



Identification of candidate genes for improving phosphorus use efficiency in a cowpea (*Vigna unguiculata* (L.) Walp.) multi-parent advanced generation inter-cross (MAGIC) population

Erik W. Ohlson · Xiaoxuan Zhang ·
Rebecca K. Hicks · Michael P. Timko 

Received: 5 November 2024 / Accepted: 25 March 2025 / Published online: 16 April 2025
© The Author(s) 2025

Abstract Cowpea (*Vigna unguiculata* (L.) Walp.) is the most important grain legume in sub-Saharan Africa where it provides a significant source of human nutrition and animal fodder to millions of low-input farmers. While adapted to a wide range of soils and generally considered climate resilient due to its drought and heat tolerance, cowpea yields are suboptimal throughout its main growing region because of numerous abiotic and biotic stress factors and low soil fertility. Among the major limitations is low available soil phosphorus (P) and, therefore, improving cowpea cultivars for growth under limiting P conditions would be of great importance and benefit to local low-input farmers. We grew a multi-parent advanced generation inter-cross (MAGIC) population of cowpea consisting of 305 F₈ recombinant inbred lines (RILs) generated from eight genetically diverse founder parents and phenotyped them for root system, shoot system, and total biomass production under normal and low phosphate

conditions in an ebb and flow hydroponic system. We then performed a genome wide association study (GWAS) to identify genes associated with phosphorus use efficiency (PUE). Two SNPs were identified that are significantly associated with shoot system growth under phosphate stress: one located on chromosome Vu09 and the other on Vu10. A third SNP was identified on Vu03 that is associated with enhanced root production under phosphate stress. Characterizing the underlying genetic basis for PUE can assist in the improvement of cowpea for better productivity across Africa.

Keywords Cowpea—*Vigna unguiculata* · Low phosphorus conditions · Phosphorus use efficiency (PUE) · QTL mapping · Single nucleotide polymorphism (SNP)

Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is an important grain legume widely grown in sub-Saharan Africa as food for human consumption and fodder for livestock (Agbicodo et al. 2009; Singh 2014). The grain is rich in protein (25%), carbohydrates (60.3%), fat (1.8%), vitamins, and minerals, providing a major source of dietary protein that nutritionally complements low-protein cereals and tubers in the diet (Ehlers and Hall 1997; Kudre et al. 2013; Singh 2014). In addition to playing a pivotal role in human

E. W. Ohlson · X. Zhang · R. K. Hicks · M. P. Timko (✉)
Department of Biology, University of Virginia,
Charlottesville, VA 22904, USA
e-mail: mpt9g@virginia.edu

E. W. Ohlson
e-mail: erik.ohlson@usda.gov

E. W. Ohlson
Corn, Soybean, and Wheat Quality Research Unit, United
States Department of Agriculture, Agricultural Research
Service (USDA-ARS), Wooster, OH 44691, USA

nutrition and health, it provides income generation for both smallholder farmers and commercial vendors (Omoigui et al. 2018). Throughout the African continent typical cowpea grain yields of smallholder farmers are only 10–20% of known yield potential due to a wide range of biotic and abiotic constraints (Widders 2012; Kamara et al. 2018), inadequate agronomy systems, lack of improved cultivars and lack of fertilizer inputs (Ajeigbe et al. 2010a,b).

Phosphorus is an essential macronutrient for plant growth and development and is needed to produce many of the key organic components of cells including nucleic acids, proteins and phospholipids. Although total phosphorus is abundant in most soils, African soils have inherently poor fertility because they have been overutilized and a consistent lack of organic inputs results in even poorer fertility over time (Bationo 2009). Commercial phosphorus fertilizers are often not available locally and when available are often too expensive for most low-input farmers (Mohammed et al. 2020) thereby not offering a reasonable solution to soil nutrient depletion. Moreover, the limited amounts of phosphorus present are not easily absorbed by plants because it exists often in forms that are generally immobile and of low availability for uptake (Thakur et al. 2014; Hinsinger 2001; Margenot et al. 2016; Ojeda-Rivera et al. 2022). Consequently, phosphorus starvation is a major constraint limiting plant growth and productivity in most African countries.

It is well established that all developmental stages of plant growth (e.g., seed development and germination, root growth, leaf and stem development, flowering) require phosphorus (Hayman 1975; Malhotra et al. 2018). Phosphorus deficiency also affects the effectiveness of nitrogen fixation of legumes, one of the main advantages of growing them in the cropping system (Abdelrahman et al. 2018; Yang et al. 2022). Since symbiotic nitrogen fixation (SNF) in nodules requires significant inputs of energy, legumes are generally considered to have a high phosphorus requirement (Sulieman and Tran 2015; Pang et al. 2018; Zhong et al. 2023). Furthermore, studies indicate that root nodules involved in SNF are strong phosphorus sinks, and therefore the growth and yield of legumes are dramatically decreased (~30–40%) under low phosphorus stress (Tesfaye et al. 2007; Valdés-López and Hernández 2008; Chen et al. 2011; Qin et al. 2012; Guo et al. 2022).

Thus, low phosphorus availability is regarded as an important constraint for legume production as well as production of other crops (Swaney et al. 2019; Chowdhury and Zhang 2021).

Over the past several decades selective breeding of plants aimed at improving their tolerance to low soil phosphorus conditions has been an effective means to overcome some of these constraints. For example, several studies have demonstrated that increasing plant phosphorus efficiency can be achieved by improving phosphorus acquisition efficiency (PAE) and/or phosphorus utilization efficiency (PUE) (Wang et al. 2010; Adem et al. 2020; Han et al. 2022).

Genotypic variation in phosphorus efficiency has been identified in cowpea (Ankomah et al. 1995; Sanguinga et al. 2000; Kolawole et al. 2000) and consequently there has been some success in identifying cowpea varieties which can tolerate low phosphorus in soils (Singh et al. 2002; Saidou et al. 2007). Although the genetic mechanism(s) underlying plant tolerance to low soil phosphorus conditions and the heritability of low soil phosphorus-tolerance are still poorly understood (Singh and Tarawali 1997) available evidence suggests that the genes controlling tolerance to phosphorus deficiency are independently heritable and can be recombined (Polle and Konzack 1990). Many studies of varietal differences in cowpea with respect to phosphorus uptake and utilization, have mainly focused on above ground traits such as yield (Kolawole et al. 2000) and biomass production (Sanguinga et al. 2000; Suzuki et al. 2021). In these cases, positive effects of phosphorus application were correlated with increased above ground biomass. In contrast, Oladiran, et al. (2012) screened cowpea genotypes for number of root whorls and total number of roots under three levels of phosphorus (0, 20 and 40 mg P kg⁻¹ soil) and found that phosphorus application significantly enhanced shoot and root dry weight and total biomass but that there was no significant relationship between root architecture and phosphorus uptake. They did however find that cowpea cultivar IT97K-414-5 was efficient in phosphorus uptake in low phosphorus soils and IT92KD-267-2 was efficient in N uptake. Certainly, a better understanding of root physiological traits that improve phosphorus acquisition under low phosphorus stress would allow selection of cowpea genotypes better adapted to low phosphorus soils (Lynch 2011).

Studies of the genetic basis and heritability of low phosphorous tolerance have shown that that crop tolerance to low soil phosphorus conditions is likely under polygenic control with individual genes contributing additively (Wissuwa et al. 2002; Araujo et al. 2005; Li et al. 2005; Ochoa et al. 2006; Hammond et al. 2009; Liang et al. 2010; Parentoni et al. 2010). In many cases, the results showed a significant correlation between root development and PUE. Using a hydroponic phenotypic screening method, Rothe (2015) identified cowpea varieties having significant phosphorus deficiency tolerance (Big John, IT97K-1069-6, IT98K-476-8, and TX2028-1-3-1) or partial tolerance (CB-46, and Golden Eye Cream). Phenotyping of F_1 , F_2 , and BC_1 populations showed that low phosphorus tolerance was heritable with significant additive effects and narrow-sense heritability. Quantitative trait locus analysis in an F_6 recombinant inbred line (RIL) population consisting of 125 individuals, derived from a cross of phosphorus deficiency tolerant IT98K-476-8 and phosphorus deficiency intolerant Aloka identified several SSR markers associated with low phosphorus tolerance on linkage group 3.

Subsequently, Ravelombola et al. (2017) examined a worldwide collection of 357 cowpea accessions for the relationship between plant growth and low phosphorus availability using 1018 single nucleotide polymorphism (SNP) markers. Not unexpectedly, they found substantial variability among the accessions in their response to low soil phosphorus and response to application of rock phosphate fertilizer. More importantly, they were able to identify 10 SNPs associated with low phosphorus tolerance and several other SNPs highly associated with response to phosphate application. Additionally, overlapping SNPs were found between low phosphorus tolerance condition and rock phosphate response indicating a high correlation between the two traits. More recently, Mohammed et al. (2020) evaluated 50 elite genotypes from African and U.S. germplasm collections for seedling root architecture and root hair length and density under low and high soil phosphorus conditions. Their results showed that increased root hair length and density were positively correlated with better PUE in low phosphorus soils.

The development of multi-parent advanced generation inter-cross (MAGIC) populations has been used to dissect the genetic architecture of

complex traits in several crops and model organisms (Kover et al. 2009; Huang et al. 2012; Gaur et al. 2012; Bandillo et al. 2013; Dell'Aquacqua et al. 2015; Pascual et al. 2015; Mangino et al. 2022) including cowpea (Huynh et al. 2018). MAGIC populations are created by inter-mating multiple elite parents for several cycles followed by single-seed descent (SSD), resulting in RILs that each carry a combination of genome blocks contributed by all founders. In the present study we have used the cowpea MAGIC population to identify SNPs that are associated with cowpea's tolerance to phosphorus deficiency and to identify candidate genes / quantitative trait nucleotides (QTN) potentially involved in enhanced PUE. We show that there are two significant QTN for PUE in cowpea that could be used as the starting point for future genetic improvement of this important agronomic characteristic in cowpea.

Materials and methods

MAGIC population development and genotyping

The cowpea MAGIC population used in this study was developed by Huynh et al. (2018) and was derived by crossing eight elite cultivars and breeding lines with high genetic diversity. Briefly, each of the parents (i.e., IT89KD-288, IT84S-2049, CB27, IT82E-18, Suvita-2, IT00K-1263, IT84S-2246, and IT93K-503-1) were intercrossed in balanced pedigree patterns to develop 8-way F_1 individuals. Seven generations of single-seed descent (SSD) were performed to develop 305 recombinant inbred lines representing each of the eight parents in approximately equal genetic proportions. The population was kindly provided by Bao-Lam Huynh and subsequently increased in the University of Virginia Department of Biology greenhouses by one generation of SSD.

Plant growth and phenotyping

The eight parents and 305 RILs comprising the cowpea MAGIC population ($n=313$) were grown in an ebb and flow (flood and drain) hydroponic system in the University of Virginia's Department of Biology greenhouses. To measure PUE, the plants were grown in deep square pots ($3.5'' \times 3.5'' \times 5''$) filled

with horticultural grade Turface MVP, a high-fired calcined clay particle. A randomized complete block design with two replicates per treatment was used. The control treatment consisted of 1×Hoagland's nutrient solution intended to imitate a fertile soil solution (31 ppm of phosphorus), and the second treatment consisted of a modified Hoagland's solution with no added phosphorus representing phosphorus limited conditions (Hoagland and Arnon 1950). The nutrient solution was replaced two weeks after planting. The pH (5.7) was monitored daily and adjusted with H₂SO₄ or NaOH as needed to ensure uniform nutrient bioavailability was maintained between treatments throughout the experiments.

Four weeks after planting, each plant was carefully uprooted, and the roots were gently cleaned by lightly agitating the roots in water. Above ground tissue was separated from the roots and each plant sample was transferred to a separate paper bag and dried at 70° C for 48 h. The weight of root system and shoot system were measured and recorded respectively; total plant weight was determined from the sum of the two measures. Several of the lines exhibited poor germination under both nutrient conditions and consequently data for some lines may not have been collected, only a single replication may exist, or data were subsequently filtered. These were excluded from analysis as noted below.

Data analysis

Phenotypic distributions were visualized using R Statistical Software (v4.4.1; R Core Team 2024) and the 'ggplot2' package (Wickham 2016). Data for root and shoot system masses within each treatment were positively skewed and were thus normalized by log transformations prior to genome-wide association study (GWAS) analyses. Outliers resulting from measurement, sampling, and tissue processing errors were identified based on deviation from the mean by greater than two standard deviations within each treatment. Lines for which only one replication was obtained were excluded from subsequent analyses. In total, two replicates were obtained for 198 lines for shoot system, 193 lines for root system, and 186 lines for whole plant system and the means for each line within each treatment were calculated. Whole plant system mass was calculated as the sum of the root and shoot masses for each line within each treatment.

Phosphorus use efficiencies were calculated for root systems, shoot systems, and whole plant systems for each line as:

$$PUE = \frac{Mass^{P-}}{Mass^{P+}} \times 100$$

where PUE is the phosphorus use efficiency, Mass^{P-} is the mass of the tissue under phosphorus limited conditions and Mass^{P+} is the tissue mass under phosphorus non-limiting conditions.

Genome-wide association study

The parental and RIL lines were previously genotyped with 51,128 SNPs using the Illumina Cowpea Consortium Array (Huynh et al., 2018; Munoz-Amatrian et al., 2017), among which 32,130 were polymorphic within the MAGIC population. A GWAS was conducted in R (4.4.1) using GAPIT (Version 3) (Wang and Zhang 2021) and the BLINK model (Huang et al. 2019). Conservative Bonferroni corrected logarithm of the odds (LOD) thresholds of 5.8 (α=0.05) and 5.2 (α=0.2) are denoted. All SNPs based on Benjamini–Hochberg analysis identified at a significance level of $p < 0.1$ were considered significantly associated with PUE and are reported. Manhattan plots were generated using 'qqman' package in R (Turner 2018).

Bioinformatic analysis

Significant SNPs were queried in Phytozome (Goodstein et al. 2012; <https://phytozome-next.jgi.doe.gov/>) using Jbrowse and BLAST and functional annotations within 25 kb of the significant SNPs are reported.

Results and discussion

Phenotypic evaluation under phosphorus deficiency

Eight parents (i.e., IT89KD- 288, IT84S-2049, CB27, IT82E-18, Suvita-2, IT00K- 1263, IT84S-2246, and IT93K-503–1) and 305 RILs constituting the cowpea MAGIC population were grown in randomized complete block design with two replicates per treatment to compare the effects of

phosphorus limited and non-limited conditions on plant growth. Phenotypic analysis was made of the plants grown in each treatment and root

system, shoot system, and whole plant PUE indices were used to quantify the relative performance of each RIL in phosphorus limited and non-limited

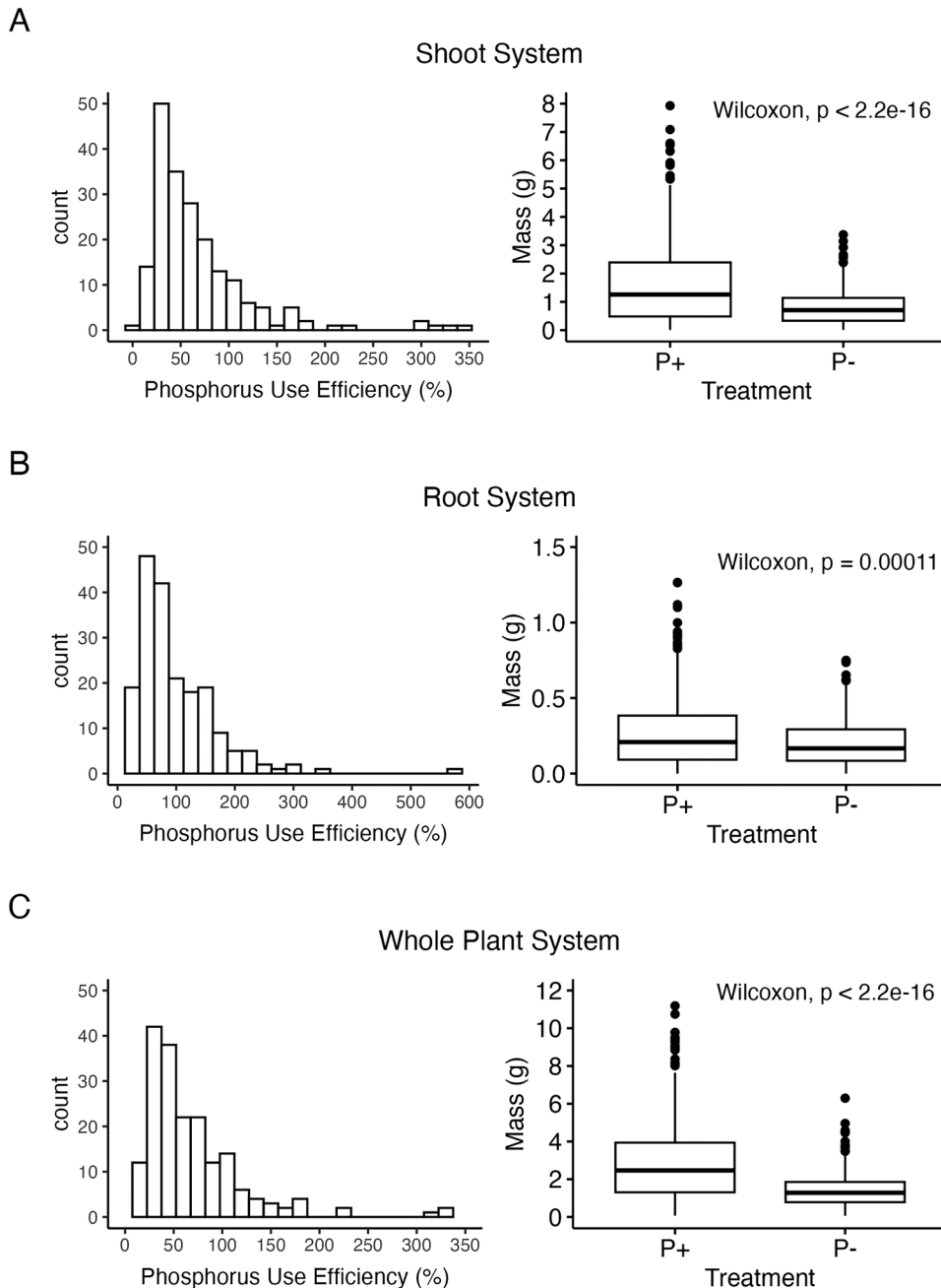


Fig. 1 Distribution of phosphorus use efficiencies (PUE) in the cowpea MAGIC population. PUE for each line was calculated as the tissue mass under phosphorus limited conditions divided by tissue mass under phosphorus non-

limiting conditions $\times 100\%$. Shown are the distributions derived from (A) root system, (B) shoot system, and (C) whole plants with boxplots showing significant differences between treatments calculated by Wilcoxon tests

conditions. For each tissue type, biomass production was significantly lower in the phosphorous limited conditions than phosphorus non-limited conditions ($P < 0.0001$) and the population distribution of PUE was positively skewed. (Fig. 1).

Parental lines produced between 21.2 – 76.7% shoot system biomass under low phosphorus conditions relative to their biomass production under phosphorus non-limited conditions and between 14.9 – 95% root biomass. In all but IT84S-2049 and IT93K-503–1, a larger proportion of total biomass production was allocated for root system development which is consistent with the expectation of enhanced root development to facilitate nutrient scavenging (Table 1). Similar observations were observed across the MAGIC population which exhibited 68% PUE for shoot system production and 98.9% for root system development. Interestingly, the MAGIC population on average outperformed nearly all parents apart from IT93K-503–1 for total plant PUE, perhaps due to favorable combinations of genes/alleles obtained from multiple parents (Table 1). The lower $PUE_{\text{Root System}}$ relative to the $PUE_{\text{Shoot System}}$ observed for IT84S-2049 and IT93K-503–1 suggests these lines do not exhibit as robust of a nutrient scavenging response relative to the other germplasm evaluated in this study under phosphorus limiting conditions.

Prior studies have found significant differences in root system structure among legumes as evidenced in differences in root fresh weight, shoot fresh and dry weights and root: shoot ratio (Mia et al. 1996). The differences found here among MAGIC genotypes, especially in root weights, is an important characteristic since extensive lateral rooting systems have been shown to be important adaptive traits that

enhance phosphorus uptake from low phosphorus soils (Zhu and Lynch 2004) and the differences may be contributing to better PUE. Overall, the PUE of the root systems was substantially better than observed for the shoot systems, suggesting the low phosphorus conditions induced a nutrient scavenging response in most of the lines.

Association mapping

GWAS was conducted to identify SNP markers associated with root, stem and whole plant indices using the Bayesian-information and linkage-disequilibrium iteratively nested keyway (BLINK) model. BLINK was selected due to its high statistical power relative to other widely used GWAS methods. In addition to its capability to incorporate principal components and kinship as covariates to reduce false positives due to population stratification, BLINK iteratively incorporates associated markers as covariates for testing markers to eliminate their association with cryptic relationships among individuals.

A total of two SNPs, on chromosomes Vu9 and Vu10, were found to be associated with shoot system PUE and one on chromosome Vu3 was found to be associated with root system PUE (Fig. 2).

Perhaps not unexpected, SNPs associated with whole plant PUE were the same as those detected for shoot system PUE since the above ground tissue comprises the largest proportion of the whole plant system mass (Table 1). From a breeding perspective, the low number of significant loci detected suggests that PUE derived from the parental lines used in this study may be relatively easy to select for using

Table 1 Phosphorus use efficiencies of parental lines and MAGIC population

Cowpea line/population	$PUE_{\text{Shoot System}}$ (%)	$PUE_{\text{Root System}}$ (%)	$PUE_{\text{Whole Plant System}}$ (%)
CB27	37.2	61.0	40.7
IT00K-1263	41.2	69.3	44.5
IT82E-18	43.1	60.9	46.3
IT84S-2049	21.2	14.9	20.3
IT84S-2246	32.4	51.4	34.5
IT89KD-288	24.0	33.4	25.1
IT93K-503–1	76.7	72.4	75.8
Suvita 2	58.0	95.1	62.9
MAGIC Population	68.6	98.9	69.2

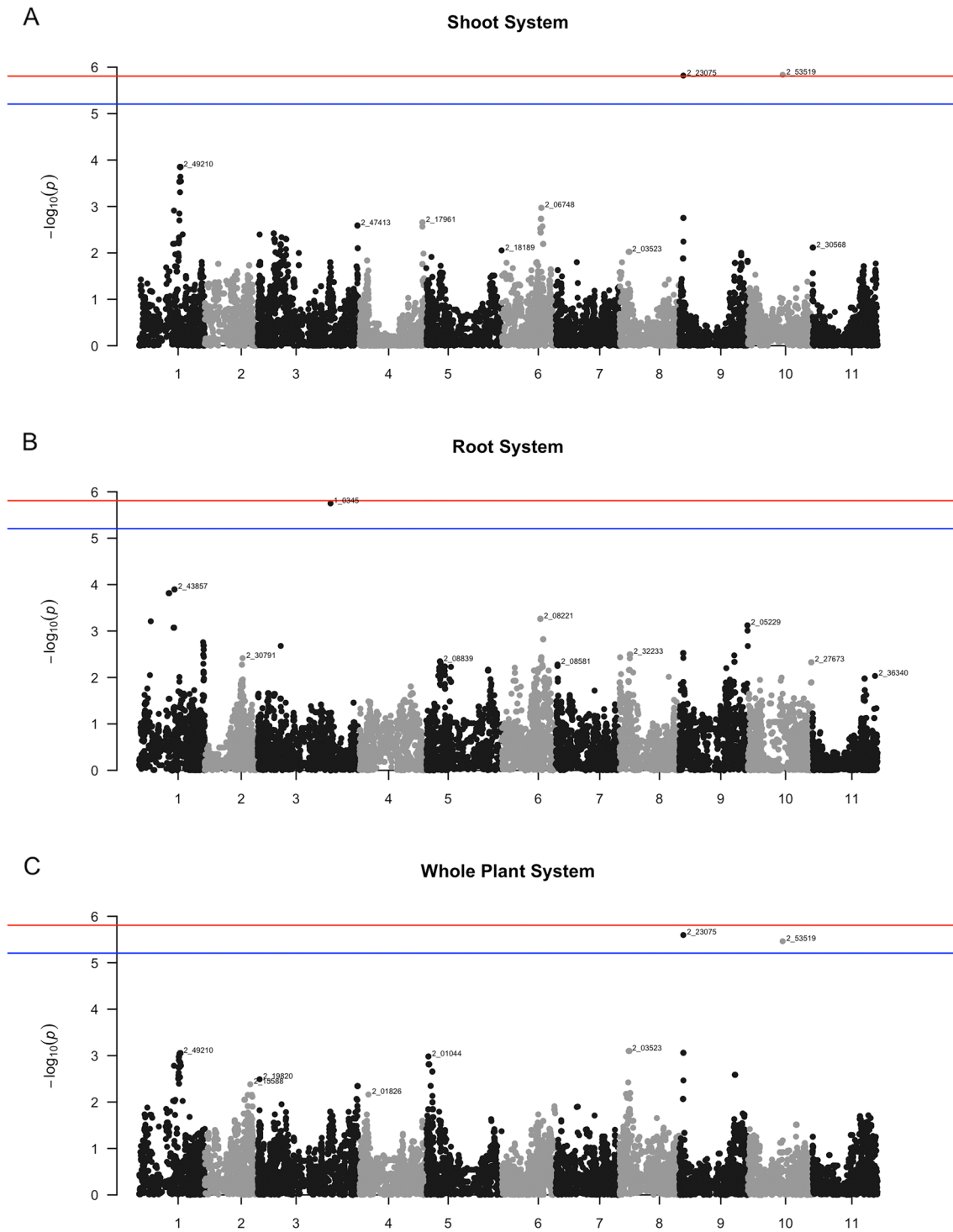


Fig. 2 Manhattan plots representing the significance of genome-wide SNPs associated with (A) shoot system phosphorus use efficiency, (B) root system phosphorus use efficiency, and (C) whole plant phosphorus use efficiency. The

Bonferroni corrected LOD thresholds of 5.8 ($\alpha=0.05$) and 5.2 ($\alpha=0.2$) are denoted. The genome-wide association study was conducted in GAPIT version 3 using the BLINK model. Annotated SNPs represent values exceeding $-\log(0.01)$

traditional or molecular breeding. Interestingly, neither of the two SNPs that were associated with shoot system PUE colocalized with the SNP associated with root system PUE, suggesting there could be a tradeoff in terms of energy allocation between the root development required for scavenging nutrients and shoot system development that is beneficial for cowpea grain and vegetable production. However, since these screenings were done under artificial hydroponic conditions a nutrient scavenging response would not necessarily result in an associated positive response in shoot biomass production. In a typical agricultural system under which cowpea production would occur, a scavenging response is likely to be positively associated with shoot biomass productivity as well.

For each SNP associated with better shoot system PUE, a beneficial response was associated with the minor allele (Fig. 3), although for the SNP 2_23075 on chromosome Vu9, each allele was present in equal proportions among the eight parents. The allele associated with the positive effect on chromosome 9 is present in IT84S-2246, CB27, IT82E-18, and Suvita-2, while only IT82E-18 conferred the beneficial allele on chromosome Vu10. For root system PUE, the minor allele was only present in CB27 and had a highly negative effect on root system biomass production under phosphorus limited conditions (Table 2).

It is now well established that plants sense phosphate deficiency and initiate signaling controlling adaptive responses necessary for phosphate acquisition. The genetic mechanisms involved in this process are not fully known. Analysis of potential gene functions associated with the QTN located on Vu03, Vu09, and Vu10 identified in the present study (Table 2) revealed that significant SNPs are within or near genes with functions associated with nutrient stress response and activation of transcriptional activities. On chromosome 9, the Vigun09g030400 gene, which is annotated as an inactive purple acid phosphatase (PAP) 28-related protein and just 212 bp from the significant SNP, is particularly intriguing based on the role of PAPs in orthophosphate mobilization under deficient conditions. Phosphorus starvation has been shown to induce expression of PAP isozymes in *Arabidopsis* which may facilitate phosphate scavenging from both extracellular and intracellular

sources (Tran et al. 2010). The SNP is also embedded in Vigun09g030500, which is predicted to encode a ATP:cob(I)alamin adenosyltransferase-like protein, a protein homologous to those responsible for catalyzing the conversion of B12 to its coenzyme form. While its possible function in relation to PUE is less obvious, it may be similarly involved in cofactor biosynthesis associated with stress response.

On chromosome Vu10, 2_53519 is just 369 bp from a predicted leucoanthocyanidin dioxygenase (LDOX) protein (Vigun10g080400), a protein family involved in flavonoid biosynthesis. Phosphate starvation causes oxidative stress in plants which could potentially be mitigated by upregulated flavonoid production. Of note, the *RtLDOX2* gene in *Reaumuria trigyna* has been shown to improve tolerance to several abiotic stresses and enhance primary root length, biomass accumulations as well as chlorophyll content under salt, drought, and ultraviolet-B detrimental conditions (Li et al. 2021). The Vigun10g080400 gene, may play a similar role in cowpea's response to phosphorus stress.

The chromosome Vu03 SNP significantly associated with root system PUE, 1_0345, is embedded in Vigun03g285000 and just 2003 bp from Vigun03g285100. Vigun03g285000 is predicted to encode a peroxidase which may be involved in responding to oxidative stress caused by phosphorus deficient conditions. Peroxidases have also been shown to alter root phenotypes, increasing lateral root density in *Arabidopsis* which might be a beneficial response for nutrient scavenging (Passaia et al. 2014). It is unclear what the role of Vigun03g285100 might be in influencing root system PUE, however phosphorus limitation induces a range of transcriptional responses in which this transcriptional regulator could be involved.

Interestingly, QTL mapping in an F₆ RIL population of 120 individuals using SSR markers by Rothe (2015) identified four significant QTL for low phosphorus tolerance on LG 3 associated with shoot system biomass production. Two QTL, *QAB.tam-3Chr.1* (14.47–39 cM) and *QAB.tam-3Chr.2* (68.58–119.48 cM), were identified from qualitative scores for low phosphorus tolerance, and a third QTL, *QLP.tam-3Chr* (72.58–120 cM), was identified from shoot dry biomasses in the low phosphorus treatment. The fourth QTL, *QPSI.tam-3Chr* (43–118.48 cM), was identified from P susceptibility

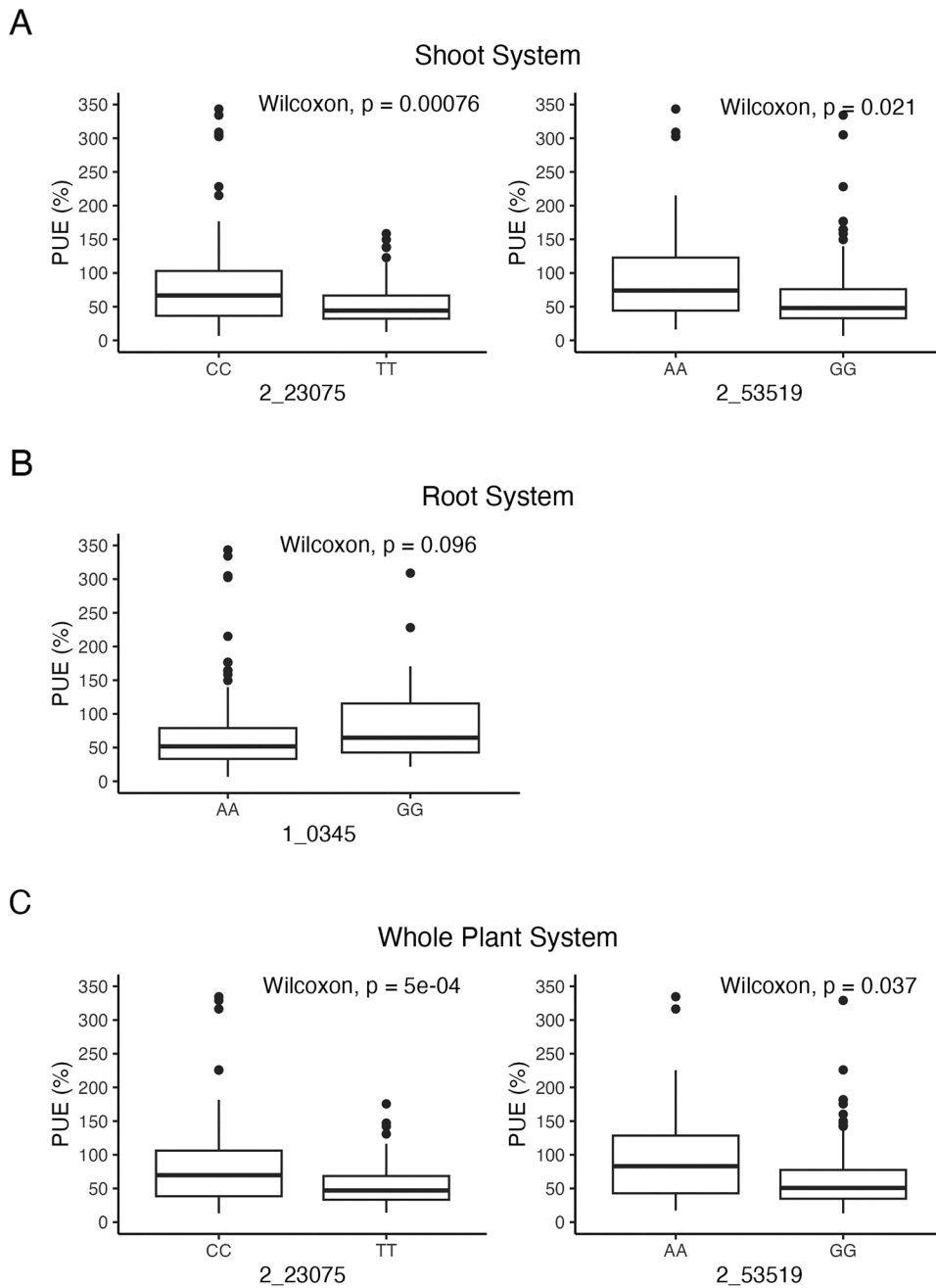


Fig. 3 Allelic effects for each quantitative trait nucleotide identified by genome wide association study analyses on phosphorus use efficiency in (A) shoot, (B) root, and (C)

whole plant systems. Significant differences between alleles were determined by Wilcoxon test

index (PSI) values. *QAB.tam-3Chr.2*, *QLP.tam-3Chr*, and *QPSI.tam-3Chr* overlap suggesting these QTL

may represent the same gene(s) for low phosphorus tolerance. However, only the *QPSI.tam-3Chr* and

Table 2 SNPs significantly associated with phosphorus use efficiency (PUE) based on analysis of shoot, root and whole plant systems

Tissue	SNP	Chr	Pos (bp)	MAF (%)	Benjamini-Hochberg Adjusted P-value	Effect ^a (%)	Gene Model [position (bp)] ^b	Function
Shoot System	2_23075	9	2,543,093	0.48	2.45E-02	16.8	Vigun09g030400 [2,536,574–2,542,881] Vigun09g030500 [2,542,881–2,545,628]	PTHR32440:SF2—INACTIVE PURPLE ACID PHOSPHATASE 28-RELATED Metallo-dependent phosphatase-like (IPR029052) Adenosylcobalamin biosynthesis, ATP:cobalamin adenosyltransferase-like
Shoot System	2_53519	10	22,266,215	0.12	2.45E-02	26.3	Vigun10g080400 [22,263,114–22,265,846]	1.14.11.19—Leucocyanidin oxygenase / Leucoanthocyanidin dioxygenase
Root System	1_0345	3	46,627,017	0.09	5.73E-02	-40.0	Vigun03g285000 [46,625,971–46,628,786] Vigun03g285100 [46,629,020–46,633,107]	PTHR31517// PTHR31517:SF3—FAMILY NOT NAMED // PEROXIDASE 35-RELATED PF12767—Transcriptional regulator of RNA polII, SAGA, subunit (SAGA-Tad1)
Whole Plant	2_23075	9	2,543,093	0.47	5.52E-02	15.6	Vigun09g030400 [2,536,574–2,542,881] Vigun09g030500 [2,542,881–2,545,628]	PTHR32440:SF2—INACTIVE PURPLE ACID PHOSPHATASE 28-RELATED Adenosylcobalamin biosynthesis, ATP:cobalamin adenosyltransferase-like
Whole Plant	2_53519	10	22,266,215	0.12	5.52E-02	24.3	Vigun10g080400 [22,263,114–22,265,846]	1.14.11.19—Leucocyanidin oxygenase / Leucoanthocyanidin dioxygenase

^a allelic effect associated with the minor allele^b The closest gene models within ± 25 kb to each SNP are reported along with their predicted function

QAB.tam-3Chr.1 confidence intervals appear to co-localize with the 1_0345 QTN. Colocalization of these shoot system QTL with the root system QTN identified in this study, suggests that these shoot system QTL may be derived from an improved nutrient scavenging capacity afforded by greater root system productivity under phosphorus deficient conditions. Based on BLAST analysis of the SSR

primer sequences used by Rothe (2015), 1_0345 is flanked by the SSR markers CLM0721 and MA135. In fact, 1_0345 falls just 143.4 kb from MA135, suggesting this SSR could serve as a valuable marker for improved phosphate scavenging via molecular breeding in labs in Africa, where SNP genotyping is less widely accessible. This study further delineates and provides additional evidence supporting the

importance of this genomic region in relation to cowpea's response to phosphorus starvation.

Conclusions

Together these results contribute knowledge to breeding cowpea for the phosphorus-deficient soils of sub-Saharan West Africa via identifying tolerant varieties and elucidating a few of the potential physiological mechanisms responsible for tolerance. These results suggest that tolerant cowpea lines via low phosphorus tolerance can be readily developed since low phosphorus tolerance is a heritable additive trait. In addition, QTL for low phosphorus tolerance in cowpea were identified that could assist in breeding for low phosphorus tolerance and future fine mapping of the loci leading to a detailed characterization of the underlying genes and mechanism of this tolerance.

Acknowledgements The authors are grateful to Tatyana Kotova and Hai Liu for their technical assistance and insightful comments.

Author contributions Conceptualization, E.W.O. M.P.T.; Data Collection and Analysis, E.W.O. R.K.H., X.Z., M.P.T.; Writing—original draft preparation, R.K.H., X.Z., M.P.T.; writing—review and editing, E.W.O., R.K.H., X.Z., M.P.T.; Supervision, M.P.T.; Funding acquisition, M.P.T. All authors have read and agreed to the published version of the manuscript.

Funding This work was funded by the Kirkhouse Trust SCIO (Grant Number GF147029).

Data availability “The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.”

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds

the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Abdelrahman M, Jogaiah S, Burritt DJ, Tran LP (2018) Legume genetic resources and transcriptome dynamics under abiotic stress conditions. *Plant Cell Environ* 41:1972–1983. <https://doi.org/10.1111/pce.13123>
- Adem GD, Ueda Y, Hayes PE, Wissuwa M (2020) Genetic and physiological traits for internal phosphorus utilization efficiency in rice. *PLoS ONE* 15:e0241842. <https://doi.org/10.1371/journal.pone.0241842>
- Agbicodo EM, Fatokun CA, Muranaka S, Visser RGF, van der Linden CG (2009) Breeding drought tolerant cowpea: constraints, accomplishments, and future prospects. *Euphytica* 167:353–370. <https://doi.org/10.1007/s10681-009-9893-8>
- Ajeigbe HA, Singh BB, Adeosun JO, Ezeaku IE (2010a) Participatory on-farm evaluation of improved legume–cereals cropping systems for crop–livestock farmers: Maize-double cowpea in Northern Guinea Savanna Zone of Nigeria. *Afri Journ Agric Res* 5(16):2080–2088
- Ajeigbe HA, Singh BB, Ezeaku IE, Adeosun JO (2010b) On-farm evaluation of improved cowpea–cereals cropping systems for crop–livestock farmers: cereals-cowpea systems in Sudan Savanna zone of Nigeria. *Afri Journ Agric Res* 5:2297–2304
- Ankomah AB, Zapata F, Danso SK, Axmann H (1995) Cowpea varietal difference in uptake of phosphorus from Gafsa phosphate rock in a low-P ultisol. *Fertilizer Res* 41:219–225
- Araujo AP, Antunes IF, Teixeira MG (2005) Inheritance of root traits and phosphorus uptake in common bean (*Phaseolus vulgaris* L.) under limited soil phosphorus supply. *Euphytica* 145:33–40
- Bandillo N, Raghavan C, Muyco PA et al (2013) Multi-parent advanced generation inter-cross (MAGIC) populations in rice: progress and potential for genetics research and breeding. *Rice* 6:11. <https://doi.org/10.1186/1939-8433-6-11>
- Bationo, A. (2009) Constraints and new opportunities for achieving a green revolution in Sub-Saharan Africa through Integrated Soil Fertility Management. UC Davis: Department of Plant Sciences. Retrieved from <https://escholarship.org/uc/item/7hr282j2>
- Chen Z, Cui Q, Liang C, Sun L, Tian J, Liao H (2011) Identification of differentially expressed proteins in soybean nodules under phosphorus deficiency through proteomic analysis. *Proteomics* 11:4648–4659. <https://doi.org/10.1002/pmic.201100231>
- Chowdhury RB, Zhang X (2021) Phosphorus use efficiency in agricultural systems: a comprehensive assessment through the review of national scale substance flow analyses. *Ecol Ind* 121:107172. <https://doi.org/10.1016/j.ecolind.2020.107172>

- Dell'Acqua M, Gatti DM, Pea G, Cattonaro F, Coppens F, Magris G et al (2015) Genetic properties of the MAGIC maize population: a new platform for high definition QTL mapping in *Zea mays*. *Genome Biol* 16:1–23. <https://doi.org/10.1186/s13059-015-0716-z>
- Ehlers JD, Hall AE (1997) Cowpea (*Vigna unguiculata* L. Walp.). *Field Crops Res* 53:187–204. [https://doi.org/10.1016/S0378-4290\(97\)00031-2](https://doi.org/10.1016/S0378-4290(97)00031-2)
- Gaur PM, Jukanti AK, Varshney RK (2012) Impact of genomic technologies on chickpea breeding strategies. *Agronomy* 2:199–221. <https://doi.org/10.3390/agronomy2030199>
- Goodstein DM, Shu S, Howson R, Neupane R, Hayes RD, Fazo J, Mitros T, Dirks W, Hellsten U, Putnam N, Rokhsar DS (2012) Phytozome: a comparative platform for green plant genomics. *Nucl Acids Res* 40(D1):D1178–D1186
- Guo Z, Cao H, Zhao J, Bai S, Peng W, Li J et al (2022) A natural uORF variant confers phosphorus acquisition diversity in soybean. *Nat Commun* 13:3796. <https://doi.org/10.1038/s41467-022-31555-2>
- Hammond JP, Broadley MR, White PJ, King GJ, Bowen HC, Hayden R, Meacham MC, Mead A, Overs T, Spracklen WP, Greenwood DJ (2009) Shoot yield drives phosphorus use efficiency in Brassica oleracea and correlates with root architecture traits. *J Exp Bot* 60:1953–1968
- Han Y, White PJ, Cheng L (2022) Mechanisms for improving phosphorus utilization efficiency in plants. *Ann Bot* 129:247–258. <https://doi.org/10.1093/aob/mcab145>
- Hayman DS (1975) Phosphorus cycling by soil microorganisms and plant roots. In: Walker N (ed) *Soil microbiology*. Butterworths, London, pp 67–92
- Hinsinger P (2001) Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: A review. *Plant Soil* 237:173–195. <https://doi.org/10.1023/A:1013351617532>
- Hoagland DR, and Arnon DI (1950) *The Water-Culture Method for Growing Plants without Soil*. 2nd Edition, Circular 347, California Agricultural Experiment Station.
- Huang BE, George AW, Forrest KL et al (2012) A multiparent advanced generation inter-cross population for genetic analysis in wheat. *Plant Biotech J* 10:826–839. <https://doi.org/10.1111/j.1467-7652.2012.00702.x>
- Huang M, Liu X, Zhou Y, Summers RM, Zhang Z (2019) BLINK: a package for the next level of genome-wide association studies with both individuals and markers in the millions. *Gigascience* 8(2):154. <https://doi.org/10.1093/gigascience/giy154>
- Huynh BL, Ehlers JD, Huang BE, Muñoz-Amatrián M, Lonardi S, Santos JRP et al (2018) A multi-parent advanced generation inter-cross (MAGIC) population for genetic analysis and improvement of cowpea (*Vigna unguiculata* L. Walp.). *Plant J* 93:1129–1142. <https://doi.org/10.1111/tpj.13827>
- Kamara AY, Omoigui LO, Kamai N, Ewansiha SU, and Ajeigbe HA (2018) Improving cultivation of cowpea in West Africa. In: Sivasankar, S., et al., Eds., *Achieving Sustainable Cultivation of Grain Legumes*, Burleigh Dodds Science Publishing Limited, vol. 2 pp. 235–252. <https://doi.org/10.19103/AS.2017.0023.30>
- Kolawole G, Tian Guang L, Singh BB (2000) Differential response of cowpea lines to aluminum and phosphorus application. *J Plant Nutr* 23:731–740
- Kover PX, Valdar W, Trakalo J, Scarcelli N, Ehrenreich IM, Purugganan MD, Durrant C, Mott R (2009) A multiparent advanced generation inter-cross to fine-map quantitative traits in *Arabidopsis thaliana*. *PLoS Genet* 5:e1000551. <https://doi.org/10.1371/journal.pgen.1000551>
- Kudre TG, Benjakul S, Kishimura H (2013) Comparative study on chemical compositions and properties of protein isolates from mung bean, black bean and bambara groundnut. *J Sci Food Agric* 93:2429–2436. <https://doi.org/10.1002/jsfa.6052>
- Li Y-D, Wang Y-J, Tong Y-P, Gao J-G, Zhang J-S, Chen S-Y (2005) QTL mapping of phosphorus deficiency tolerance in soybean (*Glycine max* L. Merr.) *Euphytica* 142(1–2):137–142. <https://doi.org/10.1007/s10681-005-1192-4>
- Li N, Wang X, Ma B, Wu Z, Zheng L, Qi Z, Wang Y (2021) A leucoanthocyanidin dioxygenase gene (*RtLDOX2*) from the feral forage plant *Reaumuria trigyna* promotes the accumulation of flavonoids and improves tolerance to abiotic stresses. *J Plant Res* 134:1121–1138
- Liang Q, Cheng X, Mei M, Yan X, Liao H (2010) QTL analysis of root traits as related to phosphorus efficiency in soybean. *Ann Bot* 106:223–234
- Lynch JP (2011) Root phenes for enhanced soil exploration and phosphorus acquisition: tools for future crops. *Plant Physiol* 156:1041–1049. <https://doi.org/10.1104/pp.111.175414>
- Malhotra H, Vandana, Sharma, S. and Pandey R (2018). Phosphorus nutrition: plant growth in response to deficiency and excess. In: *Plant nutrients and abiotic stress tolerance*, M. Hasanuzzaman et al. (eds.), *Plant Nutrients and Abiotic Stress Tolerance*, Springer Nature Singapore Pte Ltd. 2018 pp.171–19 https://doi.org/10.1007/978-981-10-9044-8_7
- Mangino G, Arrones A, Plazas M, Pook T, Prohens J, Gramazio P, Vilanova S (2022) Newly developed MAGIC population allows identification of strong associations and candidate genes for anthocyanin pigmentation in eggplant. *Front Plant Sci* 13:847789. <https://doi.org/10.3389/fpls.2022.847789>
- Margenot AJ, Singh BR, Rao IM, and Sommer R (2016) Phosphorus fertilization and management in soils of Sub-Saharan Africa, In *Soil Phosphorus, Advances in Soil Science*. (eds.) Lal R and Stewart BA (USA: Taylor and Francis Group), pp. 151–208. <https://doi.org/10.1201/9781315372327-9>
- Mia MW, Yamauchi A, Kono Y (1996) Root system structure of six food legume species. *Jpn J Crop Sci* 65:131–140
- Mohammed SB, Mohammad IF, Pangirayi TB, Vernon G, Dzidzienyo DK, Umar ML, Umar S (2020) Farmers' knowledge, perception, and use of phosphorus fertilization for cowpea production in Northern Guinea Savannah of Nigeria. *Heliyon* 6:e05207. <https://doi.org/10.1016/j.heliyon.2020.e05207>
- Muñoz-Amatrián M, Mirebrahim H, Xu P, Wanamaker SI, Luo M, Alhakami H, Alpert M, Atokple I, Batieno BJ, Boukar O, Bozdag S (2017) Genome resources for climate-resilient cowpea, an essential crop for food

- security. *Plant J* 89:1042–1054. <https://doi.org/10.1111/tbj.13404>
- Ochoa IE, Blair MW, Lynch JP (2006) QTL analysis of adventitious root formation in common bean under contrasting phosphorus availability. *Crop Sci* 46:1609–1621
- Ojeda-Rivera JO, Alejo-Jacuinde G, Nájera-González HR, López-Arredondo D (2022) Prospects of genetics and breeding for low-phosphate tolerance: an integrated approach from soil to cell. *Theor Appl Genet* 135:4125–4150. <https://doi.org/10.1007/s00122-022-04095-y>
- Oladiran O, Olajire RAC, Nnenna I (2012) Phosphorus response efficiency in cowpea genotypes. *J Agric Sci* 4:81–90
- Omoigui L, Kamara A, Batieno BJ, Lorlamen T, Kouyate Z, Yirzagla J, Diallo S and Garba U (2018) Guide to cowpea production in west Africa. *Tropical Legume III (TLIII)*, International Institute of Tropical Agriculture, ISBN 978–978–131–357–8, 2018.
- Pang J, Ryan MH, Lambers H, Siddique KH (2018) Phosphorus acquisition and utilisation in crop legumes under global change. *Curr Opin Plant Biol* 45:248–254. <https://doi.org/10.1016/j.pbi.2018.05.012>
- Parentoni SN, de Souza Jr CL, de Carvalho Alves VM, Gama EE, Coelho AM, De Oliveira AC, Guimaraes PE, Guimaraes CT, Vasconcelos MJ, Patto Pacheco CA, and Meirelles WF, (2010) Inheritance and breeding strategies for phosphorous efficiency in tropical maize (*Zea mays* L.). *Maydica* 55:1–15
- Pascual L, Desplat Bevan E, Aurore H, Laure D, Jean-Paul B, Bouchet Quang H, Betty L, Philippe C, Mathilde V (2015) Potential of a tomato MAGIC population to decipher the genetic control of quantitative traits and detect causal variants in the resequencing era. *Summ Plant Biotechnol J* 13(4):565–577. <https://doi.org/10.1111/pbi.12282>
- Passaia G, Queval G, Bai J, Margis-Pinheiro M, Foyer CH (2014) The effects of redox controls mediated by glutathione peroxidases on root architecture in *Arabidopsis thaliana*. *J Experi Bot* 65:1403–1413. <https://doi.org/10.1093/jxb/ert486>
- Polle EA, Konzack CF (1990) Genetics and breeding of cereals for acid soils and nutrient efficiency. In: Baligar VC, Dunea RR (eds) *Crops are Enhancers of nutrient use*. Academic Press, pp 81–121
- Qin L, Zhao J, Tian J, Chen L, Sun Z, Guo Y et al (2012) The high-affinity phosphate transporter GmPT5 regulates phosphate transport to nodules and nodulation in soybean. *Plant Physiol* 159:1634–1643. <https://doi.org/10.1104/pp.112.199786>
- R Core Team (2024) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria
- Ravelombola W, Qin J, Shi A, Lu W, Weng Y, Xiong H, YaNg W, Bhattarai G, Mahamane S, Payne WA, Miller JC Jr, Scheuring D (2017) Association mapping revealed SNP markers for adaptation to low phosphorus conditions and rock phosphate response in USDA cowpea (*Vigna unguiculata* (L.) Walp.) germplasm. *Euphytica* 213:183. <https://doi.org/10.1007/s10681-017-1971-8>
- Ravelombola W, Shi A, Huynh BL (2021) Loci discovery, network-guided approach, and genomic prediction for drought tolerance index in a multi-parent advanced generation intercross (MAGIC) cowpea population. *Hortic Res* 8:24. <https://doi.org/10.1038/s41438-021-00462-w>
- Rothe JC (2015) Breeding for tolerance of cowpea to low phosphorus soil conditions through physiological and genetic studies. Ph.D. Dissertation, Texas A&M University, College Station, TX.
- Saidou A, Abaidoo R, Singh B, Iwuafor E, Sangina N (2007) Variability of cowpea breeding lines to low phosphorus tolerance and response to external application of Phosphorus. In: Bationo A, Waswa B, Kihara J, Kimetu J (eds) *Advances in Integrated Soil Fertility Management in sub-Saharan Africa: Challenges and Opportunities*. Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-5760-1_38
- Sanguinga N, Lyasse O, Singh BB (2000) Phosphorus use efficiency and nitrogen balance of cowpea breeding lines in a low P soil of the derived savanna zone in West Africa. *Plant Soil* 220:119–128
- Singh BB (2014) *Cowpea, The food legume of the 21st century*. Crop Science Society of America, Madison, WI USA, pp 38–68
- Singh BB, and Tarawali SA (1997) Cowpea and its improvement: key to sustainable mixed crop/livestock farming systems in West Africa. In: Renard C (ed) *Crop Residues in Sustainable Mixed Crop/Livestock Farming Systems*, CAB in Association with ICRISAT and ILRI, Wallingford, UK, pp. 79–1007.
- Singh BB (2002) Recent genetic studies in cowpea. In: Fatokun CA, Tarawali SA, Singh BB, Kormawa PM, Tamo M (eds) *Challenges and Opportunities for Enhancing Sustainable Cowpea Production*. Intl Inst Tropical Agric, Ibadan, Nigeria, pp. 3–13
- Suliman S, Tran LS (2015) Phosphorus homeostasis in legume nodules as an adaptive strategy to phosphorus deficiency. *Plant Sci* 239:36–43. <https://doi.org/10.1016/j.plantsci.2015.06.018>
- Suzuki K, Fatokun C, Boukar O (2021) Effect of phosphorus application on the performance of some cowpea lines. *Agronomy J* 114:727–738. <https://doi.org/10.1002/agi2.20878>
- Swaney DP, Howarth RW (2019) Phosphorus use efficiency and crop production: Patterns of regional variation in the United States, 1987–2012. *Sci Total Environ* 685:174–188. <https://doi.org/10.1016/j.scitotenv.2019.05.228>
- Tesfaye M, Liu J, Allan DL, Vance CP (2007) Genomic and genetic control of phosphate stress in legumes. *Plant Physiol* 144:594–603. <https://doi.org/10.1104/pp.107.097386>
- Thakur D, Kaushal R, Shyam V (2014) Phosphate solubilizing microorganisms: role in phosphorus nutrition of crop plants - A review. *Agric Rev* 35(3):159–171
- Tran HT, Hurley BA, Plaxton WC (2010) Feeding hungry plants: The role of purple acid phosphatases in phosphate nutrition. *Plant Sci* 179:14–27. <https://doi.org/10.1016/j.plantsci.2010.04.005>
- Turner S (2018) qqman: an R package for visualizing GWAS results using Q-Q and manhattan plots. *J Open Source Softw.* <https://doi.org/10.21105/joss.00731>
- Valdés-López O, Hernández G (2008) Transcriptional regulation and signaling in phosphorus starvation: what about

- legumes? J Integr Plant Biol 50:1213–1222. <https://doi.org/10.1111/j.1744-7909.2008.00758.x>
- Wang J, Zhang A (2021) GAPIT version 3: boosting power and accuracy for genomic association and prediction. Genomics Proteomics Bioinformatics 19:1–12
- Wang X, Shen J, Liao H (2010) Acquisition or utilization, which is more critical for enhancing phosphorus efficiency in modern crops? Plant Sci 179:302–306. <https://doi.org/10.1016/j.plantsci.2010.06.007>
- Wickham H (2016) ggplot2: Elegant graphics for data analysis. Springer-Verlag, New York. ISBN 978–3–319–24277–4, <https://ggplot2.tidyverse.org>.
- Widders IE (2012) Cowpea: A solution to global challenges. In: Innovative research along the cowpea value chain. eds. Boukar O, Coulibaly O, Fatokun CA, Lopez K. and Tamò M. International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, xi–xviii.
- Wissuwa M, Wegner J, Ae N, Yano M (2002) Substitution mapping of Pup1: a major QTL increasing phosphorus uptake of rice from a phosphorus-deficient soil. Theor Appl Genet 105:890–897
- Yang J, Lan L, Jin Y, Yu N, Wang D, Wang E (2022) Mechanisms underlying legume–rhizobium symbioses. J Integr Plant Biol 64:244–267. <https://doi.org/10.1111/jipb.13207>
- Zhong Y, Tian J, Li X, Liao H (2023) Cooperative interactions between nitrogen fixation and phosphorus nutrition in legumes. New Phytol 237:734–745. <https://doi.org/10.1111/nph.18593>
- Zhu J, Lynch JL (2004) The contribution of lateral rooting to phosphorus acquisition efficiency in maize (*Zea mays* L.) seedlings. Funct Plant Biol 31:949–958. <https://doi.org/10.1071/FP04046>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.