index and shoot P content of POT1 inoculated Arabidopsis plants were increased significantly, compared with non-inoculated plants (Fig. S12 and Table S11). However, plants grown under no P condition still showed growth defects when compared with phosphate-sufficient plants.

We also examined whether POT1 colonization could promote growth of leafy vegetable, Bok Choy (*B. rapa* subsp. *parachinensis*) under different phosphate sources by growing in vermiculite (Figure 6A and Table S12). After four weeks, Bok Choy plants grown in low P, tricalcium phosphate (CaP), aluminium phosphate (AlP), iron phosphate (FeP) and hydroxyapatite (HA) conditions without POT1 exhibited stunted growth, whereas biomass of POT1 inoculated plants were increased as indicated by fresh shoot weight, number of leaves and leaf area index (Fig. 6B, 6C, 6D and S13). Moreover, the total phosphate content was higher in all the inoculated plants under insoluble phosphate and low phosphate conditions, compared with non-inoculated Bok Choy (Fig. 6E). When no P condition was simulated in vermiculite soil, the shoot fresh weight, number of leaves and leaf area index of plants were increased in plants treated with POT1 compared to non-inoculated plants (Fig. S14). However, the biomass of the plants still decreased seven-fold compared to plants grown under full P condition (Table S13).

3.9. Growth promotion by POT1 under local soil conditions

We investigated whether the POT1 functions in local soil at Lim Chu Kang in Singapore on Bok Choy and Rice growth. Firstly, we analyzed the pH and macro/micro-nutrient content of local soil. Our results showed the pH of Lim Chu Kang soil was 6.5, which was higher than pH 5.7 of commercial soil (Fig. S15A), which was in the range of highest phosphate availability (Havlin, 2020) (Fig. S15B). Additionally, the phosphate content of commercial soil was 4-fold higher than that of Lim Chu Kang soil (Fig. 15C). However, the phosphate content in Lim Chu Kang soil (50 mg/kg) indicated that there were still sufficient for plant growth (Philip et al., 2021). We also analyzed other macro/micro-nutrients such as nitrogen, potassium, calcium, magnesium, iron, sodium, copper, manganese, zinc and boron in commercial and Lim Chu Kang soil (Fig. S16A, S16B). Notably, the content of copper, manganese and zinc in Lim Chu Kang soil was higher than that of commercial soil (Table S14).

For four weeks, we grew Bok Choy plants in Lim Chu Kang soil with/without POT1 (Fig. S17). We found that POT1 improved the growth conditions of the plants, as indicated by the increase in shoot fresh weight, number of leaves, leaf index in POT1 inoculated plants (Fig. 7A, 7B, 7C, 7D, Table S15). Moreover, the total phosphorus content in the leaves of POT1-inoculated plants was higher than that of non-inoculated plants (Fig. 7E, Table S15). We also confirmed that POT1 promoted the growth of Rice, a monocot plant, in Lim Chu Kang soil (Fig. 8 and S18). The co-cultivation of Rice plants with POT1 resulted in increased plant height (Fig. 8A, 8B), number of tillers (Fig. 8C) and shoot phosphate content (Fig. 8D) compared with plants grown without POT1 inoculation. Furthermore, the increased tiller number was accompanied by an increase in seed number (Fig. 8E) and seed dry weight (Fig. 8F) were also increased by POT1 inoculation (Table S16).

In addition, we tested the effect of POT1 on plant growth under Lim Chu Kang soil condition using heat-killed POT1 as a negative control. We found that shoot fresh weight, number of leaves and leaf area index of Bok Choy were significantly higher in live POT1 inoculated plants as compared to heat-killed POT1 and uninoculated plants (Fig. S19, Table S17). Similarly, in the Rice growth test, heat-killed POT1 was non-functional, as indicated by shorter plant height, lower tiller numbers and reduced seed weight/ number compared with live POT1 (Fig. S20, Table S18). These results suggest that the phosphate-solubilizing capability of POT1 helps plants absorb phosphate sources from soils containing insoluble phosphate, thereby increasing plant growth.

3.10. Comparison of five *Penicillium* strains on phosphate solubilizing activity and Arabidopsis growth

Tricalcium phosphate solubilization capability of POT1 was compared to other *Penicillium* strains such as *P. bilaiae, P. chrysogenum* , *P. janthinellum* and *P. simplicissimum* (Fig. S21). The soluble phosphate concentrations in the medium of *P. chrysogenum* , *P. janthinellum* and *P. simplicissimum*cultured under CaP were 1.69 mg/mL, 1.84 mg/mL and 1.97 mg/mL, respectively. The highest solubilizing activity was shown in *P. bilaiae* (3.44 mg/mL), followed by POT1 (3.06 mg/mL) (Table S19). Based on these results, we also compared growth promotion activity of POT1 with that of other *Penicillium* strains such as *P. bilaiae* ,*P. chrysogenum* , *P. janthinellum* and *P. simplicissimum* under P-deficient conditions (Fig. S22A). All strains showed longer primary root length than that of control, which was without any fungal inoculation. However, when we compared among the 5*Penicillium* strains, the primary root length of plants inoculated with POT1 was the longest (Fig. S22B). Similarly, shoot fresh weight in POT1 inoculated plants (2- fold) was the highest among all the*Penicillium* strains (Fig. S22C). Unexpectedly, there was no difference in shoot fresh weight between plants inoculated with *P. simplicissimum* and control plants. Moreover, in the case of plants co-cultivated with *P. janthinellum* , 2.56- fold decrease in shoot fresh weight was observed. The root fresh weight of plants inoculated with all fungi strains was higher than that of non-inoculated plants, and the highest root fresh weight was shown in *P. chrysogenum*strain (Fig. S22D, Table S20).

To investigate the plant growth by phosphate solubilizing activity of each fungus, we tested Arabidopsis growth in vermiculite using an insoluble phosphate source, which is calcium phosphate (CaP) (Fig. S23A). The shoot fresh weight of Arabidopsis inoculated with POT1 was higher than that of other strains under insoluble phosphate condition (Fig. S23B). Furthermore, we measured anthocyanin accumulation, which is a typical phosphate deficiency response in plants. The lowest anthocyanin content was observed in plants inoculated with POT1 and*P. simplicissimum* (Fig. S23C, Table S21). Altogether, POT1 showed better activity than other *Penicillium* strains.

4. Discussion

In our study, we identified that the newly isolated fungal strain,*P. olsonii* TLL1 (POT1), has a high ability to solubilize phosphate, and promotes root elongation in Arabidopsis (Fig. 1). Even if the phosphate source is completely omitted from the growth medium, the length of the primary root is longer with POT1 inoculation.

The STOP1 transcription factor is a crucial checkpoint in the root development to phosphate deficiency stress in Arabidopsis. Our study shows that STOP1 protein level is decreased by POT1 inoculation under full P and low P conditions. It implies that the reduction of STOP1 protein leads to a low ALMT1 transcript level. Thus, the longer primary root length observed with POT1 is due to a decrease in the protein and transcript levels of *STOP1* and *ALMT1* , respectively (Fig. 2 and 3A). STOP1 stability is reported to be post-translationally regulated by RAE1, an E3 ubiquitin ligase, thereby controlling primary root length (Sawaki et al., 2009; Zhang et al., 2019).

Moreover, we confirmed that POT1 inhibited callose and NO accumulation in root cells (Fig. 3B, 3C). Despite being grown under low phosphate condition, Arabidopsis roots elongate normally due to the inhibition of callose and NO accumulation with root colonization of POT1. As negative factors of primary root elongation, callose deposition and NO accumulation inhibit primary root growth in the root apical meristem and entire root cells, respectively. These metabolites can be degraded and scavenged by beta-1,3 glucanases and flavonoids (Vanacker et al., 1995; Levy et al., 2007; Muller et al., 2015; Wang et al., 2010; Burch-Smith and

Zambryski, 2012).

The secretion of β-1,3 glucanases by endophytic fungi is well documented as an antagonistic activity against invading fungal pathogens (Markovich and Kononova, 2003). Endophytic fungal communities with phosphate solubilizing activity associated with the rhizosphere of healthy maize and rice plants secrete β-1,3 glucanases, chitinases and amylases (Potshangbam et al., 2017). Besides, *Aspergillus nidulans*and *Aspergillus oryzae* are reported to secrete flavonoids in cultures (Qiu et al., 2010). Taken together, we suggest that POT1 might control STOP1 protein stability and secrete beta-1,3 glucanases and flavonoids in low phosphate environments. Thus, further studies are required to confirm the mechanism of post translational regulation as well as the secretion of beta-1,3 glucanases and flavonoids by POT1 under phosphate-limiting conditions.

Phosphate fixation in soil depends on edaphic factors such as pH, organic matter content, the presence of exchangeable cations such as Fe, Al, Ca, and Mg, and clay content, which contribute to phosphate insolubility. The phosphate added as agrofertilizer to the soil reacts with cation ions such as Al, Ca and Fe and becomes fixed in the soil, which is inaccessible for uptake by the plant roots (Penn and Camberato, 2019). Hence, phosphate-solubilizing fungi (PSF) could be widely employed as a potential solution to improve phosphate uptake and use efficiency.

Phosphate solubilizing fungi thrive in the rhizosphere and establish symbiotic associations with the root systems of plants. Endophytic fungi such as *Penicillium, Aspergillus, Trichoderma, Piriformospora* , and *Curvularia* are among the active PSF species involved in phosphate cycling (Mehta et al., 2019). The *P. guanacastense*JP-NJ2 strain has a high ability to solubilize aluminium phosphate (Qiao et al., 2019), whereas *Penicillium rugulosum* IRMF94 strain dissolved larger concentrations of calcium and iron phosphate (Blanco and Reyes, 2018). The solubilization of recalcitrant phosphate sources by *Aspergillus spp.* and *Penicillium spp.* generally decreased the liquid medium pH, for the solubilization of insoluble phosphate (Acevedo et al., 2014). Here, we confirmed the phosphate-solubilizing activity of POT1 in insoluble phosphate complexes such as tricalcium phosphate, aluminium phosphate, iron phosphate and hydroxyapatite (Fig. 4). Among these, the highest solubilizing capability is observed in culture media containing tricalcium phosphate (Fig. 4D).

Organic acid secretion is the widely reported mechanism of phosphate solubilization, where PSFs break down insoluble phosphates by secreting organic acids onto soil surfaces (Rawat et al., 2021). Malic acid was most competent to solubilize CaP, followed by citric, formic and succinic acids (Gaind, 2016). It has been reported that tartaric acid and citric acid were dominant in *Penicillium oxalicum* P4, while malic and citric acid dominated in *A. niger* P85 cultures when solubilizing tricalcium phosphate (Yin et al., 2015). The commercial strain, *P. bilaiae,* has been shown to produce oxalic and citric acids (Cunningham and Kuiack, 1992). We have shown that in low P conditions, POT1 highly exudates gluconic acid while malic acid and citric acid are reduced.

The use of microbial inoculants has gained acceptance over chemical fertilizers as they are eco-friendly and help to improve soil health. The application of fungal species as biofertilizers to arable land to improve soil quality is emerging as an eco-friendly approach in modern agriculture (Kour et al., 2020). *P. bilaiae* was widely used as a biofertilizer, and several studies conducted in growth chambers and the field increased the biomass, phosphate uptake and grain yield in wheat (*Triticum aestivum* L.) (Kucey, 1988), canola (*Brassica napus* L.) (Kucey and Leggett, 1989), bean, pea (Vessey and Heisinger, 2001), and lentil (Wakelin et al., 2007). Among the *Penicilliumgenus, P. bilaji* , *P. italicum* , *P. albidum* , *P. frequentans* , *P. simplicissimum* , *P. rubrum* , *P. expansum* , *P. oxalicum, P. citrinum* have been commercially used as biofertilizers for the mobilization of P, Mn, Zn, Fe, Co, Cu, and Mo and biotic and abiotic stress tolerance (Odoh et al., 2020). *P. bilaiae* was reported to be effective in calcareous soil compared to moderately acidic soil (Sanchez-Esteva et al., 2016). In our study, we have confirmed that plant growth conditions in Arabidopsis, Bok Choy and Rice in vermiculite and Singapore's local soil, which has low phosphate availability, are improved due to the P-solubilizing activity of POT1 (Fig. 5-8).

In addition, we showed that POT1 was remarkably effective on root growth promotion and phosphate

solubilizing activity in phosphate-deficient medium and insoluble phosphate-contained soil when compared to other*Penicillium* strains such as *P. bilaiae* , *P. chrysogenum* , *P. janthinellum* and *P. simplicissimum* . Thus, we suggest that POT1, which showed better activity than other*Penicillium* strains, can be used as a high potential biofertilizer for both monocot and dicot crop growth as well as the different types of soil conditions, which are upland and paddy soil. Furthermore, combinations of various beneficial fungal strains are required for the evaluation of plant growth and viability tests among fungal strains. Overall, this study provides useful information for developing more efficient biofertilizers than the existing ones.

5. Conclusion

The optimal usage of chemical fertilizers is necessary to maintain ecosystem function and develop sustainable agriculture. Thus, application, research and methodology developments of biofertilizers need to be emphasized on improving effective, stable, nontoxic microbiome strains for promoting plant growth. Using POT1, a potential biofertilizer, our study provides information on the combined action of both phosphate-solubilizing function and inhibitor removal of primary root elongation, especially under phosphate-limiting conditions. Therefore, these findings will support the development of biofertilizer for improved phosphate use efficiency and apply to different types of plants, such as monocot and dicot plants, increasing the yield even under low or no phosphate condition as well as different conditions of soil, such as upland and paddy soil. Our research would contribute towards a better solution for sustainable agriculture with reduced fertilizer cost, especially phosphate, increasing its use efficiency as well as increased crop yield.

Declaration of Conflict of Interest

This research finding has been filed with the Intellectual Property Office of Singapore (IPOS), Application no. 10202300767S. This patent was filed by Suraby Erinjery Jose, Naweed I. Naqvi, Rajani Sarojam, Zhongchao Yin, Bong Soo Park. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgments

The work is supported by Temasek Life Sciences Laboratory core funding (3085).

Data accessibility statement

The sequences of the barcode markers used for construction of the tree are available in the GenBank database under the following accession numbers: OQ678019 (*ITS*), OQ700986 (*TEΦ1α*), OQ678118 (*SSU*), OQ678119 (*LSU*), and OQ700985 (*MCM7*).

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Figures and Figure Legends

Fig. 1

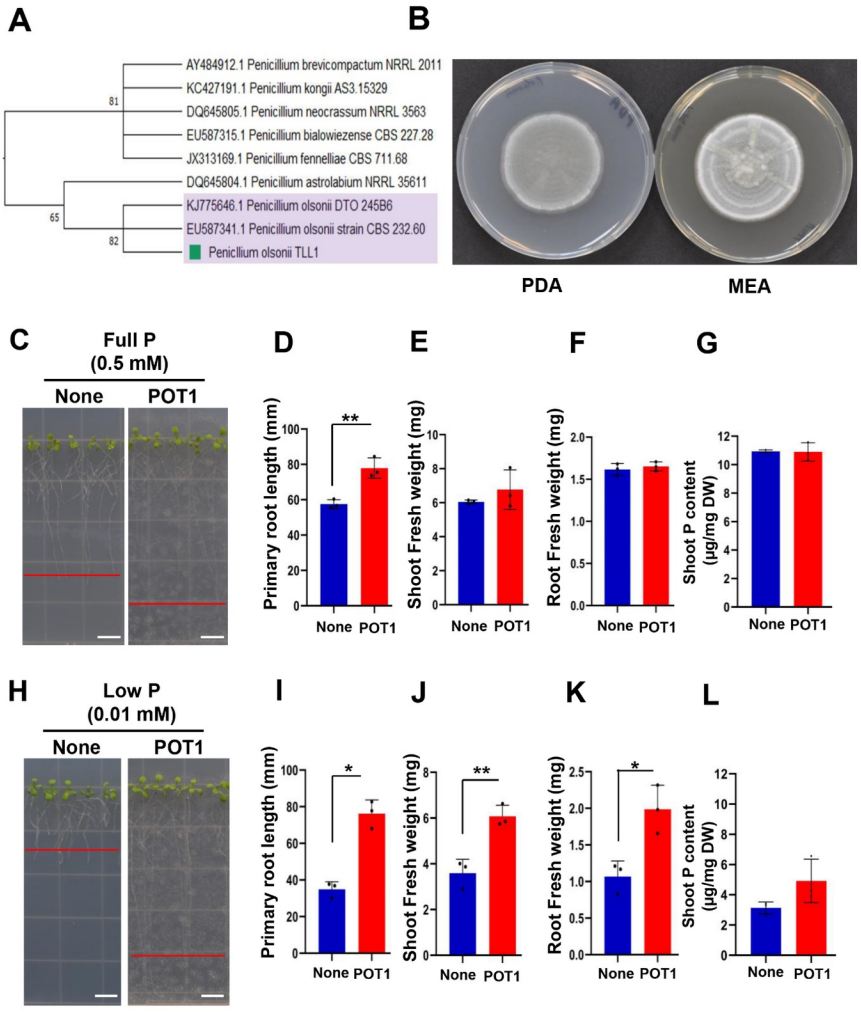

Fig. 1. Identification of phosphate solubilizing fungus and growth promotion of Arabidopsis POT1 under P-sufficient and P-limiting conditions.

(A) Phylogenetic tree generated from maximum likelihood (ML) analysis of phosphate-solubilizing fungus POT1 based on ITS sequence data. Boot strap values on 1000 replications are shown at the nodes of the tree.

(B) Colony morphology of phosphate-solubilizing fungus POT1. POT1 was cultured on (left) PDA and (right) MEA for seven days. Scale bar represents 1 cm.

(C) Arabidopsis plants grown in full P conditions with and without POT1. Four-day-old plants were transferred to full P media pre-cultured with and without POT1 under *in vitro* conditions for 10 days. Scale bar represents 1 cm. The bar diagrams show D) primary root length, E) Shoot fresh weight, F) root fresh weight of Arabidopsis Col-0 plants co-cultivated with POT1 in full P conditions for 10 days. The bar diagram shows combined data from three independent experiments (n=3; unpaired two-tailed t-test, ***P<0.001; **P<0.01; *P<0.05). G) shoot P content of Arabidopsis plants co-cultivated with and without POT1 after

four weeks.

(H) Arabidopsis plants grown in low P conditions with and without POT1 Four-day-old plants were transferred to full P media pre-cultured with and without POT1 under *in vitro* conditions for 10 days. Scale bar represents 1 cm. The bar diagrams show I) primary root length, J) Shoot fresh weight, K) Root fresh weight of Arabidopsis WT plants co-cultivated with POT1 in low P conditions for 10 days. The bar diagram shows combined data from three independent experiments (n=3; unpaired two-tailed t-test, ***P<0.001; **P<0.01; *P<0.05). L) Shoot P content of Arabidopsis plants co-cultivated with and without POT1 after four weeks. The concentration of total phosphorus content in Arabidopsis shoots were significantly increased after growth in the presence of POT1 in low P under *in vitro* conditions (n=3; unpaired two-tailed t-test, **P<0.01).

Fig. 2

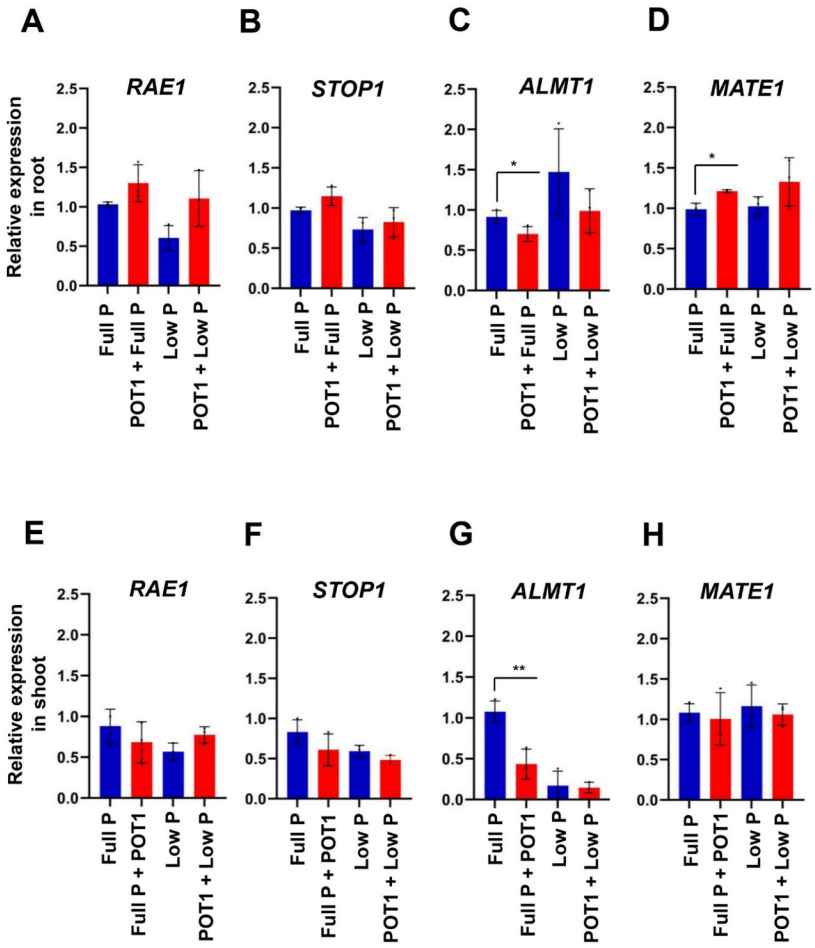

Fig. 2. Gene expression analysis.

Relative expression of A) RAE1 B) STOP1, C) ALMT1 and D) MATE1 in roots of Arabidopsis when treated with and without POT1 under full P and low P conditions. Relative expression of E) RAE1 F) STOP1,

G) ALMT1 and H) MATE1 in shoots of Arabidopsis when treated with POT1 under full P and low P conditions. Expression levels are shown relative to the mean expression of actin. Relative expression levels are normalized to that of full P conditions. The bar diagram shows combined data from three independent experiments (n=3; unpaired two-tailed t-test, **P<0.01; *P<0.05).

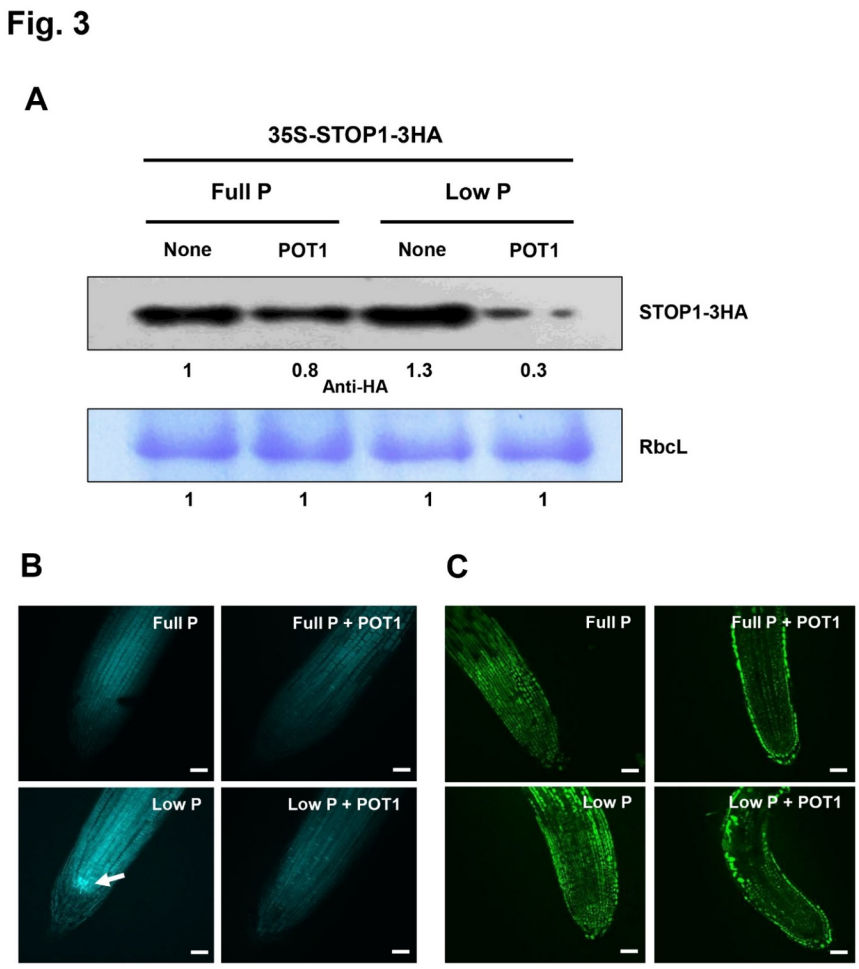

Fig. 3. Effects of POT1 inoculation on STOP1 accumulation, callose deposition and NO accumulation in Arabidopsis.

A) Transgenic Arabidopsis plants expressing 35S:STOP1-3HA were grown under full P and low P with and without POT1 inoculation. STOP1 protein levels were analyzed by western blots using anti-HA antibody. Stained gel bands of large subunit of Rubisco (RbcL) were used as control. POT1 inoculation destabilizes STOP1 protein in Arabidopsis STOP1-overexpression lines under P-sufficient and P-limiting conditions.

(B) Callose deposition in Arabidopsis roots under full P and low P conditions inoculated and uninoculated conditions with POT1. Four-day-old plants were transferred to full P media pre-cultured with and without POT1 under *in vitro* conditions for 10 days. Callose accumulation was analyzed in 1.5 cm root tips using aniline blue staining. The white arrowhead points to callose accumulation in the stem cell niche in phosphate limiting conditions. Three independent experiments were performed, and one representative experiment is shown. Scale bar 100 μm.

(C) Nitric oxide (NO) accumulation in Arabidopsis roots under full P and low P conditions under inoculated and uninoculated conditions with POT1. Four-day-old plants were transferred to full P media pre-cultured with and without POT1 under *in vitro* conditions for 10 days. NO accumulation was analyzed in 1.5 cm root tips using DAF-FM DA staining. Inoculation with POT1 reduced NO accumulation in roots. Scale bar 100 μm.

Fig. 4

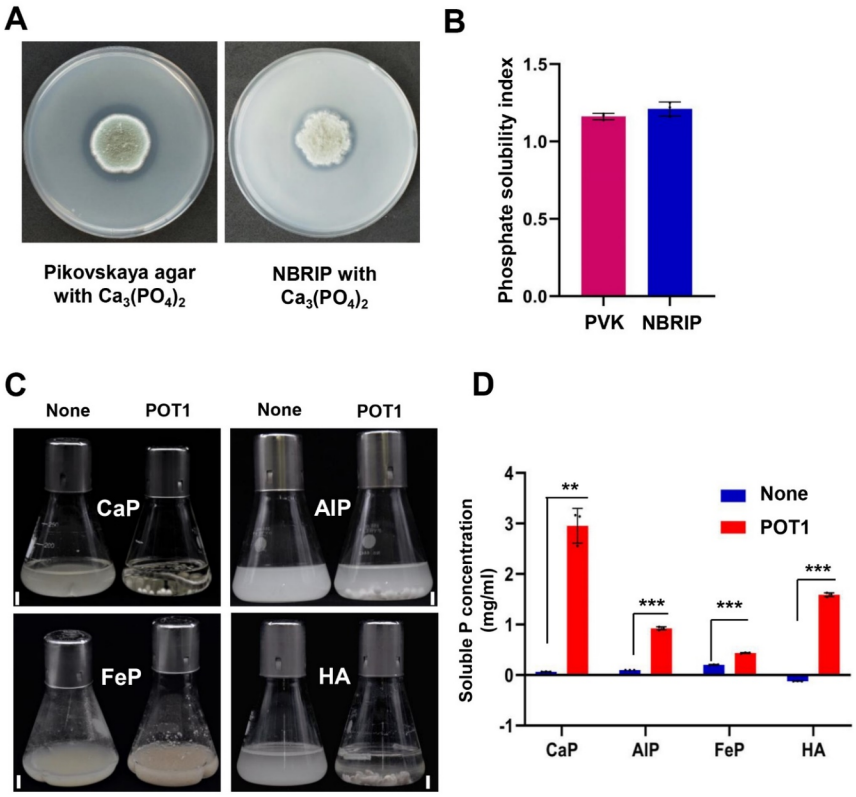

Fig. 4. Insoluble phosphate solubilization activity of POT1 under different recalcitrant P sources.

(A) POT1 producing dissolved circular zones of phosphorus when cultured on Pikovskaya agar and NBRIP media amended with CaP for seven days. (B) A bar diagram showing phosphate solubility indices of POT1 when cultured in Pikovskaya agar and NBRIP media amended with CaP for seven days. (C) Phosphate-solubilizing capability of POT1 in different inorganic phosphorus compounds such as tricalcium phosphate (CaP), aluminium phosphate (AlP), iron phosphate (FeP) and hydroxyapatite (HA) after fourteen days. Scale bar represents 1 cm. (D) Soluble P concentration produced by POT1 in different inorganic insoluble phosphates such as CaP, AlP, FeP and HA. POT1 spore suspension was inoculated in 100 mL modified Pikovskaya media amended with CaP, AlP, FeP and HA for two weeks and the soluble phosphate concentration was measured using phosphomolybdneum spectrophotometry. Every treatment included three biological replicates, each of which contained three technical replicates (n=3; unpaired two-tailed t-test, ***P<0.001; **P<0.01; *P<0.05). The error bars show standard deviation.

Fig. 5

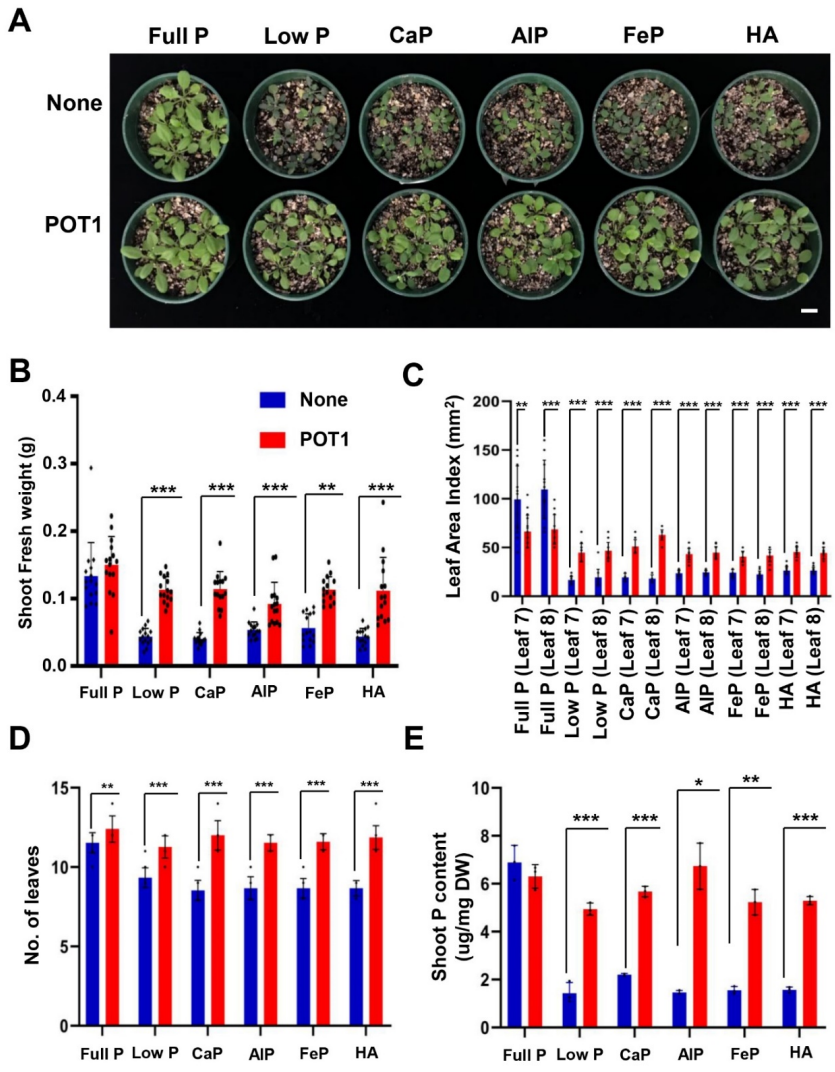

Fig. 5. POT1 promotes growth of Arabidopsis under insoluble phosphate and phosphate limiting conditions.

(A) A representative image of *A. thaliana* WT plants grown in full P, low P, CaP, AlP, FeP and HA as sole source of P with and without POT1. Ten-day-old plants were transferred to nutrient-poor vermiculite soil pre-inoculated with and without POT1 and were harvested after two weeks. The scale bar represents 1 cm. The bar diagrams represent (B) Shoot fresh weight, C) Leaf area Index, D) Number of leaves, E) Shoot phosphate content of *A. thaliana* WT plants grown in full P, low P, CaP, AlP, FeP and HA with and without POT1 in vermiculite for two weeks. The bar diagram shows combined data from fifteen independent experiments (n=15; unpaired two-tailed t-test, ***P<0.001; **P<0.01; *P<0.05). E) shoot P content of Arabidopsis plants co-cultivated with and without POT1 after four weeks. The concentration of total phosphorus content in Arabidopsis shoots were significantly increased after growth in the presence of POT1 in vermiculite soil (n=3; unpaired two-tailed t-test, **P<0.01).

Fig. 6

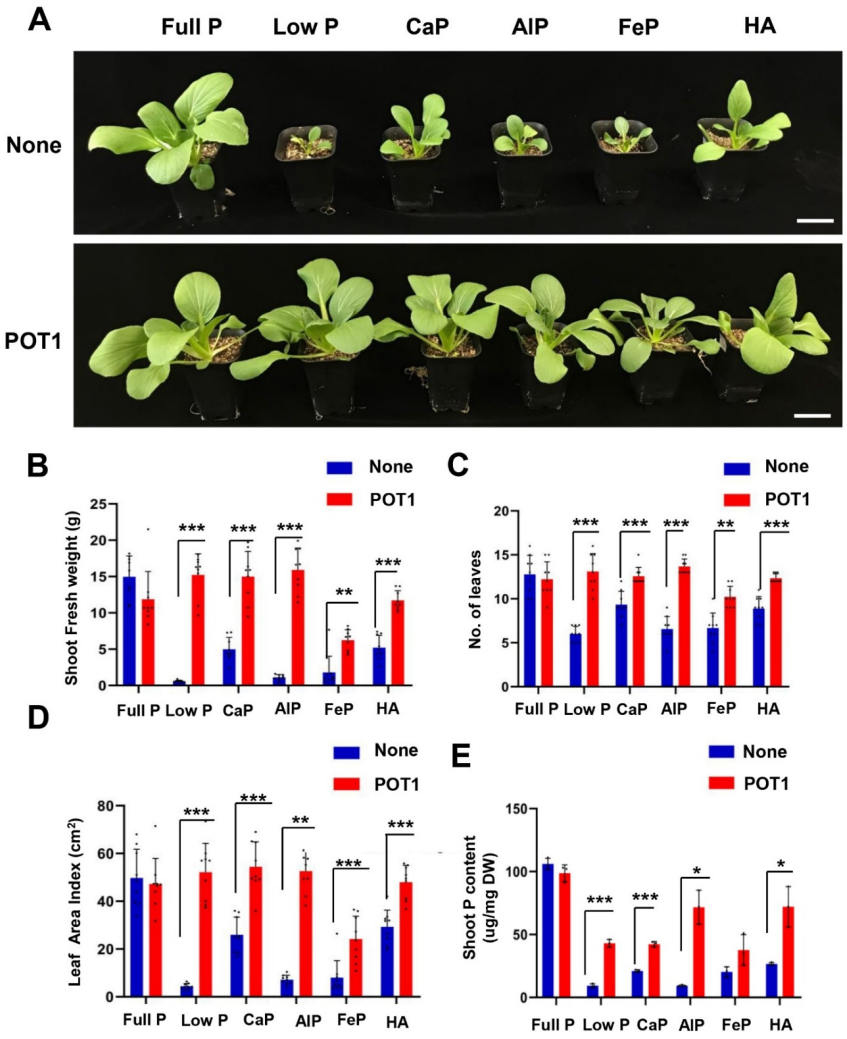

Fig. 6. POT1 promotes growth of leafy vegetable Bok Choy under insoluble phosphate and phosphate limiting conditions.

(A) A representative image of Bok Choy plants grown in full P, low P, CaP, AlP, FeP and HA as sole source of P with and without POT1. Ten-day-old Bok Choy plants were transferred to nutrient-poor vermiculite soil pre-inoculated with and without POT1 and were harvested after four weeks. Scale bar represents 5 cm. The bar diagrams show B) Shoot fresh weight, C) number of leaves D) leaf area index of 4th leaf of Bok Choy plants co-cultivated with POT1 in full P, low P, CaP, AlP, FeP and HA conditions after four weeks. The bar diagram shows combined data from nine independent experiments (n=9; unpaired two-tailed t-test, ***P<0.001; **P<0.01; *P<0.05). E) shoot P content of Bok Choy plants co-cultivated with and without POT1 after four weeks. The concentration of total phosphorus content in Bok Choy shoots were significantly increased after growth in the presence of POT1 in vermiculite soil (n=3; unpaired two-tailed t-test, **P<0.01).

Fig. 7

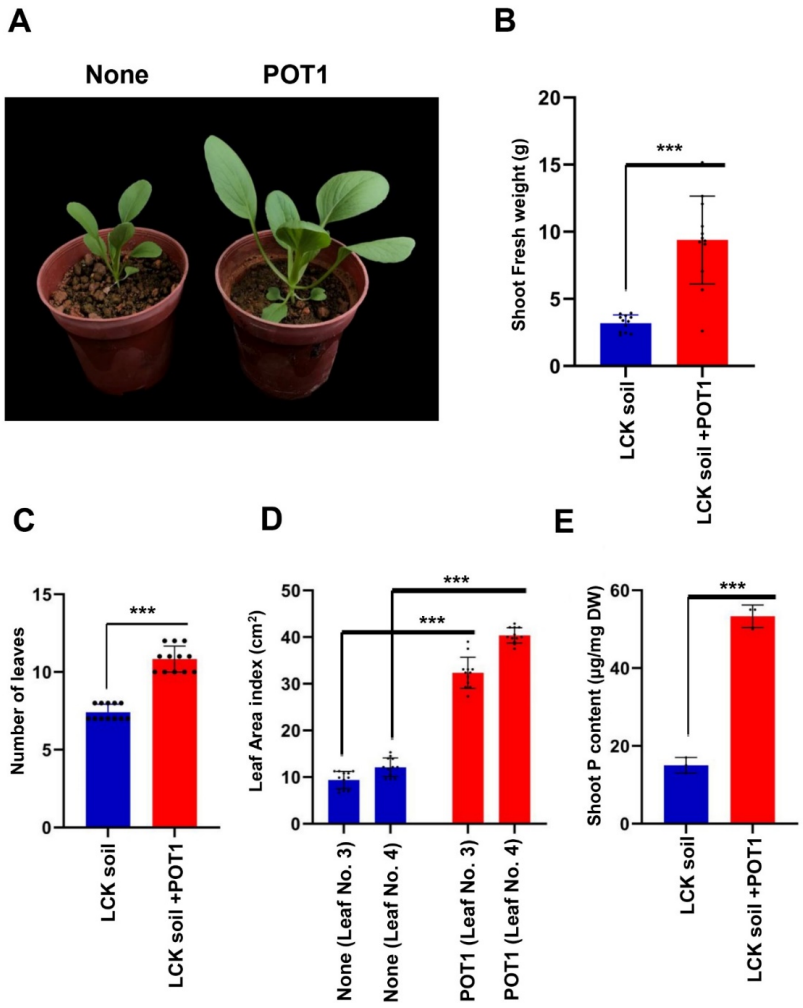

Fig. 7. POT1 promotes plant growth index of leafy vegetables in Singapore local soil.

(A) A representative image of Bok Choy plants grown in Lim Chu Kang soil with and without POT1. Ten-day-old Bok Choy plants were transferred to Lim Chu Kang soil pre-inoculated with and without POT1 and harvested after four weeks. Scale bar represents 5 cm. The bar diagrams show B) Shoot fresh weight, C) number of leaves, D) leaf area index of 3rd and 4th leaf of Bok Choy plants co-cultivated with POT1 after four weeks. The bar diagram shows combined data from twelve independent experiments (n=12; unpaired two-tailed t-test, ***P<0.001; **P<0.01; *P<0.05). (E) shoot P content of Bok choy plants co-cultivated with and without POT1 after four weeks. The concentration of total phosphorus content in Bok Choy shoots were significantly increased after growth in the presence of POT1 in LCK soil (n=3; unpaired two-tailed t-test, **P<0.01).

Fig. 8

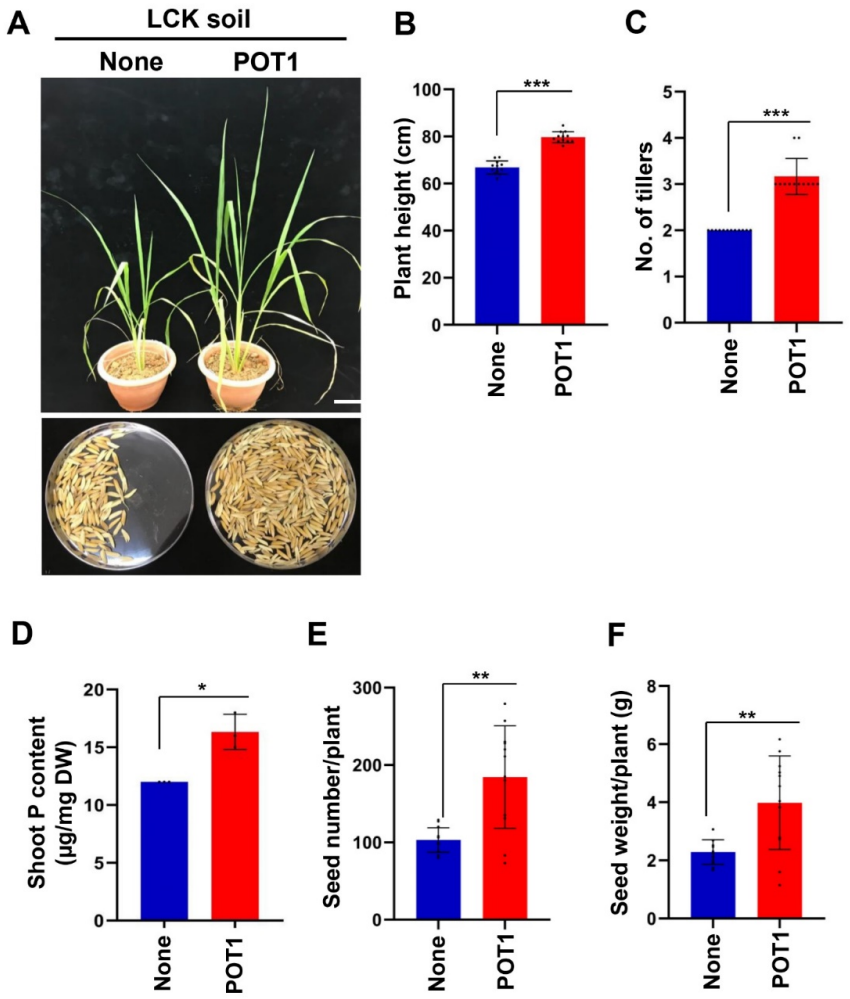

Fig. 8. POT1 promotes plant growth index of Rice in Singapore local soil.

A) A representative image of Rice grown in Lim Chu Kang soil with and without POT1 after six weeks. Scale bar represents 10 cm. Fourteen-day old Rice seedlings were transferred to LCK soil pre-inoculated with and without POT1 and were grown for three months. The bar diagrams show B) plant height, C) number of tillers, D) shoot phosphorus content after six weeks and, E) seed number per plant, F) seed weight per plant of co-cultivated with and without POT1 in LCK soil. The bar diagram shows combined data from twelve independent experiments (n=12; unpaired two-tailed t-test, ***P<0.001; **P<0.01; *P<0.05).