

**Table S1.** Selected chemical properties of the Gorbea series soils used in the rhizobox experiment

All analytical techniques were carried out according to the Normalisation and Accreditation Commission of the Chilean Soil Science Society (Sadzawka *et al.*, 2006). ECEC, Effective cation exchange capacity. <sup>A</sup> Extractable by Olsen method. <sup>B</sup> Determined by soil oxidation with sodium hypobromite. <sup>C</sup> Determined by Hedley fractionation procedure. <sup>D</sup> Measured in H<sub>2</sub>O. <sup>E</sup> Walkley– Black method. <sup>F</sup> Extracted by 1 M ammonium acetate. <sup>G</sup> Extracted by 1M potassium chloride.



**Table S2**. Primer sequences used in the real-time qPCR analysis.

**Table S3.** Effects of Pi starvation in Crac and Tukan grown in hydroponics. Plants were grown in Pi-sufficient conditions (200 μM) for 2 weeks, and then half of them subjected to Pi deprivation (10 μM) for 3 weeks. Values (%) of growth rate, root system architecture, and Pi acquisition were calculated as the difference between individual plants in both conditions. Asterisks indicate the significance of differences between the genotypes in the same condition, as determined by Student's t test analysis: \* *P*<0.05, \*\*\* *P*<0.001.





Fig. S1. Growth performance wheat plants under different experimental conditions. (A) Hydroponically and (B) pot grown plants for phenotyping experiments; and (C) hydroponically grown plants for molecular analysis.



**Fig. S2.** Rhizobox experiment to analyze the effect of Pi starvation in Crac (dark bars) and Tukan (light bars). Plants were grown for 8 weeks in a high P-fixing acidic soil without Pi addition. (A) Visual representation of rhizobox growth system; (B) Shoot Pi accumulation; (C) Shoot Pi concentration; (D) Shoot and root biomass production; (E) Root-to-shoot ratio; (F) Total root length; (G) Root area; (H) Average root diameter; and (I) Root efficiency in Shoot Pi accumulation. Data represent the means of five independent replicates  $(\pm SE)$ . Asterisks indicate the significance of differences between the genotypes, as determined by Student's t test analysis: \* *P*<0.05, \*\* *P*<0.01, \*\*\* *P*<0.001.





**Fig. S3.** Expression levels of *TaPHO2* alleles of Crac (dark bars) and Tukan (light bars) plants grown in nutrient solution with Pi (+Pi; 150  $\mu$ M) and without Pi for the last week (-P). (A) *TaPHO2 1A*; (B) *TaPHO2 1B*; and (C) *TaPHO2 1D*. Expression levels were referenced to the expression of the housekeeping gene *TahnRNPQ*. Bars represent the means of five independent replicates  $(\pm SE)$ . Small letters indicate differences between genotypes in the same condition and capital letters indicate differences in the genotype between conditions, as determined by Student's t test (*P*<0.05).



AIN Met EATALVLLPHSHSGLTARAPPCVGGSSAMet KR B 35 SYVRRIKRSSTVRGVMetARPQEATLARVPAPAPTRPV 71 RETAAATTTTKTVYHDTWFDNLAIGYLSRKLQEASGIK 109 G K H G Y Q G L I E A A V A I S R I F R L D T Q C E V V A G A L E R 143 A Met PSYIVT Met IKV Met Met PPSKFSREYFAAFTTIFFP 176 WLVGPCEVRESEVDGTREKNVVYIPKCRFLESTNCV 212 G Met C T N L C K I P S Q K F Met Q D S L G V S V Y Met S P N F E 242 D Met SCE Met IFGQQPPEDDPALKQPCFSTKCIAKQDY 276 **GVNC** Stop 281

**Fig. S4**. Phylogenetic analysis of *D27* sequences from *Aegilops tauschii* (Atau), *Arabidopsis thaliana* (At), *Brachypodium distachyon* (Bd), *Oryza sativa* (Os), *Sorghum bicolor* (Sb), *Zea mays* (Zm), and *Triticum aestivum* (A); and Amino acidic sequence of *TaD27* (B). In red, sequence encoding for DUF4033 superfamily. The tree was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches (Fellsenstein, 2009). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.*, 2004) and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA7 (Kumar *et al.*, 2016).



B 36 **SFRDGRAVGAHRQIESEAYKAARRHGKVCYREFSEV** 72 PKPDSFKSFVGQLASLFSGNSLTDNSNTGVVRLGDG 108 RVLCLTETVKGSIVVDPDTLDTVSKFEYEDKLGGLIH 145 SAHPIVTDTEFWTLIPDLIRPGYVVAR Met DAGSNERQ 181 FVGRVDCRGGPAPGWVHSFPVTENYVVVPE Met PLR 215 Y C A A N L L R A E P T P L Y K F Q W H L E S G S Y Met H V Met C K A S 249 GKI 252

**Fig. S5**. Phylogenetic analysis of partial *CCD8* sequences from *Aegilops tauschii* (Atau), *Arabidopsis thaliana* (At), *Brachypodium distachyon* (Bd), *Oryza sativa* (Os), *Sorghum bicolor* (Sb), *Zea mays* (Zm), and *Triticum aestivum* (A); and Partial amino acidic sequence of *TaCCD8* (B). In red sequence encoding for RPE65 superfamily. The tree was constructed as described in Figure S4.



**Fig. S6.** Transparent tray containing the root systems of a single Crac (A) and Tukan (B) plant grown for 5 weeks in pots filled with sand/vermiculite and exposed to low Pi stress (10 µM). Root systems from individual plants were cut before the analysis in order to minimize overlaps in the scanner.

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