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PHOSPHORUS-STARVATION TOLERANCE 1 (OsPSTOL1) is prevalent in upland rice and enhances root growth and hastens low phosphate signaling in wheat

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Abstract

PHOSPHORUS-STARVATION TOLERANCE 1 (*OsPSTOL1*) is a variably present gene that benefits crown root growth and phosphorus (P) sufficiency in rice (*Oryza sativa*). To explore the ecophysiological importance of this gene, we performed a biogeographic survey of landraces and cultivars, confirming that functional *OsPSTOL1* alleles prevail in low nutrient and drought-prone rainfed ecosystems, whereas loss-of-function and absence haplotypes predominate in control-irrigated paddy varieties of east Asia. An evolutionary history analysis of *OsPSTOL1* and related genes in cereal, determined it and other genes are kinase-only domain derivatives of membrane-associated receptor like kinases. Finally, to evaluate the potential value of this kinase of unknown function in another Gramineae, wheat (*Triticum aestivum*) lines overexpressing *OsPSTOL1* were evaluated under field and controlled low P conditions. *OsPSTOL1* enhances growth, crown root number, and overall root plasticity under low P in wheat. Survey of root and shoot crown transcriptomes at two developmental stages identifies transcription factors that are differentially regulated in *OsPSTOL1* wheat that are similarly controlled by the gene in rice. In wheat, *OsPSTOL1* alters the timing and amplitude of regulators of root development in dry soils and hastens induction of the core P-starvation response. *OsPSTOL1* and related genes may aid more sustainable cultivation of cereal crops.

Alek T. Kettenburg, Miguel A. Lopez, and Kalenahalli Yogendra provided equal contributions.

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KEYWORDS

biogeography, gene history, *Oryza sativa*, phosphate nutrition, root development, sustainability, *Triticum aestivum*

1 | INTRODUCTION

To feed a growing global population the production of major grain crops must double while simultaneously increasing sustainability (Ray et al., 2013; Zhao et al., 2017). The essential nutrients nitrogen (N) and phosphorus (P) are critical for optimal growth and yield but their overuse is highly damaging to the environment (López-Arredondo et al., 2014; Yu et al., 2022). Although an estimated 18% of global crop land receives insufficient P fertilisation, approximately 46% receives an excess that leads to eutrophication of aquatic ecosystems (Carpenter 2008; Kleinman et al., 2015; MacDonald et al., 2011). These environmental challenges, along with the rising cost of P fertiliser and low bioavailability of P in many soils has made the improvement of P use efficiency of crops a critical need (Alewell et al., 2020). This might be achieved through introduction of genes that enhance plasticity of developmental or metabolic processes that limit loss of quality and yield under P or other nutrient deficiencies.

The rice (*Oryza sativa L.*) *PHOSPHORUS-STARVATION TOLERANCE 1* (*OsPSTOL1*) gene was identified in the upland *aus* landrace Kasalath and found to increase P uptake and yields in P deficient soil by promoting early crown root growth (Gamuyao et al., 2012). *OsPSTOL1* lies within an approximately 90 kb insertion-deletion (indel) region on chromosome 12 that is absent from the Nipponbare reference genome (*japonica* subspecies) (Chin et al., 2011). Introduction of the Kasalath (K) allele of *OsPSTOL1* into varieties that lack it by breeding or transgenesis confers increased tolerance to P deficiency (Gamuyao et al., 2012; Wissuwa & Ae 2001b). Surveys of small germplasm collections of rice find that *OsPSTOL1* is present in varieties grown in rainfed upland regions (Chin et al., 2011), where soil nutrient availability is generally lower and drought is prevalent (Haefele et al., 2014). *OsPSTOL1* homologues are found in *Oryza* species with AA genomes, with most maintaining both gene presence and absence alleles (Pariasca-Tanaka et al., 2014), suggesting it is under balancing selection (Vigueira et al., 2016) and therefore may be an adaptive gene.

OsPSTOL1 falls within a subfamily of receptor-like kinases (RLKs), but was designated a receptor-like cytoplasmic kinase (RLCK) due to the lack of the characteristic N-terminal signal peptide and transmembrane domains of RLKs (Gamuyao et al., 2012). Homologues of *OsPSTOL1* are recognisable in other Gramineae crops, including *Zea mays* (maize) (Azevedo et al., 2015), *Triticum aestivum* (wheat) (Milner et al., 2018), and *Sorghum bicolour* (sorghum) (Hufnagel et al., 2014). *OsPSTOL1* homologues in maize and sorghum that also lack a N-terminal signal peptide domain colocalize with quantitative trait loci for root morphology and P uptake (Azevedo et al., 2015; Hufnagel et al., 2014), suggesting that functions of *OsPSTOL1*-like genes may be conserved; yet their origins and distinctions in structure and expression remain unclear. The serine/threonine kinase activity of OsPSTOL1 was confirmed in vitro (Gamuyao et al., 2012), although its role in root plasticity and P sufficiency is an open question, whether in rice or when expressed in another monocot crop.

Here, we survey *OsPSTOL1* presence/absence and allelic variation across more than 3000 re-sequenced landrace and varietal genomes and assess the biogeographical and dispersal history of these alleles (Gutaker et al., 2020; Sun et al., 2017). We find that the K allele of *OsPSTOL1* is uncommon in paddy and control-irrigated accessions and overrepresented in those of rainfed ecosystems that are prone to drought. Wheat is also a predominantly rainfed crop, but its genes underlying P uptake and root architecture are understudied (Oono et al., 2013b; Walkowiak et al., 2020). To explore translatability of the adaptive function of *OsPSTOL1*, we examine the impact of its ectopic expression in wheat and identify increases in growth under P-replete and limiting conditions in multiple environments, including the field. Evaluation of *OsPSTOL1* effects on roots and crowns demonstrates that *OsPSTOL1* influences root architecture and yield traits. *OsPSTOL1* expression in wheat also influences transcripts of regulators of developmental plasticity that are important under drought and in low P sensing.

2 | MATERIALS AND METHODS

2.1 | Determination of genotypic, geographic, and cultivation ecosystem information of rice varieties

Haplotype information of *OsPSTOL1* and neighbouring loci were determined using vcf files and fastq files obtained from the 3000 rice genome project (Wang et al., 2018b), Rice Pan-Genome Browser (Sun et al., 2017) and Gutaker et al. (Gutaker et al., 2020). Country-of-origin, subspecies, rice growing ecosystem, and SNP group information was mined from the Rice Pan-Genome Browser (Sun et al., 2017) and (Gutaker et al., 2020). Countries with fewer than 10 varieties sampled were discounted. Regional data on rice growing ecosystem distribution were from (Haefele et al., 2014). P fertiliser application data were from (Roser & Ritchie 2013), updated in 2017. See Supporting Information: Methods for details of genome and allele evaluation.

2.2 | *OsPSTOL1* homologues, phylogenetic tree construction, and protein domain prediction

OsPSTOL1 orthologs have been reported in some *Oryza* (Neelam et al., 2017; Pariasca-Tanaka et al., 2014). To identify *OsPSTOL1*-like

genes in other species, BLASTP was performed with the K-allele of OsPSTOL1 as a query against proteomes of related species. Gaps were trimmed with trimAl (Capella-Gutiérrez et al., 2009) using default settings. The tree was constructed using RAxML (Kozlov et al., 2019), with default settings and 1000 bootstraps. Protein domains were predicted using hmmscan (Finn et al., 2011), ScanProsite (de Castro et al., 2006), and Phobius (Kall et al., 2007). See Supporting Information: Methods for more details.

2.3 | Generation of *pUbi::OsPSTOL1* wheat lines

The coding sequence of *OsPSTOL1* (*OsPUPK46-2*; BAK26566.1) of the Kasalath accession was synthesised, ligated into *pENTR/D-TOPO* (Invitrogen), validated by sequencing, and transferred by Gateway LR recombination (Invitrogen) into the T-DNA vector *pMDC99* (Curtis & Grossniklaus, 2003), which contains a *Zea mays Ubiquitin1* (*Ubi1*) promoter (JX947345.1) to drive gene near-constituive expression, and a *35 S CaMV* terminator. *Agrobacterium*-mediated transformation of wheat, cv. Fielder, with *Ubi::OsPSTOL1* was conducted (Ishida et al., 2015). Two independent *OsPSTOL1* lines (T_3–T_5) were compared to a null segregant from the T_1 generation. See Supporting Information: Methods for detail on transgene evaluation. Plasmids and wheat genetic material are available from S.R.

2.4 | Evaluation of wheat under greenhouse and field conditions

Evenly sized seeds were imbibed for 4 h and placed in the dark at 4°C for 3 days before transplanting into pots (diameter 150 mm; height 150 mm) filled with a 2.0 L of cocopeat soil (South Australian Research and Development Institute, Adelaide, Australia). Plants were grown in The Plant Accelerator (Australian Plant Phenomics Facility, University of Adelaide, Australia (−34.971353 S; 138.639933 E) with natural light and 22°C daytime and 15°C night temperatures. Grain protein content and total grain nitrogen (N) was measured using a N analyser (Rapid N exceed®, Elementar). Chlorophyll content was measured using a SPAD-502Plus (Konica Minolata Inc.).

Field evaluation was at Glenthorne Farm, South Australia (−35.056923 S 138.556224 E) between May and December 2018. T_5 null segregant seeds were used as the control. The fertilised plot (50 g m^2 High Phosphate Super Phosphate (RICHGRO) and 20 g m^2 Soluble Nitrogen Urea (RICHGRO)) was planted, managed and evaluated weekly as described (Regmi et al., 2020), with irrigation by rainfall. Blitzem Snail and Slug Pellets (Yates, Auckland, New Zealand) were applied at a rate of 5 h m^2. Biomass was measured as the average of all plants per genotype within a plot (two plots per genotype). For grain nutrient analysis, plants were divided into four groups of eight, and the grain from each group was pooled as one biological replicate and nutrients measured using microwave digestion followed by ICP-OES.

2.5 | Evaluation of wheat under controlled P nutrition

Seedlings of average size and growth were transplanted into each pot containing a pre-washed mix of 70% (v/v) #20 silica sand (Gillibrand) and 30% (v/v) Green Grades Profile™. Profile™ contains 10% (v/v) aluminium oxide, which acts as a solid-state buffer for P (Lynch et al., 1990). Prewashing with industrial tap water was performed to reduce the starting P content to 25 µM. Pots were sub-irrigated with 3 L of industrial water supplemented with additional nutrients as follows: for high [Pi] (HP), the solution contained 436 µM KH_2PO_4, and for low [Pi] (LP) this was substituted with 440 µM KCl (Table S1c). Plants were harvested at 36 and 50 DAS, representing Zadoks Stage 23 and Stage 31, respectively. Each pot (5–6 plants) was pooled as a biological replicate, with four replicates per treatment and sampling time. For evaluation of root systems, pre-germinated seeds were transplanted into PVC pipes (10.16 cm diameter, 40 cm height) filled with the prewashed mix of 70% (v/v) sand and 30% (v/v) Profile™ and drip irrigated daily. For HP, the solution contained 436 µM KH_2PO_4 and for LP, 5 µM KH_2PO_4 and 335 µM KCl. At 28 DAS, root systems were recovered. See Supporting Information: Methods for more detail. For nutrients, Pi concentration of approximately 150 mg fresh tissue was extracted (Yamaji et al., 2017) and measured by the molybdate blue colorimetric method (Murphy & Riley, 1962). Total P of 15–25 mg of dry tissue was determined by persulfate digestion using a 10 mL reaction of 5% (v/v) sulphuric acid and 6% (w/v) potassium persulfate (Jeffries et al., 1979). N in dry tissue was measured using a Flash EA1112 N analyser at the UC Riverside Environmental Sciences Research Laboratory.

2.6 | mRNA-seq library construction, sequencing, read processing, and analysis

Three biological replicates were used. TRizol (Invitrogen) extracted total RNA was processed and used to generate libraries for mRNA-sequencing as described (Reynoso et al., 2019). Sequences were aligned using hisat2 (Kim et al., 2015) and wheat, IWGSC v1.1. For publicly available RNA-seq data, reads were aligned to these genomes: *Oryza sativa* IRGSP-1, *O. rufipogon* OR_W1943, or v1.0 *O longistaminata*. For mapping transcripts to *OsPSTOL1* alleles or the transgene in wheat, the region from 5000 bp upstream to 500 bp downstream of the start and stop codons was added as a separate chromosome [GenBank AB458444.1, K-allele, N22; GenBank SAMN04568482, representative J-allele; *O. rufipogon* W0141 for *OrPSTOL1* (Zhao et al., 2018); *O. longistaminata* KN540838.1_FG002 scaffold KN540838.1: 24,114-24,980 for *OlPSTOL1*]. Alignments were visualised using the Integrated Genome Viewer browser v2.7 (Narang et al., 2010). Differentially regulated gene (DRG) transcripts were identified by use of the limma-voom package (Law et al., 2014) with raw read counts normalised with voom by the quantile method. Genes with less than 5 counts per million (CPM) in at least three samples were excluded; this included *TaPSTOL1/TraesCS5A02G067800LC*. Log_2 Fold Change (Log_2FC), adjusted *p* values

(adj.*p*.Val), and false discovery rate (FDR) were calculated. Clustering was performed (Abu-Jamous & Kelly 2018) using Z-score normalisation of Log_2FC values, followed by Gene Ontology (GO) (H Backman & Girke 2016), using the wheat GO definitions from BioMart (Smedley et al., 2009) (Ensembl Release 50). Transcription factors were subsetted using the Plant Transcription Factor Database (Jin et al., 2017). Wheat orthologs of rice genes were found using BioMart. See Supporting Information: Methods for details. Data are available at NCBI Gene Expression Omnibus, https://www.ncbi.nlm.nih.gov/geo/ under accession GSE185875.

2.7 | Statistical analyses and visualisation

The stats package of R (R Core Team 2017) was used for analysis of variance (ANOVA), linear modelling, and Chi-square Goodness of fit testing. Graphs were made with ggplot2 (Wickham 2016), ggradar (Bion 2019), and UpSet packages (Lex et al., 2014).

3 | RESULTS

3.1 | *OsPSTOL1* was lost in a subsection of rice varieties after the temperate-tropical split in *japonica*

A systematic comparison of *OsPSTOL1* alleles of the genotypes represented in the 3000 Rice Genome collection (Sun et al., 2017) identified two sub-groups (Figure 1a–c; Table S2a–d). One group has the characterised *OsPSTOL1 K*-allele of *O. sativa* Kasalath and includes accessions of widely distributed AA genome wild species, including *O. rufipogon* (Asia), *O. glumaepatula* (South America) and *O. longistaminata* (west Africa) (Stein et al., 2018). The second group has the uncharacterised *O. sativa* J-alleles that are also widely distributed in *O. barthii* (Africa), *O. glaberrima* (Africa), and *O. meridionalis* (Australia) (Pariasca-Tanaka et al., 2014; Stein et al., 2018; Vigueira et al., 2016). The widely dispersed extant descendant of the progenitor of domesticated rice, *O. rufipogon*, has both J- and K-alleles. Our analysis reveals that *OsPSTOL1* was disrupted on multiple occasions by loss-of-function mutations or interstitial chromosomal deletion. In addition to the characterised deletion of the gene in Nipponbare (Heuer et al., 2009) (henceforth the Absent haplotype), we find premature stop codon variants of the K-allele (KStop1 and KStop2) (Figures 1b,c and S1), and the *OsPSTOL1* ortholog of *O. meridionalis*. In summary, both domesticated *O. sativa* and AA genome wild *Oryza* possess J- and K-alleles, as well as allele variants resulting in truncation and gene loss (Table S2b,c) (Neelam et al., 2017; Pariasca-Tanaka et al., 2014). It is not known if the J- and K-alleles are distinct in terms of gene regulation or protein function, but a survey of transcriptomes from a panel of 38 diverse varieties (Lou et al., 2017) finds K-allele genotypes maintain statistically higher transcript abundance in both shallow and deep roots (Figure 1d).

The similar broad species distribution of *PSTOL1* presence and Absence haplotypes within *Oryza* species indicates selection for variants with high transcript accumulation as well as non-functionality including gene loss. This trans-*Oryza* genetic variation could reflect adaptive or undesirable roles in particular environments. To begin to address this, we considered *OsPSTOL1* variation based on the geographic and collection attributes of 1438 *O. sativa* landraces (Gutaker et al., 2020; Wang et al., 2018b). A total of 64% of the landraces have *OsPSTOL1*: functional J-alleles predominate in *japonica* and aromatic landraces (17%) and K-alleles predominate in *aus* and *indica* landraces (43%) (Figure 1e). Landraces with the Absent haplotype predominate in East Asia (Figure 1f).

To evaluate the provenance of *OsPSTOL1* variation, we leveraged the reconstruction of the historic distribution of landraces across Asia generated by Gutaker et al. (2020), who identified eight and six modern geographic subpopulations of *japonica* and *indica* rice, respectively (Figure 2a,b). *OsPSTOL1* presence is near ubiquitous in the genetically distinct *japonica* subpopulations, except in landraces of Laos and Taiwan/Japan (Figure 2a,c,d). Of the temperate *japonica*, the Taiwan/Japan population consists almost entirely of lowland irrigated paddy landraces, whereas a J-allele group, containing Korea/Japan landraces, is predominantly grown in upland farms, where soils are more aerobic than irrigated paddies (Kirk et al., 2014). Based on the landrace dispersal pattern, *OsPSTOL1* was lost in the temperate Taiwan/Japan lineage after the temperate-tropical split of the *japonicas*, an estimated 4100 yr BP (Figure 2d).

To clarify the origin of J-alleles in the Korea/Japan population, we tracked dispersal at the haplotype level by following nucleotide variation at the flanking loci: *OsPUPK20-2* (*LOC_Os12g26380*) and *OsPUPK67-1* (*LOC_Os12g26470*) (Figure S2a–c; Table S2f). Using a lower-case letter to designate the alleles of the flanking genes, the J-allele of Korea/Japan and Philippines are linked to *PUPK20-2-m* and *PUPK67-1-a*, whereas those of Taiwan/Japan are bordered by the more distant *PUPK20-2*-e and *PUPK67-1-h*. This indicates that the Korea/Japan J-alleles were introgressed from a tropical population that is related to the extant Philippines 2 population (Figure S2a–c), possibly through selection. Our survey also clarifies that K-alleles in *japonica* were introgressed from *indica* landraces, indicating unaccounted admixing (Figure S2a–d). All *japonica* landraces with the Absent haplotype have the *PUPK20-2-a* or *e* allele as well as *PUPK67-1-h*, characteristic of the temperate subpopulation (Figure S2a–d). This confirms the Absent haplotype of the *japonicas* originates from a single deletion event. Reflective of the high levels of admixing in *indica* accessions (Gutaker et al., 2020), we find no clear dispersal pattern of *OsPSTOL1* alleles in the *indica subspecies*, although the more highly expressed K-allele prevails in all but the China/Taiwan subpopulation (Figure 2b,e,f).

3.2 | *OsPSTOL1* gene presence prevails in rainfed and low nutrient growing systems lacking controlled irrigation

The loss of *OsPSTOL1* in the irrigated Taiwan/Japan *japonicas* and restoration by introgression in upland rainfed landraces of Korea/Japan suggests that this gene may be advantageous in specific cultivation ecosystems. To begin to address this, we considered

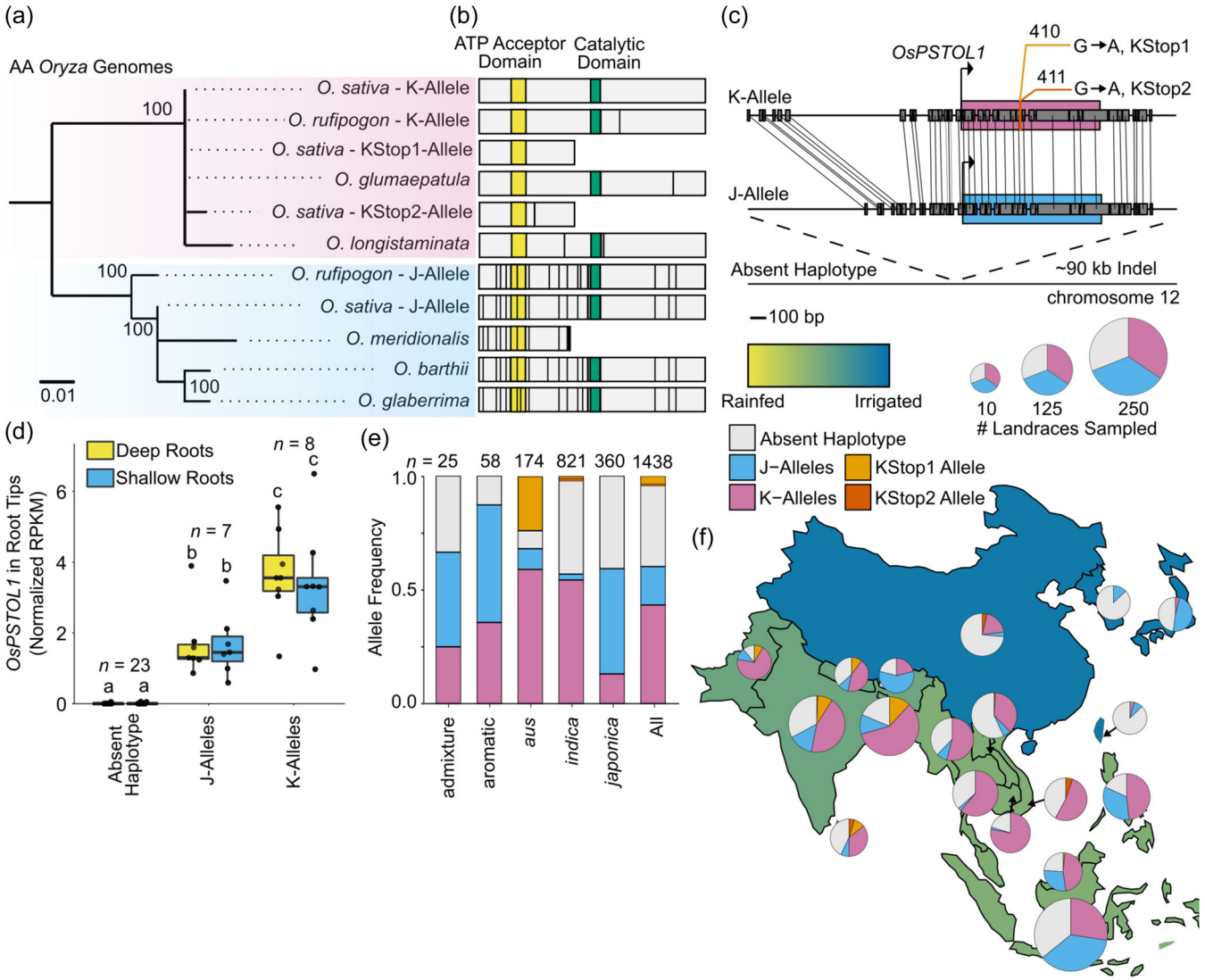

FIGURE 1 ***OsPSTOL1*** Haplotype variation and geographic distribution. (a) Phylogenetic tree based on PSTOL1 amino acid sequences within AA genome *Oryza*. The K-allele group is highlighted magenta, while the J-allele group is highlighted blue. Scale bar indicates amino acid substitutions per position. Node labels indicate the percentage of 1000 bootstraps. (b) Predicated protein models of PSTOL1 proteins within *Oryza*. Vertical black lines indicate a non-synonymous mutation relative to the K-allele of *O. sativa* (Kasalath landrace). (c) Genome alignment of *OsPSTOL1* haplotypes in *O. sativa*. Black rectangles indicate segments of common sequence. Coloured rectangles represent coding sequences. Arrow facing right indicates the start of translation. The sequence from Kasalath was used for the K-allele, that from N22 for the J-allele and that from Nipponbare for the Absent haplotype. (d) Abundance of *OsPSTOL1* mRNA in root tips of shallow and deep roots from a diversity panel of rice varieties (Lou et al., 2017). Letters indicate significant differences (ANOVA followed by Tukey's HSD, $p < 0.05$). Boxplot boundaries represent the first and third quartiles; a horizontal line divides the interquartile range, median. (e) Distribution of *OsPSTOL1* haplotypes in landraces by subspecies. (f) Geographic distribution of *OsPSTOL1* haplotypes in landraces. Pie charts are scaled to the number of landraces sampled from each country. Colour of each region indicates the proportion of cultivated rice land that is rainfed. [Color figure can be viewed at wileyonlinelibrary.com]

OsPSTOL1 distribution in landraces known to be cultivated in irrigated lowlands, rainfed lowlands, or uplands. The latter two ecosystems are more prone to drought. This identifies significant enrichment of J-alleles in *japonica* landraces from the rainfed uplands ($p < 0.05$) and depletion in lowland irrigated paddy accessions ($p < 0.05$) (Figure S3a). In *indica*, K-alleles are depleted in the irrigated lowlands ($p < 0.05$) but have similar frequency in the rainfed lowlands and uplands.

To investigate the hypothesis further, we examined the 3000 Rice Genomes that include landraces and varieties of Asian cultivated rice and considered country-based cultivation environment data. Consistent with the biogeographical survey of landraces, the lowest frequency of *OsPSTOL1* (5.92%) is found in the lowland irrigated paddy landraces of Taiwan/Japan *japonica* and their descendants (single-nucleotide polymorphism group JG7) (Figure S3b; Table S2b). The KStop2 allele is nearly exclusive to *indica* in China, consistent

with the dispersal of *indica* landraces (Figure 3a,b; Figure S3c). Cambodia, where 88% of the rice is rainfed (FAO 2011a) and droughts are common (Chhinh & Millington 2015), has the highest *OsPSTOL1* and K-allele presence within Asia (Figure 3a,b). By contrast, neighbouring Vietnam, which has the highest rate of irrigated rice in the region (FAO 2011b), has low *OsPSTOL1* presence (Figure 3a,b). Laos is exceptional, with the lowest *OsPSTOL1* presence in southeast Asia, despite cultivation of a similar percentage of irrigated rice as Cambodia (FAO 2011c). Outside of Asia, functional J and K-alleles predominate in west Africa and Madagascar, where rice is predominantly rainfed and soil nutrients are generally poor (Figures 3a,b and S3c) (Haefele et al., 2014). Yet, both functional

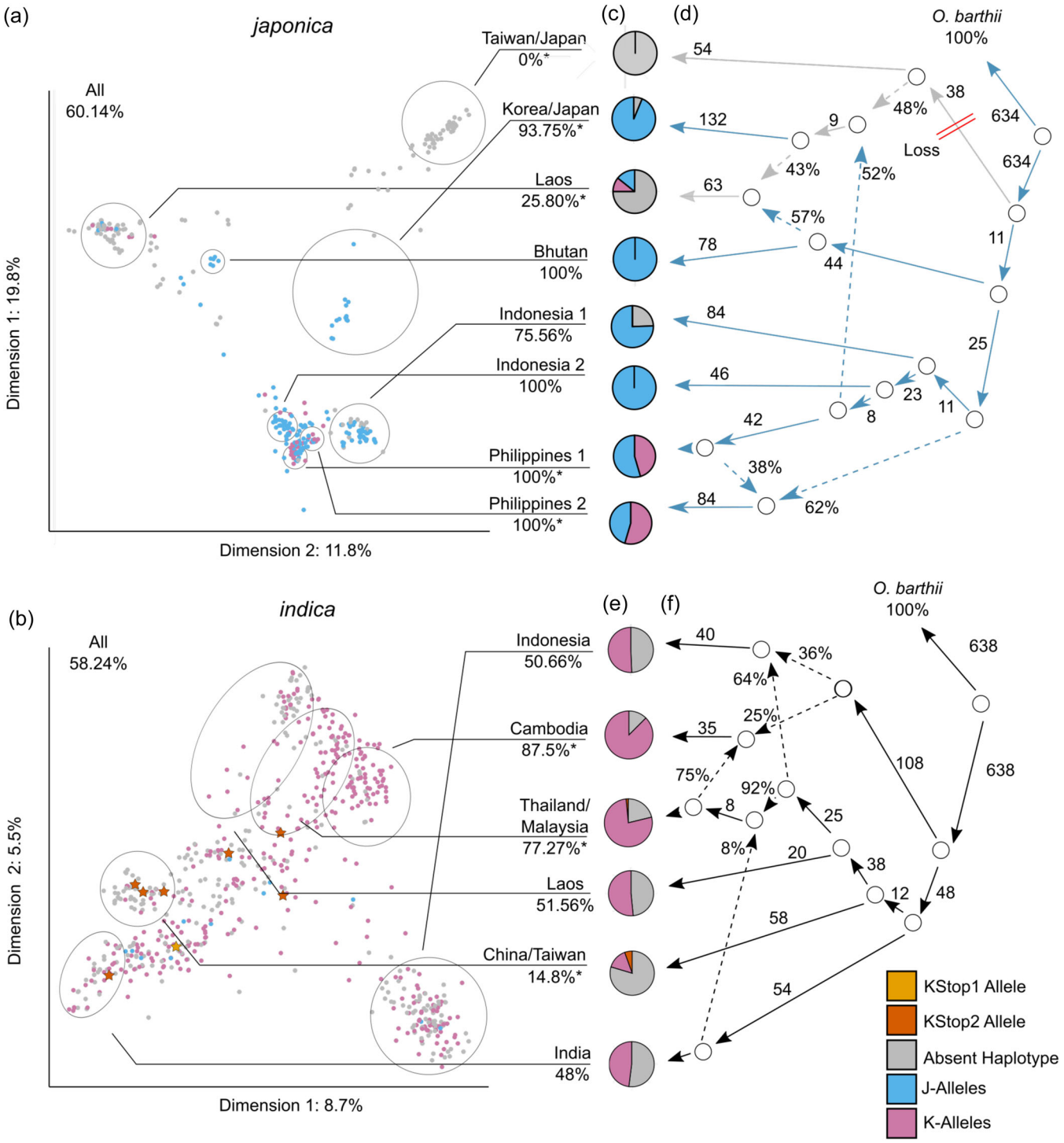

FIGURE 2 (See caption on next page)

and deletion haplotypes are found in varieties on other continents where irrigation and fertilisation are well controlled. We do not know if this distribution reflects selection or dispersal routes of rice from Asia to Europe (Spengler et al., 2021) and Asia and Africa to the Americas.

Given the somewhat mixed results in terms of cultivation ecosystem and *OsPSTOL1* allelic variation, we performed a regression analysis to quantitatively assess allele bias in rainfed versus control irrigated cultivation ecosystems. This determines that global frequency of functional K-alleles negatively correlates with the proportion of irrigated rice ($p = 0.00023$), J-alleles show no correlation ($p = 0.69$), and the deletion/loss-of-function variants (Absent, KStop1, and KStop2) show a positive correlation with irrigation ($p = 0.00087$) (Figure 3c–e). Notably, deletion/loss-of-function alleles positively correlate with P fertiliser application rate ($p = 0.038$) (Figure S4c), whereas J-alleles show a weak negative correlation ($p = 0.034$), and K-alleles show no correlation ($p = 0.42$) (Figure S4a,b). A functional *OsPSTOL1* allele may be beneficial in ecosystems where P is scarce, and drought is common.

3.3 | *OsPSTOL1-like* genes have protein domains characteristic of extracellular signal recognition kinases, yet *OsPSTOL1* and several other family members lack these domains

Next, we identified genes related to *OsPSTOL1* in other Gramineae crops based on conservation of the approximately 31 kDa kinase domain. A combined phylogenetic and protein domain analysis identified *OsPSTOL1*-like RLK and RLCK genes: 27 multi-module and four solo kinase-domain genes, including *OsPSTOL1* and *LOC_Os8g24630*, maize *Zm00001d039923* and wheat *TaPSTOL1/TraesCS5A02G067800LC* (Milner et al., 2018) (Figure 4a,b). A larger number of *OsPSTOL1*-like genes in wheat was identified using less stringent criteria (Abbas et al., 2022). The multi-module RLKs are larger (36-101 kDa) due to an N-terminal extension preceding the kinase domain. Nearly all begin with a signal peptide followed by one or more domains that bind or remodel extracellular carbohydrates. These N-terminal domains are followed by a transmembrane domain and then the kinase domain. This architecture resembles RLKs that recognise pathogen associated molecular patterns (PAMPs) and activate intracellular signalling (Cayrol et al., 2016; Zipfel 2014). Four multi-module *OsPSTOL1*-like genes of sorghum (*SORBI_3003G080700*, *SORBI_3003G263300*, *SORBI_3003G263400*, *SORBI_007G032900*) stand out for having SNPs associated with root architecture and P uptake (Bernardino et al., 2019; Hufnagel et al., 2014). Collectively, these multi-modal Gramineae genes are expressed in roots and leaves, induced by pathogens or PAMPs, and in some cases by P deficiency (Figure S5d).

Our analysis also uncovers evidence that the kinase-only PSTOL1-like RLCKs of rice and wheat arose from multi-module transmembrane signalling RLKs. The alignment of mRNA-seq reads from multiple *Oryza* (*O. sativa*, *O. rufipogon* and *O. longistaminata*) to the *OsPSTOL1* region led us to identify a 5′ exon and intron of variable length just upstream of the annotated AUG start codon (Figures 4c–f and S6c–f). Notably, this 5′ region has high sequence identity to a transmembrane domain that is in-frame with the kinase domain but apparently lacks an AUG start codon in all these species. The *OsPSTOL1 K allele* is strongly expressed in the crown region (the base of the shoot where crown roots emerge) (Figure S5e), consistent with the reported crown root primordia expression of *pOsPSTOL1::uidA(GUS)* in rice (Gamuyao et al., 2012). Although GUS staining was undetectable in other root types or shoot tissues, *OsPSTOL1* mRNA is present in shoots, developing leaves, panicles, roots, and root tips of varieties with a K-allele. This discrepancy between *in planta* GUS staining and mRNA accumulation data may reflect the region of the *OsPSTOL1* K-allele used to drive *uidA*, which based on our revised annotation consists of 272 bp of promoter, followed by exonic and intronic sequence. This is strong evidence that *OsPSTOL1* arose from a gene encoding a membrane-anchored kinase.

FIGURE 2 Distribution of *OsPSTOL1* in discrete geographic subpopulations of rice landraces. *OsPSTOL1* SNP variation data are superimposed onto a multidimensional scaling analysis by geographic region and deduced ancestries of 225 *japonica* and 690 *indica* landraces exactly as reported by Gutaker et al. (2020). (a and b) *japonica* (a) and *indica* (b) landraces projected onto the first two dimensions after multidimensional scaling of genomic distance data. Colours indicate *OsPSTOL1* alleles. KStop1- and KStop2-alleles are indicated as stars, whereas other alleles as circles. (a) Gutaker et al. (2020) clustered *japonica* genotypes using *k*-medoids ($k = 9$ subpopulations) and filtered using silhouette parameters, which resulted in $k_d = 8$ discrete subpopulations, contained within grey circles. Taiwan/Japan accessions are cultivated under irrigated lowland conditions. Subpopulation names of correspond to predominant geographic location of individual accessions in each subpopulation. (b) Gutaker et al. (2020) clustered the *indica* genotypes using k-medoids ($k = 7$ subpopulations) and filtered resulting in $k_d = 6$ discrete subpopulations contained within grey circles. (c and e) Pie charts representing the allelic composition of each discrete subpopulation of *japonica* (c) and *indica* (e) subgroups. Percentages indicate the frequency of full J- and K-alleles within each population. An asterisk indicates the frequency of *OsPSTOL1* presence alleles is significantly different than expected by *Chi*-square test ($p < 0.05$). (d) (Gutaker et al., 2020) admixture graph for the $k = 9$, $k_d = 8$ *japonica* subpopulations, rooted with *Oryza barthii* as an outgroup. *PSTOL1* is found in all *O. barthii* varieties surveyed (Pariasca-Tanaka et al., 2014). Graph represents topology that is consistent between models for all lower values of *k*. Two red slashes indicate the predicted loss of *OsPSTOL1* in the temperate lineage. Blue arrows indicate predicted routes of dispersal of the J-allele group, while grey arrows represent predicted routes of dispersal of the Absent haplotype. (f) (Gutaker et al., 2020) admixture graph for $k = 7$, $k_d = 6$ indica subpopulations of (c), rooted with *O. barthii* as an outgroup. (d) and (f) Solid arrows represent the predicted uniform ancestries, associated values are estimated scaled drift (f_2) and dashed lines represent mixed ancestries, with estimated proportion of ancestry as a percent. [Color figure can be viewed at wileyonlinelibrary.com]

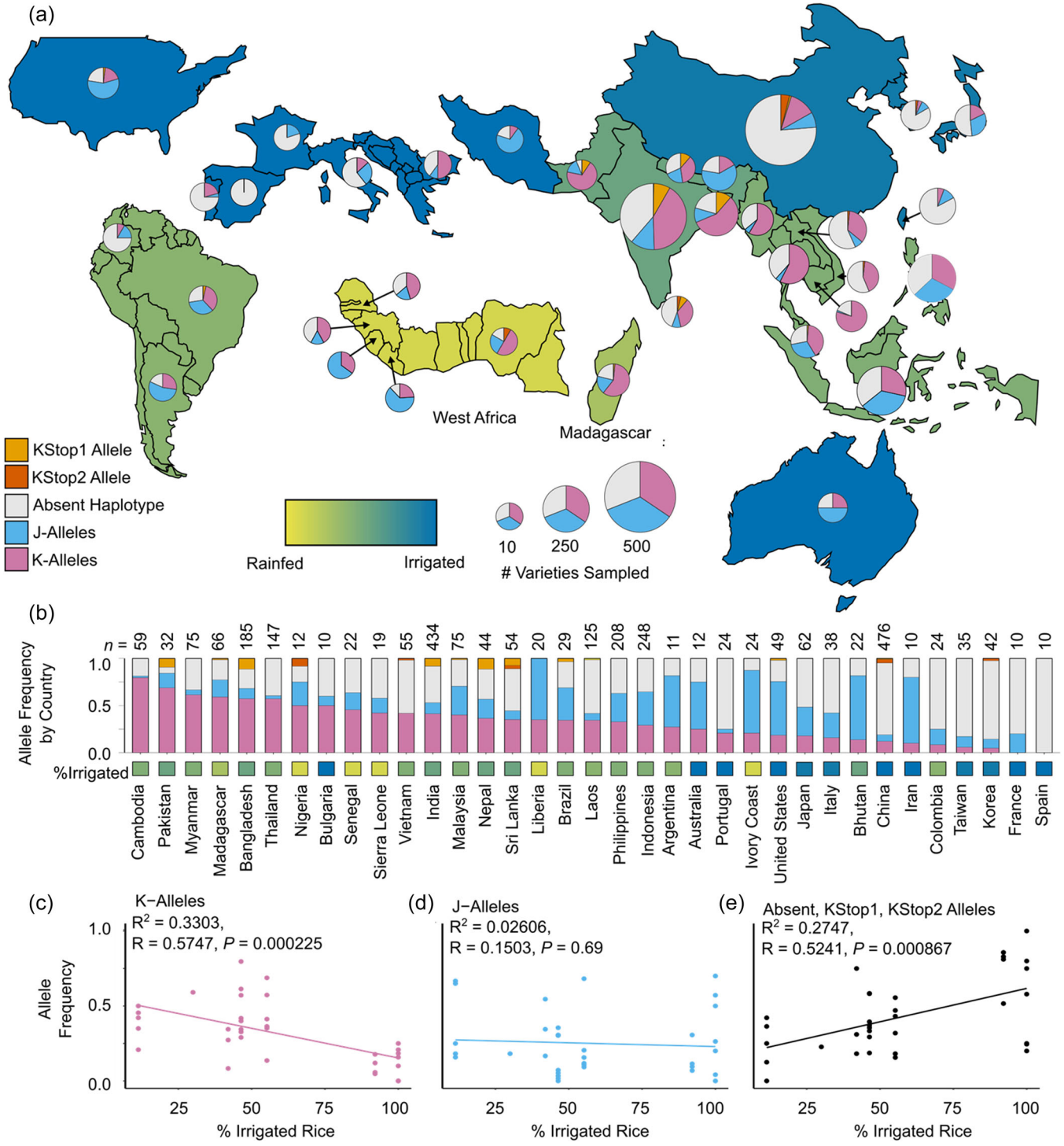

FIGURE 3 Geographic distribution of *OsPSTOL1* in the 3000 rice genomes data set. (a) and (b) Distribution of *OsPSTOL1* haplotypes based on the country where the variety was collected. Size of pie charts is proportional to the number of varieties sampled. Colour of regions indicates the proportion of cultivated rice land that is irrigated relative to rainfed (lowland or upland). (c–e) Correlation between the frequency of K-alleles (c), J-alleles (d), and absent haplotype (e), and the proportion of irrigated rice in a country (Haefele et al., 2014). R^2, R, and p-value, and best-fit line calculated using linear regression. [Color figure can be viewed at wileyonlinelibrary.com]

Consistent with the conclusion that OsPSTOL1 and related RLCKs are derived from RLKs, the single-copy wheat gene *TaPSTOL1* lies directly downstream from a GH18 domain-encoding gene. Notably, a GH18 domain remains contiguous with the kinase domain in the *TaPSTOL1* homologue of *Triticum urartu* (*TRIUR3_01194*), the AA genome progenitor of bread wheat (Figure S6a,b). *TaPSTOL1* overexpression and knockdown by RNAi influences root and shoot growth, flowering time, but not P use efficiency (Milner et al., 2018).

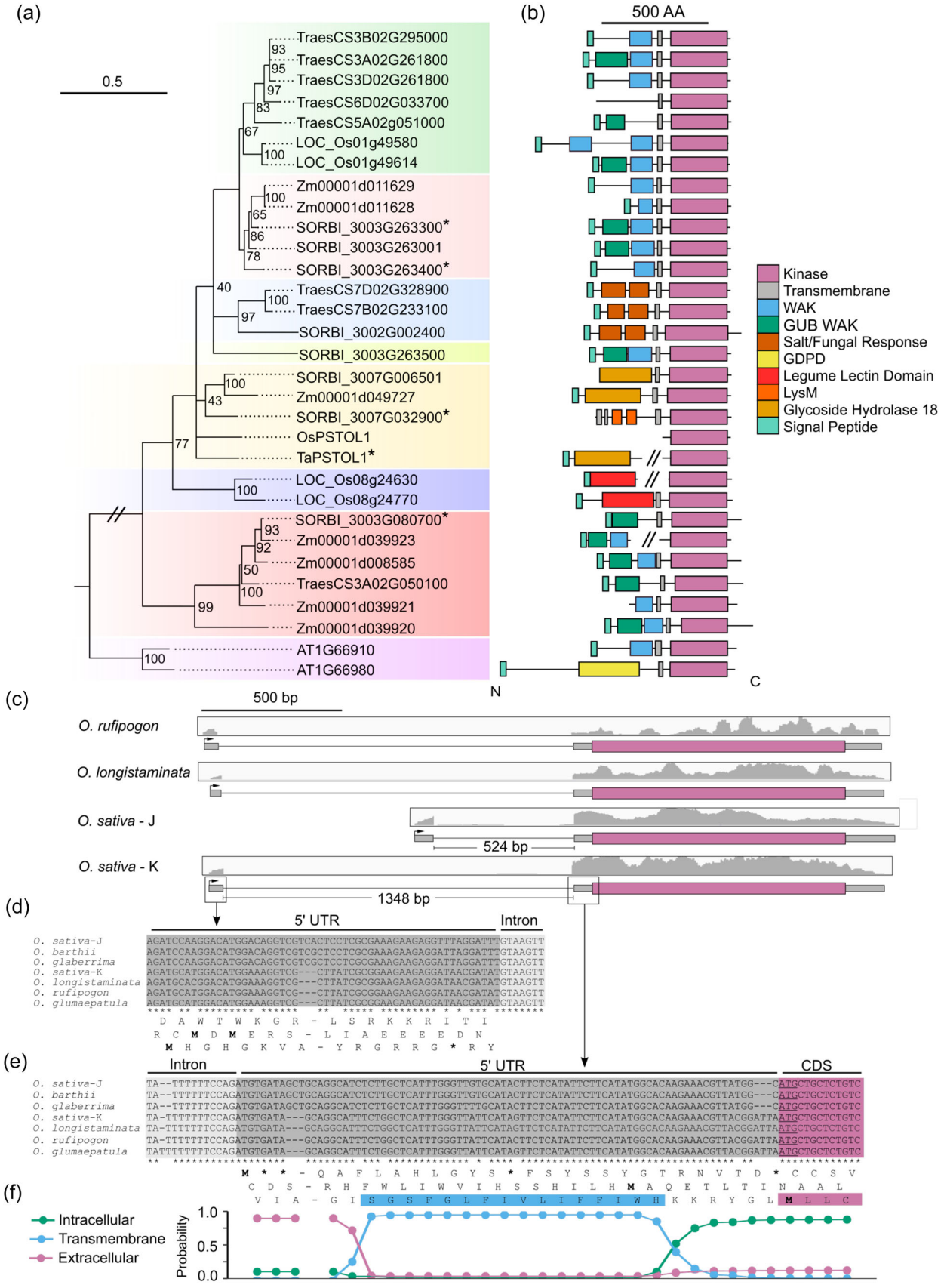

FIGURE 4 (See caption on next page)

Although *pTaPSTOL1*::*uidA* transcriptional fusions display GUS staining in root hairs and tips (Milner et al., 2018), *TaPSTOL1* transcripts are below the threshold of detection in the Wheat Transcriptome Atlas (Ramírez-González et al., 2018) (Figure S5b), suggesting its expression and activity may be limited to specific cells, conditions, or accessions. Unlike the situation of gene presence and absence found for *OsPSTOL1*, *TaPSTOL1* appears to be maintained as an intact single-copy gene on chromosome 5A in bread wheat, as confirmed by targeted gene amplification or genome resequencing (Bayer et al., 2022; Milner et al., 2018). As *OsPSTOL1* has agronomic benefits in rice and related proteins of sorghum and wheat influence an overlapping set of traits, we hypothesised that *OsPSTOL1* expressed in wheat may be valuable under standard and low P cultivation.

3.4 | Ectopic expression of *OsPSTOL1* in bread wheat benefits performance traits

To test our hypothesis, we established transgenic lines (cv. Fielder) that express the *OsPSTOL1* cDNA under the control of the near-constitutive maize *Ubiquitin1* (*Ubi*) promoter. Following initial screening of transgenics, the growth and performance of two independent events (*pUbi*::*OsPSTOL*1.1 and 1.2) was evaluated relative to a null segregant (Null) in the greenhouse and a rainfed field trial under replete P conditions (Figure 5). In the greenhouse, we observe a significant benefit in growth and reproduction phenotypes of the transgenics, including greater shoot biomass ($p < 0.04$), grain yield ($p < 0.011$), and earlier flowering ($p < 1.04 \times 10^{-7}$) (Figures 5b and S7a,b,f). *OsPSTOL1* wheat displays more rapid early growth, reaching maturity before the null segregant (Figure 5a). Other traits including 1000 grain weight, grain protein content, total grain nitrogen, heads, tillers, height, and leaf chlorophyll content, were increase in only a single transgenic or unaffected (Figures 5 and S7a–l). These results demonstrate ectopic *OsPSTOL1* provides an overall benefit to production without an impact on seed quality traits.

Next, the lines were evaluated in a rain-fed wheat field trial under standard cultivation (P replete) to discern any variation in performance. The results demonstrate a trend of increased shoot biomass (11%–16%) and seed number (6%–9%) for both transgenics in each replicated plot without an effect on 1000 grain weight (Figures 5c and S8a–f). The 13% increase in grain yield of PSTOL1.1, although not different from the null at $p < 0.05$, exceeds the 1% yield gain per annum typically gained by breeding (Dixon et al., 2009; Rahman et al., 2021; Reynolds et al., 2021; Tester & Langridge 2010). Grain nutrient content was not significantly affected (Figure S9). Above average rainfall during vegetative growth followed by below average rainfall just before and at the heading (Figure S10) or variability in the soil microenvironment may have contributed to the trait variability between individual plants and lack of statistical significance. These data indicate that *OsPSTOL1* improves agronomic traits in wheat, but transgenic event by environment interactions influence the overall benefit.

FIGURE 4 *OsPSTOL1* has 5' non-coding sequence resembling the transmembrane domain of other ***OsPSTOL1*-like genes.** (a) Phylogenetic tree of *OsPSTOL1*-like kinase proteins in rice *(Oryza sativa, Os)*, sorghum (*Sorghum bicolour, SORBI*), maize *(Zea mays, Zm)*, bread wheat *(Triticum aestivum, Traes)*, and *Arabidopsis thaliana (At)*. Alignment was based on the amino acid sequence of the kinase domain of the K-allele. For genes with multiple transcript isoforms, the longest deduced protein model was used. Node labels indicate the percentage of 1000 bootstraps. *TaPSTOL1* (TraesCS5A02G067800LC/Traes_5AS_AA3DC6A5F). *OsPSTOL1* K-allele used (BAK26566.1). Scale bar indicates amino acid substitutions per position. The tree was constructed by the maximum likelihood method. *SORBI_3003G080700*, *SORBI_3003G263300*, *SORBI_3003G263400*, and *SORBI_007G032900* were formerly annotated as *Sb03g006765*, *Sb03g0031680*, *Sb03g031690*, and *Sb07g002840*, respectively (Bernardino et al., 2019; Hufnagel et al., 2014). Genes marked with an asterisk influence growth or development. **b**, Protein models of *OsPSTOL1*-like proteins. Two diagonal lines between two separate segments indicate the aligned kinase domain was downstream of an annotated gene that has high sequence identity to genes near it on the phylogenetic tree. WAK, wall-associated receptor kinase; GUB WAK, wall associated receptor kinase galacturonan-binding (Kanneganti & Gupta 2008; Kohorn & Kohorn 2012); GDPD, glycerolphosphodiesterase; LysM and glycoside hydrolase 18 (GH18) legume lectin domains involved in recognition of carbohydrates from microbes (Lannoo & Van Damme 2014; Mesnage et al., 2014; Wang et al., 2019). The proteins with N-terminal domains are receptor-like kinases, whereas OsPSTOL1 and others lacking these N-terminal domains are classified as receptor-like cytoplasmic kinases (Gamuyao et al., 2012). (c) Alignment of RNA-seq reads from publicly available datasets to the K-allele of *OsPSTOL1* locus in *O. rufipogon* and *O. longistaminata*, and the J- and K-alleles of *O. sativa*. mRNA-seq derived from root tissue was used for *O. rufipogon*, *O. longistaminata* and the K-allele of *O. sativa*. RNA-seq derived from the crown was used for the J-allele group of *O. sativa*. Gene structure illustrated as a magenta box for the predicted coding sequence, grey box indicates the predicted 5' untranslated region (UTR), and a black horizontal line for an intron deduced from RNA-sequencing reads are supported by canonical intron splice donor and acceptor sites. RNA sequence reads were visualised with a genome browser and reads mapped in grey within a black horizontal box, placed above the gene structure diagram. (d and e) Nucleotide sequence alignment of exon 1 and the 5′ end of the intron positioned within the 5′ UTR (**b**) and the 3′ end of the intron and 5′ end of exon 2 that precedes the start codon (c) of *OsPSTOL1*. Translation of the three reading frames for *O. sativa* (Kasalath) is shown. * indicates stop codon. Blue highlight indicates the putative transmembrane domain sequence now within the 5′ UTR of the gene. Magenta highlight indicates the start of the translated PSTOL1 protein. (f) Transmembrane domain prediction calculated using Phobius for the amino acid sequence in the third frame of (e). *OsPSTOL1* mRNA translation is predicted to initiate downstream of this domain, given the absence of any 5′ AUG or alternative start codon. See Table S1a for details of the data sets and accessions surveyed. [Color figure can be viewed at wileyonlinelibrary.com]

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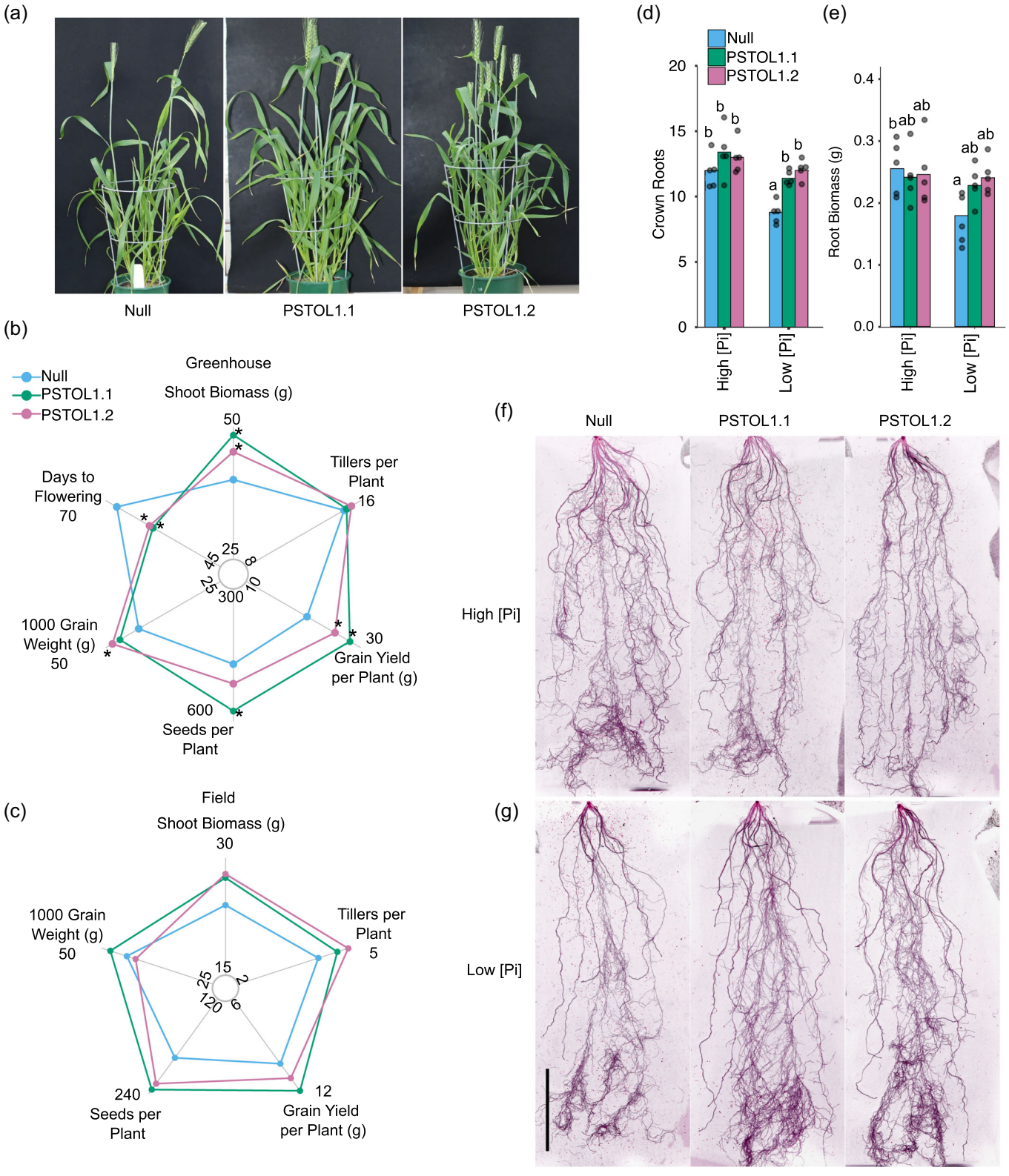

FIGURE 5 (See caption on next page)

3.5 | *OsPSTOL1* effects are influenced by P nutrition and developmental stage

Given the performance of PSTOL1.1 and PSTOL1.2 lines under replete P conditions, we sought to determine their performance under P deficiency under controlled greenhouse conditions. We chose to manipulate P availability using a buffered aluminium oxide and sand mixture, shown to more accurately simulate low P stress than unbuffered hydroponics and provide greater reproducibility than comparisons with P replete and deficient soil (Hanlon et al., 2018; Vejchasarn et al., 2016). Both lines develop more crown roots and maintain greater root biomass compared to the null segregant under low Pi nutrition (LP, 25 µM), but are indistinguishable when grown under replete P (HP, 436 µM) (Figure 5d–e). P depletion enhances fine root development in all three genotypes at 36 days after sowing (DAS), but this was visibly more pronounced in the transgenics (Figure 6a,b). By 50 DAS under LP, shoot fresh biomass trends higher in PSTOL1.1 and is significantly higher in PSTOL1.2 (Figure 6d). Neither P nor N content are statistically distinguishable between genotypes, although both transgenics trend higher in P (30%) and N (8% [PSTOL1.1] and 29% [PSTOL1.2]) (Figure 6d,e). P content and concentration are lower under LP and decline with age, presumably due to incorporation into shoot tissues, but are indistinguishable between genotypes (Figure S11a–d). The slight difference in phenotypes of the two transgenic lines may reflect distinction in the level of the *OsPSTOL1* mRNA in root and crown tissue (Figure S12) or spatial-temporal variation in gene activity determined by the transgene insertion site.

The root and crown transcriptomes of the three lines were profiled by RNA-seq for the two conditions at 36 and 50 DAS in biological triplicate. Multidimensional scaling plots demonstrate separation by developmental stage, P availability and genotype (Figure S14a,b). Even at this whole transcriptome scale the root transcriptome of the transgenics and Null separate under LP and HP at 36 DAS (panel a, Dimension 2) and in crowns under LP at 50 DAS (panel b, Dimension 2). A total of 1751 elevated and 1658 reduced differentially regulated genes (DRGs) are shared by the two independent transgenic events ($p < 0.05$, $|\log_2 FC| \geq 1$) across the tissues, stage and treatments (Table S3a). Therefore, we sought genes that were consistently distinguishable in the transgenics relative to the null segregant, identifying a greater number of DRGs under LP than HP, with the maximum number in the crown transcriptomes after prolonged cultivation under LP (50 DAS) (Table S3b). This is consistent with greater distinctions in phenotypic traits at 50 DAS. A clustering analysis of the DRGs exposes the degree of uniformity in *OsPSTOL1* effect in the independent transgenic events, as well as distinctions between condition and stage (Figure 6f,g).

To consider common DRGs in both wheat and rice OsPSTOL1 transgenics, we compared the wheat data to the microarray data set generated from *35 S::OsPSTOL1* IR64 rice and its null segregant under low P nutrition (Gamuyao et al., 2012). Of the 23 DRGs identified in rice, 11 correspond to wheat orthologs of DRGs in OsPSTOL1 wheat under at least one condition (Figure S14 and Table S3c). Orthologs of the transcription factor *DELAY OF ONSET OF SENESCENCE* (*OsDOS*) that limits senescence, S-adenosyl methionine decarboxylase (*SAMDC2*) that is involved in polyamine biosynthesis, and several others stand out as similarly regulated in OsPSTOL1 transgenics of both species, indicating similar downstream effects.

3.6 | Transcription factors related to root development, drought stress, and flowering are differentially regulated in roots and crowns of OsPSTOL1 wheat

Transcription factors were subset from the DRG clusters to gain insight into developmental or environmental response regulation conferred by *OsPSTOL1*. Due to limited study of transcription factors in wheat, we focused our analysis on the known function of the orthologous genes in rice. A number of the DRG are transcription factors associated with root development under standard or water deficit conditions (Figures 6f,g and S15a,b; Table S3d,e). These include orthologs of *OsHOX12/14*, involved in vasculature development (Shao et al., 2018), with *OsHOX12* mRNA enriched in cells of the quiescent center and developing protoxylem (Reynoso et al., 2022). Another example is *OsNAC122/10* associated with root diameter and drought tolerance (Jeong et al., 2010). Homologues of *OsCRL1*, involved in lateral root and crown initiation (Inukai et al., 2005), are downregulated in OsPSTOL1 roots at 36 DAS but upregulated in the crown at 50 DAS. In sum, the transgenics differentially regulate numerous mRNAs associated with root initiation.

FIGURE 5 *OsPSTOL1* in wheat can enhance growth in greenhouse and field. (a) Representative images of two independent *pUbi::OsPSTOL1* transgenic lines and a null segregant at Zadoks stage 65. (b) and (c) Yield traits of *pUbi::OsPSTOL1* transgenic lines and their null segregant in a greenhouse (b) and a field (c) under nutrient replete conditions. For the field, plants were divided into two groups and the average biomass of each group was recorded. The biomass reported is the average of the two plots. Greenhouse: Null, $n = 10$; PSTOL1.1, $n = 7$; PSTOL1.2. Field: Null, $n = 24$; PSTOL1.1, $n = 22$; PSTOL1.2, $n = 21$. (d) Crown root number and **e**, root biomass of the two independent *pUbi::OsPSTOL1* transgenic lines and their null segregant at 28 DAS cultivated under high and low [Pi] conditions in 40 cm tall pots ($n = 5$) in the greenhouse. (f) and (g) Representative images of the roots of two independent *pUbi::OsPSTOL1* transgenic lines and their null segregant at 28 DAS grown in high (f) and low (g) [Pi] conditions in 40 cm tall pots. Colour has been inverted for visualisation. Scale bar, 10 cm. Means significantly different between genotypes are indicated by different letters ($p < 0.05$, analysis of variance (ANOVA) with Tukey's honest significant difference (HSD) test). [Color figure can be viewed at wileyonlinelibrary.com]

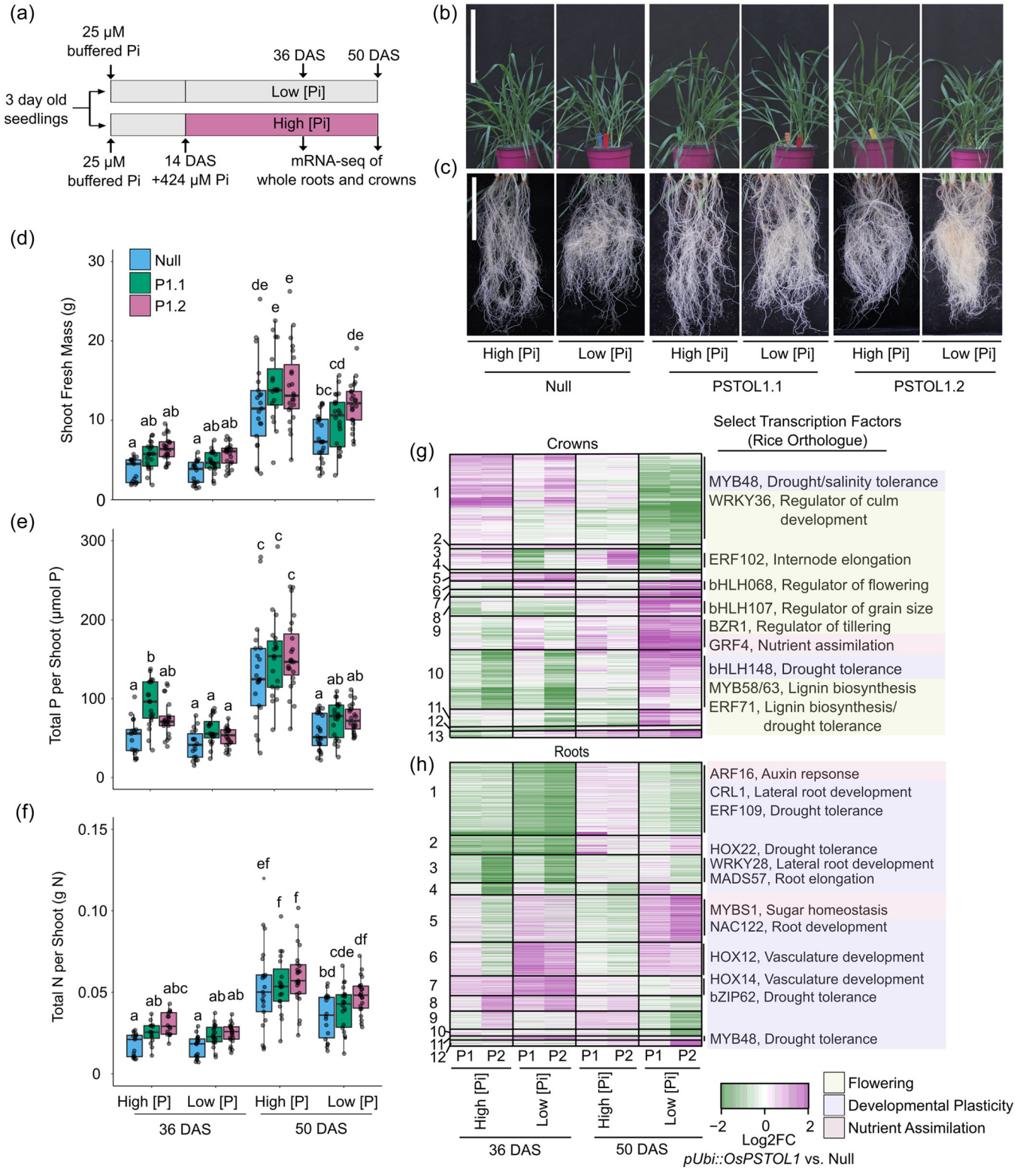

FIGURE 6 (See caption on next page)

Other DRGs encode Sugars Will Eventually Be Exported Transporters (SWEET), Trehalose-6-phosphate synthases (TPS) and phosphatases (TPP) that regulate the allocation of carbohydrates from photosynthetic source organs to sink tissues, such as roots, crowns or developing seed (Paul et al., 2018) (Figure S16; Table S3f). *SWEET* mRNAs are higher in the transgenic roots under HP particularly at 50 DAS, suggesting there may be greater resource allocation to continue root development. Thus, OsPSTOL1 may directly or indirectly influence the partitioning of carbohydrates from source leaves to roots.

OsPSTOL1 transgenics showed small but beneficial alterations in a number of developmental traits including a reduction in days to flowering coupled with greater shoot biomass and grain yield (Figure S7a,b,f). Indeed, OsPSTOL1 significantly influences transcript levels of orthologs of a number of developmental regulators in both transgenics (Figures 6f,g and S15). These include wheat orthologs of *OsbHLH068*, *OsBZR1*, *OsGRF4*, Os*HOX12*, and *OsbHLH107*, associated with internode elongation, tillering, nutrient assimilation and yield (Li et al., 2018), flowering, and panicle development in rice (Chen et al., 2017; Gao et al., 2016; Shao et al., 2018; Tong et al., 2009; Yang et al., 2018). The differential regulation of genes involved in reproduction are of interest as P deficiency typically delays flowering in rice and wheat (Rodríguez et al., 1998; Ye et al., 2019). The DRGs include several orthologs of genes implicated in ABA signalling and drought resilience, most of which show strong upregulation at 50 DAS in crowns. The elevation of these regulators could reflect the promotion or an advancement in development by ectopic OsPSTOL1 expression. Of particular note is the down-regulation of orthologs of *OsARF16* and *OsWRKY28*, involved in changes in root architecture under P deficiency (Shen et al., 2013; Wang et al., 2018a). This led us to consider that signatures of P sensing or mobilisation may be altered in OsPSTOL1 transgenics.

3.7 | Evolutionarily conserved core low P response pathway is induced earlier in P-starved OsPSTOL1 wheat

The prior DNA microarray analysis of *35S*::*OsPSTOL1* and *OsPSTOL1* near isogenic lines of rice provided no indication of an effect on P sensing or response after prolonged P deficiency (Gamuyao et al., 2012; Pariasca-Tanaka et al., 2009). Taking advantage of the inventory of low P responsive genes of rice (Oono et al., 2013a), we observe progressive elevation of the orthologous low marker genes in roots at 36 DAS, followed by greater induction at 50 DAS in roots and crowns (Figure S17a,b; Table S3g,h). A subset of these mRNAs rises early in transgenic roots at 36 DAS (clusters 4, 8, and 9, Figures 7a and S17b). The precocious elevation of these transcripts led us to systematically analyze genes in the low P signalling and response pathway (Figure 7). Our survey starts with the PHR MYB-type transcription factors that control downstream low P responses. We observe that *PHR3* orthologs are elevated in roots at 36 DAS, consistent with upregulation of downstream low P response genes. However, PHRs are tightly controlled by SPXs, which post-translationally repress their activity (Poirier 2019; Wang et al., 2009). Early and pronounced upregulation of *SPX*s suggests that PHR upregulation is counterbalanced in the transgenics. PHR activity also serves to limit levels of P transporters. PHRs upregulated *IPS1*, a noncoding RNA that acts as a molecular sponge of *miR399* to limit accumulation of *PHO2* (*PHOSPHATE 2*) mRNA, encoding an E3 ligase that catalyzes the degradation of the P transporters. The greater reduction in *PHO2* mRNA, despite a significant elevation of *IPS1* particularly in PSTOL1.2, suggests that either the level or spatial distribution of *IPS1* is insufficient to curb *miR399* activity. It also suggests that turnover of P transporter may be reduced in the transgenics.

These data indicate both elevation and dampening of the typical P-starvation response in the transgenics. On one hand, PHR-regulated genes are associated with P recycling and remobilisation are significantly elevated at 36 DAS in both lines (Figure 7; Table S2h). These include enzymes of sulfolipid and galactolipid metabolism reported to be low P-induced in wheat (Oono et al., 2013b; Walkowiak et al., 2020). Remarkably, there is precocious activation of a low P response signature in roots growing under P replete conditions at 36 DAS in the PSTOL1.2 line. This includes significant precocious elevation of 10 P transporter mRNAs (Figure S17c). On the other hand, despite the changes in *PHR* mRNAs and the *miRNA399-IPS1-PHO2* module components at 36 DAS, mRNAs encoding many known P transporter orthologs are not differentially regulated under LP in the wheat transgenics as

FIGURE 6 *OsPSTOL1* modestly modifies developmental and P nutrition effects on shoot phenotypes and transcriptional regulator mRNA levels in the shoot crown and roots. (a) Schematic of the experiment. Three day-old seedlings germinated on plates were transferred to sand culture with 25 μM buffered Pi. Fourteen days after sowing (DAS) high [Pi] (HP) pots were watered every 2 days with fertiliser containing 424 μM Pi, whereas low [Pi] LP pots were watered with fertiliser containing 0 μM Pi. Root systems and crown regions were harvested at 36 and 50 DAS for mRNA-seq. (b and c) Representative photos of shoots and roots at 36 DAS. Scale bar, 10 cm. (d–f) Shoot fresh mass (d), total shoot P content (e), and total shoot N content (f) from the mRNA-seq experiment tissue; $n = 20$. Means significantly different between genotypes are indicated by different letters ($P < 0.05$, analysis of variance (ANOVA) with Tukey's honest significant difference (HSD) test) Boxplot boundaries represent the first and third quartiles; a horizontal line divides the interquartile range, median. (g) and (h) Heatmaps showing differentially regulated genes (DRGs; $p < 0.05$, log2FC | ≥ 1) of at least one condition, DAS, tissue or genotype between *pUbi*::*OsPSTOL1* transgenic line and the null segregant in crowns (g) and roots (h). P1, PSTOL1.1; P2, PSTOL1.2. Clusters were generated with the Clust package. Selected transcription factors within individual clusters are identified by the rice ortholog name at right. [Color figure can be viewed at wileyonlinelibrary.com]

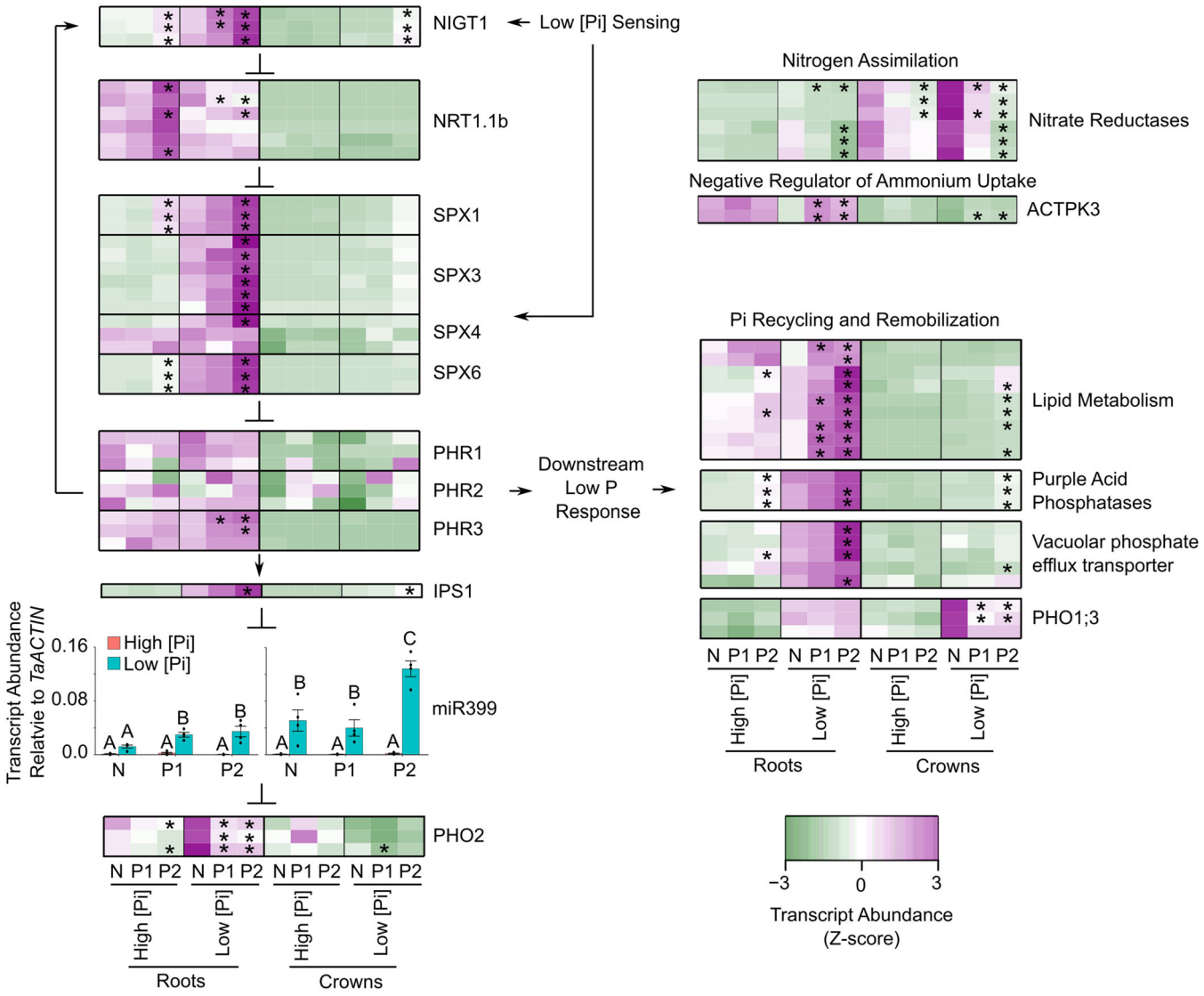

FIGURE 7 *OsPSTOL1* wheat precociously elevates core low P response pathway and Pi recycling transcripts under P deficiency. Transcript abundance normalised by Z-score of the core low P response pathway and downstream genes at 36 DAS in roots and crowns in *pUBI::OsPSTOL1* transgenics (P1 = *pUBI::OsPSTOL1.1*; P2 = *pUBI::OsPSTOL1.2*) and their null segregant (N). Each row is one gene; homologues are grouped. An asterisk indicates significantly different than the null segregant at the same P status and organ ($p < 0.05$). miR399 transcript was measured with qRT-PCR relative to *TaACTIN*. $n = 4$. Means significantly different between genotypes and treatments within roots or crowns are indicated by different letters ($p < 0.05$, analysis of variance (ANOVA) with Tukey's honest significant difference (HSD) test). Genes shown are differentially regulated in at least one transgenic line in at least one condition in *pUbi::OsPSTOL1* transgenic lines compared to null segregant ($p < 0.05$), with differentially regulated genes indicated with an asterisk. [Color figure can be viewed at wileyonlinelibrary.com]

observed in *OsPSTOL1* rice (Gamuyao et al., 2012). Finally, *PHO1;3* mRNA, encoding the P starvation-induced transporter involved in root to shoot partitioning of P is significantly lower in the transgenic crowns.

The activation of the low P pathway is reciprocally joined by the inhibition of N uptake (Hu et al., 2019). Consistent with possible heightened perception of P depletion, mRNAs encoding orthologs of the N response sensor and transporter (transceptor) *NRT1.1b* and N assimilation enzymes (nitrate reductases) are lower in roots and crowns of both transgenics under P starvation (Figure 7). *NIGT1s*, encoding a negative regulator of *NRT1.1b*, are upregulated in OsPSTOL1.2 under HP and LP at 36 DAS. This is consistent with elevated *SPX* but not that of *PHR* and *IPS1* mRNAs. It is possible that SPX levels are limited by a NRTL.1B-interacting protein 1 E3 ligase, as in P-deprived rice (Hu et al., 2019). These results indicate that *OsPSTOL1* accentuates low P sensing under replete and low P. OsPSTOL1 may also influence N assimilation. Hence, *OsPSTOL1* may predispose roots to acquire and remobilise P resources proactively while counterbalancing of N uptake. Shoot contents of N and P were not clearly affected (Figure 6d,e). It is likely that the transcriptome signatures are primarily from young and actively growing fine roots that are prevalent in the trangenics.

Notably, endogenous *TaPSTOL1* transcripts were below the threshold of detection in roots and crowns of P deficient and replete plants. The *TaPSTOL1* promoter is active in root hairs and tips of roots under low P hydroponic culture (Milner et al., 2018). Possibly, TaPSTOL1 transcripts accumulated in a few specific cells, and therefore may appear undetectable in bulk tissue transcriptomics.

4 | DISCUSSION

OsPSTOL1 is variably present in both domesticated rice and its wild relatives due to multiple independent gene mutation or deletion events (Pariasca-Tanaka et al., 2014; Vigueira et al., 2016), demonstrating it is nonessential but could be beneficial in certain environments. Low frequency of a functional *OsPSTOL1* in east Asia is at least partially due to a gene deletion that occurred after the tropical-japonica split. It is not clear if this loss reflects selection or the genetic bottleneck that the founders of the temperate population passed through (Gutaker et al., 2020). Our biogeographical survey of landraces and accessions confirm *OsPSTOL1* is depleted in *japonica* and *indica* landraces adapted to irrigated lowlands but is enriched in *japonica* varieties cultivated in rainfed uplands prone to low nutrient availability and drought (Haefele et al., 2014). To date, studies showing a benefit of *OsPSTOL1* with low P input were performed under aerobic (nonwaterlogged) conditions, with an increased benefit under simultaneous water deficiency (Chin et al., 2010; Gamuyao et al., 2012; Pariasca-Tanaka et al., 2009; Wissuwa & Ae 2001a, b), common in rainfed ecosystems after seedling establishment (Haefele et al., 2014). We find that rice varieties of west Africa and Madagascar have high frequencies of *OsPSTOL1*, where cultivation is almost exclusively rainfed and soil nutrient quality is often low (Haefele et al., 2014). Thus, although a cost of the presence of OsPSTOL1 in paddy cultivation has not been tested, this gene is likely to provide an adaptive benefit in nutrient poor and dry aerobic soils.

Our phylogenetic analyses indicate *OsPSTOL1* is truncated to a solo kinase domain relative to related RLK genes found across Gramineae crops, which typically possess a N-terminal region associated with molecular interactions with microbes, followed by transmembrane and kinase domains. Microbes or PAMPs rather than P availability induce many of these genes. Our transcript revaluation finds that *OsPSTOL1*possesses the conserved transmembrane sequence within its 5′ UTR, confirming its origin by truncation but also raising the possibility that use of a non-AUG start codon could result in synthesis of a kinase with a N-terminal transmembrane domain. Indeed, genetic variation in four sorghum Os*PSTOL1*-like genes correlate with root architecture and low P tolerance (Bernardino et al., 2019; Hufnagel et al., 2014); unlike *OsPSTOL1* and *TaPSTOL1*, these genes have N-terminal and transmembrane domains and display transcript induction by pathogens. This emphasises that this family of modular genes may be valuable subjects for improving root architecture to enhance nutrient acquisition, including P and N, in multiple Gramineae, motivating our evaluation of *OsPSTOL1* in bread wheat.

Through heterologous expression in wheat, we show that *OsPSTOL1* acts on evolutionarily conserved pathways that influence developmental and environmental responses. Modest benefits to seed number and yield, were evident in *OsPSTOL1* wheat cultivated with fertilisation in the greenhouse and rainfed field, without a reduction in 1000 grain weight, which can occur when seed number increases due to finite C and N resources delivered to seed. This indicates greater efficiency in nutrient acquisition or mobilisation. These phenotypes are accompanied by elevated levels of transcription factors associated with growth, flowering, and yield in the crown tissue. Under P deficiency, root and shoot growth is enhanced in the transgenics relative to the null segregant, along with a trend towards greater P and N content in shoot tissue under both deficient and replete P conditions that increases towards bolting. Notably, cumulative benefits on P uptake in *OsPSTOL1* rice have only been observed late in development, typically after 70 days (Pariasca-Tanaka et al., 2009; Wissuwa & Ae 2001b).

To better understand the molecular function of OsPSTOL1 in wheat, we monitored root and crown transcriptomes during development and in response to P nutrition. We find that *OsPSTOL1* wheat precociously amplifies the core low P sensing and response of roots, enhancing early elevation of transcripts associated with P remobilisation and recycling. After prolonged P deficiency, OsPSTOL1 wheat roots and crowns have modified levels of mRNAs encoding transcription factor that control nutrient assimilation, sugar homoeostasis and reproductive development. The transcriptome data suggest OsPSTOL1 may limit N uptake at 36 DAS under low Pi, however N accumulation consistently tracks with biomass accumulation in the transgenics, possibly due the production of fine roots that increase total root surface area. Higher resolution analyses, such as single cell RNA-sequencing and spatial transcriptomics will be necessary to decipher the complex role of OsPSTOL1 in low P sensing and response indicated by the organ-level transcriptome study.

The *OsPSTOL1* effect includes elevated expression of mRNAs encoding transcription factors involved in root development and drought tolerance. Among these genes are several *HOX*s, also differentially regulated in *OsPSTOL1* rice transgenics (Gamuyao et al., 2012), suggesting these developmental regulators are conserved downstream targets of OsPSTOL1 and may determine the plasticity associated with vigour in dry and low nutrient ecosystems. This and the precise cellular function of OsPSTOL1 deserve further investigation, as contrasting root system architecture is generally associated with robust P acquisition by shallow roots with dense hairs (Lynch 2011) and water acquisition under deficit by thin deep lateral roots (Klein et al., 2020). It seems likely that the selection of *OsPSTOL1* in low nutrient rainfed ecosystems with recurrent dry periods reflects appropriate regulation of developmental plasticity and P sensing that favour nutrient and moisture capture.

5 | CONCLUSIONS

This study provides new insight into the roles of *OsPSTOL1* as an adaptive gene by linking information on its biogeographical distribution across rice subspecies and ecosystems and clarifying its evolution and that of the similar solo kinase-domain wheat gene

TaPSTOL1. We show ectopic expression of *OsPSTOL1* in wheat influences root systems and yield traits, as well as adaptive gene regulation. It will be worthwhile to evaluate the agronomic benefits of OsPSTOL1 in elite cultivars of wheat. It will also be useful to address potential interactions in terms of drought, mycorrhizal fungi, and the acid soil syndrome that commonly co-occur with P deficiency.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The gene transcript ata are available at NCBI Gene Expression Omnibus, https://www.ncbi.nlm.nih.gov/geo/under_accession_GSE185875

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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