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INTEGRATED ANALYSIS OF GENOTYPIC PLASTICITY FOR ENHANCED PHOSPHORUS USE EFFICIENCY IN CHICKPEA (*Cicer arietinum* L.)

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ABSTRACT

Phosphorus (P) limitation is a major constraint in crop productivity, emphasizing the need for genetic improvement of phosphorus use efficiency (PUE) for sustainable agriculture. During 2024-25, thirty-three chickpea genotypes were evaluated under low and adequate P conditions to assess genotypic plasticity and identify the efficient genotypes using PUE index, principal component analysis (PCA), and multi-trait genotype-ideotype distance index (MGIDI). The PUE index ranked genotypes across the contrasting P regimes, PCA summarized trait covariation to highlight efficient multivariate profiles, and MGIDI integrated multiple traits to estimate the distance from an ideal genotype. Two-way ANOVA revealed significant genotype, treatment, and their interaction effects. Under low P conditions, shoot phosphorus concentration declined by 45.6%, while phosphorus assimilation efficiency increased by 82.7%, and root dry weight by 62.7%, indicating adaptive biomass allocation to the roots. Strong positive trait associations were observed across both P regimes, with biomass and P traits varying co-ordinately. Across all the methods, genotypes IG 5860, Pusa 3022 (BG 3022), and PG 0515 consistently emerged as superior genotypes. Notably, no cultivar surpassed the efficient check IG 5860, reinforcing its value as a donor for PUE breeding. These convergent rankings validate the integrated approach as a reliable strategy for prioritizing chickpea genotypes under low P stress.

Keywords: Chickpea, phosphorus, PCA, PUE, PUE index, MGIDI

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a winter pulse largely grown in the semi-arid parts of southern Asia and sub-Saharan Africa (Thudi *et al*., 2023). Due to its cultivation in marginal lands, with limited available soil moisture, the crop faces multiple biotic and abiotic challenges including low phosphorus (P) stress, preventing it from attaining its true yield potential (Thakro *et al*., 2023). P is crucial for plant growth, development, reproduction and energy production, as it is a key component of ATP, nucleic acids, cellular membranes and supports enzymatic reactions in metabolic pathways (Ramtekey *et al*., 2021). Legumes have a higher demand for P compared to other crop species, as they utilize substantial amounts of this macronutrient during key activity of nitrogen fixation (Mitran *et al*., 2018). The low availability and mobility of P in soils hinder plant growth, and although P fertilizers are used, only 20 - 30% is absorbed by plants in the first year (Sun *et al*., 2018). The heavy usage of P fertilizer not only triggers devastating eutrophication in aquatic ecosystems but also accelerates the depletion of finite rock phosphate reserves (Zak *et al*., 2018). Therefore, developing high yielding P efficient

cultivars with improved P use efficiency (PUE) that rely on reduced fertilizer use is a critical step toward preserving global P stocks and addressing environmental problems (Han *et al*., 2022).

The two main components of PUE are P acquisition efficiency (PAqE) which refers to the ability of the plants to take up the P and P assimilation efficiency (PAsE), which is the plant's capacity to effectively utilize the absorbed P for physiological and metabolic processes to enhance growth and productivity and typically evaluated as the amount of P utilized per unit biomass (Dissanayaka *et al*., 2018). However, an important gap remains in understanding how chickpea genotypes establish and sustain growth in early seedling stage under P limitation. This stage is particularly critical because successful establishment in P deficient marginal soils depends on early root development, biomass accumulation and efficient P use before plants progress to later growth phases. Accordingly, the screening at 35 days under hydroponic conditions offers a robust and controlled means to identify genotypes with early adaptive capacity, while minimising the confounding effects of soil variability and enabling precise assessment of biomass and P-related traits. These adaptive responses are closely associated with genotypic plasticity, which refers to the ability of a genotype to produce different phenotypes in response to environmental variation, while maintaining the same genetic constitution (Nicotra *et al.,* 2010). Under P deficient conditions, plasticity is commonly manifested through modifications in root system architecture, biomass partitioning, nutrient acquisition and physiological processes that improve P acquisition and assimilation (Lynch, 2011). Such plastic responses are increasingly recognised as key determinants of adaptation to nutrient limited environments, and have been documented across several crop species, including legumes, where substantial genotypic variation in P acquisition and assimilation has been reported (Pang *et al.,* 2018). Consequently, understanding genotypic plasticity provides valuable insight into the mechanisms underlying PUE and facilitates the identification of superior genotypes for breeding under low P environments. The present study was aimed to assess the genotypic plasticity of chickpea under varying P levels using a hydroponic system and to identify P efficient genotypes using three complementary approaches, *viz*., PUE index, principal component analysis (PCA), and multi-trait genotype-ideotype distance index (MGIDI), insights from which would be helpful in chickpea breeding programmes. Since no single index fully captures the multidimensional nature of PUE, these methods were combined to obtain a more comprehensive and less biased evaluation of genotypes across the correlated traits. To the best of our knowledge, this is the first study in chickpea to integrate PUE index, PCA and MGIDI as complementary screening tools for evaluating PUE associated biomass traits at the seedling stage.

MATERIALS AND METHODS

Experimental materials and growth environment

The present study, employing a hydroponics-based screening during 2024-25, evaluated 33 chickpea genotypes (31 cultivars and 2 check landraces). Genotype IG 5860 was used as efficient check and ILC 10456 as inefficient check, both sourced from the ICARDA. The remaining 31 test genotypes were obtained from diverse national and international breeding programmes to capture a broad genetic base. Specifically, the panel included 16 genotypes [Pusa Green 112 (BGD 112), Pusa 72, Pusa 362, Pusa 1103, Pusa 5023, Pusa 3022 (BG 3022), BGD 111-1, Pusa 2085, BG 1053, BGD 103, Pusa 372, BG 3043, Pusa Chickpea 10216 (BGM 10216), Pusa Vijay (BGM 10217), Pusa Chickpea Manav (BGM 20211), and Pusa JG 16] sourced from ICAR-IARI, New Delhi. Four genotypes *viz*., JG 14, JG 11, JG 16, and JG 315 were sourced from JNKVV, Jabalpur. The study also included genotype ICCV 10 from ICRISAT, Patancheru; RSG 888 from SKNAU, Jobner, GNG 1581 from SKRAU, Bikaner; DCP 92-3 from ICAR-IIPR, Kanpur, KWR 108 from CSAUAT, Kanpur; C 235 from PAU, Ludhiana; FG 212 from ANDUAT, Ayodhya, and PG 0515 from MPKV, Rahuri. The panel also included Indian germplasm collection T-39-1 along with two Australian cultivars, RUPALI and

Genesis 836. The seeds were supplied by the Division of Genetics, ICAR-IARI, New Delhi. The seeds were treated with carbendazim 50 WP (Bavistin 50 WP) @ 1 g L^{-1} and subsequently rolled in germination paper to promote germination. Seven-day-old seedlings of consistent size were transplanted into a nutrient medium, with the entire experiment conducted under controlled conditions using two distinct setups: one receiving adequate P (1000 µM) and the other receiving low P (10 µM). The nutrient solution composed of $Ca(NO_3).4H_2O$ (4 mM), KNO_3 (6 mM), $MgSO_4.7H_2O$ (4 mM), $NH_4H_2PO_4$ (1 mM), H_3BO_3 (0.01 mM), $MnCl_2.4H_2O$ (0.002 mM), $ZnSO_4.7H_2O$ (0.0003 mM), $CuSO_4.5H_2O$ (0.0002 mM), $Na_2MoO_4.2H_2O$ (0.00008 mM), $Co(NO_3)_2.6H_2O$ (0.025 mM), NaOH (0.16 mM), and Fe-EDTA (0.1 mM). The experiment was conducted under controlled growth conditions with a daytime temperature of 25±2ºC and a night time temperature of 15±2ºC, a relative humidity of 50 ± 5%, a photoperiod of 10 h light and 14 h darkness at an intensity of 425 ± 450 µ mol m^{-2} s^{-1} (photosynthetic photon flux density; PPFD), and an ambient CO_2 concentration of 380-400 ppm. The pH of the solution was maintained at 6.5 and monitored regularly.

Data collection

Data was recorded on 35-day-old seedlings, with shoots and roots separated for measurement. Shoot fresh weight (SFW, g $plant^{-1}$) and root fresh weight (RFW, g $plant^{-1}$) were determined after which samples were oven-dried at 62ºC for 72 h. Subsequently, shoot dry weight (SDW, g $plant^{-1}$), root dry weight (RDW, g $plant^{-1}$), and total dry weight (TDW, g $plant^{-1}$) were noted. The shoot P concentration (SPC, mg $plant^{-1}$) and root P concentration (RPC, mg $plant^{-1}$) were analysed using the vanadomolybdate yellow method following digestion with an HNO_3 and $HClO_4$ solution (Kitson and Mellon, 1944). PAqE and PAsE were calculated with the help of following formulas (Ramtekey *et al*., 2021; Rajamanickam *et al*., 2024).

$$\text{PAqE (mg plant}^{-1}\text{)} = \text{P concentration} \times \text{TDW}$$

$$\text{PAsE (\%)} = [\text{TDW/ P concentration}] \times 100$$

Statistical analysis

The experiment was conducted in a completely randomised design with 5 replications per treatment under both low P (LP) and adequate P (AP) conditions. Two-way analysis of variance (ANOVA) was performed using 'inti' package R statistical software (R Core Team 2025; *https://www.r-project.org/*). Data for SFW, RFW, RDW, SPC, PAqE, and PAsE were log-transformed to meet model assumptions, as these traits exhibited non-normality or heteroscedasticity on the original scale. Model assumptions were validated by examining normality of residuals graphically using quantile-quantile (QQ) plots (Wilk and Gnanadesikan, 1968) and statistically using the Shapiro-Wilk test (Shapiro and Wilk, 1965) while homogeneity of variances was assessed using Levene's test (Levene, 1960). The percent change in the mean of the i^{th} trait in response to P stress created due to low P supply was calculated using the following formula (Vu *et al.,* 2021).

$$\text{Percent change in mean of } i^{th} \text{ trait} = \frac{\overline{LP_i} - \overline{AP_i}}{\overline{AP_i}} \times 100$$

where $\overline{LP_i}$ is the mean of the i^{th} trait across all "n" genotypes under low P conditions and $\overline{AP_i}$ is the mean of the i^{th} trait across all "n" genotypes under adequate P conditions. A negative percent change indicates a reduction in the trait mean under P stress relative to the adequate P treatment, whereas a positive value denotes a stress induced enhancement.

Pearson correlation coefficient was calculated using "metan" package (version 1.19.0). Principal component analysis (PCA) and hierarchical clustering were performed utilizing the "factoextra" package (version 1.0.7). The statistical analysis of multitrait genotype ideotype distance index (MGIDI) was computed using "metan" package (version 1.19.0). All analyses were conducted in the R statistical software using RStudio as the interface. (R Core Team 2025; *https://www.r-project.org/*).

Classification of genotypes based on PUE index

Genotypes were classified as efficient, moderately efficient, or inefficient based on a PUE index calculated as described below (Joshi *et al.,* 2023).

$$PUE\ Index = \frac{(SFW)_{LP}X(SFW)_{AP}}{(\overline{SFW})^2_{AP}} + \frac{(RFW)_{LP}X(RFW)_{AP}}{(\overline{RFW})^2_{AP}} + \frac{(SDW)_{LP}X(SDW)_{AP}}{(\overline{SDW})^2_{AP}} +$$
$$\frac{(RDW)_{LP}X(RDW)_{AP}}{(\overline{RDW})^2_{AP}} + \frac{(TDW)_{LP}X(TDW)_{AP}}{(\overline{TDW})^2_{AP}} + \frac{(SPC)_{LP}X(SPC)_{AP}}{(SPC)^2_{SP}} + \frac{(RPC)_{LP}X(RPC)_{AP}}{(\overline{RPC})^2_{AP}} +$$
$$\frac{(PAqE)_{LP}X(PAqE)_{AP}}{(\overline{PAqE})^2_{AP}} + \frac{(PAsE)_{LP}X(PAsE)_{AP}}{(\overline{PAsE})^2_{AP}}$$

The description of the PUE index is as follows. For example, in the above formula $(SFW)_{LP}$ refers to the SFW value of a particular genotype in low P condition, $(SFW)_{AP}$ refers to the SFW value of the same genotype in adequate P condition and $(\overline{SFW})^2_{AP}$ refers to the mean value of all the genotypes of the trait SFW in adequate P condition. The summation of all the trait indices resulted in a cumulative stress tolerance index, termed the PUE index. After computing the PUE index for each genotype, mean ($\overline{x}$) and standard deviation (s) were determined. Genotypes with PUE index < ($\overline{x}$ − 0.5 s) were categorized as inefficient, those with PUE index > ($\overline{x}$ + 0.5 s) were classified as efficient, and those falling between ($\overline{x}$ − 0.5 s) and ($\overline{x}$ + 0.5 s) were considered moderately efficient.

RESULTS AND DISCUSSION

This study investigated PUE in 33 chickpea genotypes under controlled hydroponic conditions at the seedling stage. Hydroponic system was used to overcome the limitations associated with soil-based assessment of root traits at the seedling stage, as it allowed precise control over nutrient supply, and eliminated the confounding soil factors. Further, it ensured that plant responses reflect P availability alone, thereby provided more reliable and accurate early-stage PUE screening. This approach has successfully been adopted for PUE evaluation in mungbean (Kothari *et al.*, 20223), lentil (Ramtekey *et al.,* 2021), wheat (Rajamanickam *et al.,* 2024), maize (Liang *et al.,* 2025), and rice (Zhang *et al.,* 2025). In present study, two distinct P levels, adequate P (1000 µM) and low P (10 µM), were used to create a biologically meaningful contrast between adequacy and deficiency, and maximize the differences in plant responses, consistent with treatment concentrations established for chickpea hydroponic P stress experiments (Alloush, 2003). Intermediate P concentrations were not used because they often mask genotype specific responses by producing overlapping phenotypes, thereby lowers the discriminatory power of screening system. Screening at 35-day seedling stage is consistent with hydroponic P stress studies in rice (Zhang *et al.,* 2025), maize (Tang *et al.,* 2019), and chickpea has also been successfully phenotyped in semi-hydroponic systems for trait-based selection (Chen *et al.,* 2017). Early-stage screening is not merely a methodological convenience, but an agronomic necessity, because genotypes that fail to establish adequate biomass under P limitation at seedling stage are unlikely to express useful PUE in terms of yield, as successful early establishment is essential for higher productivity. Accordingly, the traits measured in this study included biomass related parameters, namely, SFW, RFW, SDW, RDW, and TDW, together with P related measurements, including SPC, RPC, PAqE, and PAsE which collectively reflect both the ability of genotypes to establish biomass and their efficiency in acquiring and assimilating P under contrasting P supply conditions.

Analysis of variance (ANOVA)

After log transformation, residual diagnostics indicated that normality was adequately met for seven of the nine traits, whereas RDW and PAqE still showed some deviation from normality. Homogeneity of variances was satisfied for all traits based on Levene's test. Two-way ANOVA was retained for these traits because of its moderate robustness to non-normality in balanced designs (Schmider *et al.*, 2010; Blanca *et al.*, 2017). QQ plots of residuals further supported approximate normality across all traits (Fig. 1). Two-way ANOVA revealed highly significant effects of genotype, P treatment, and

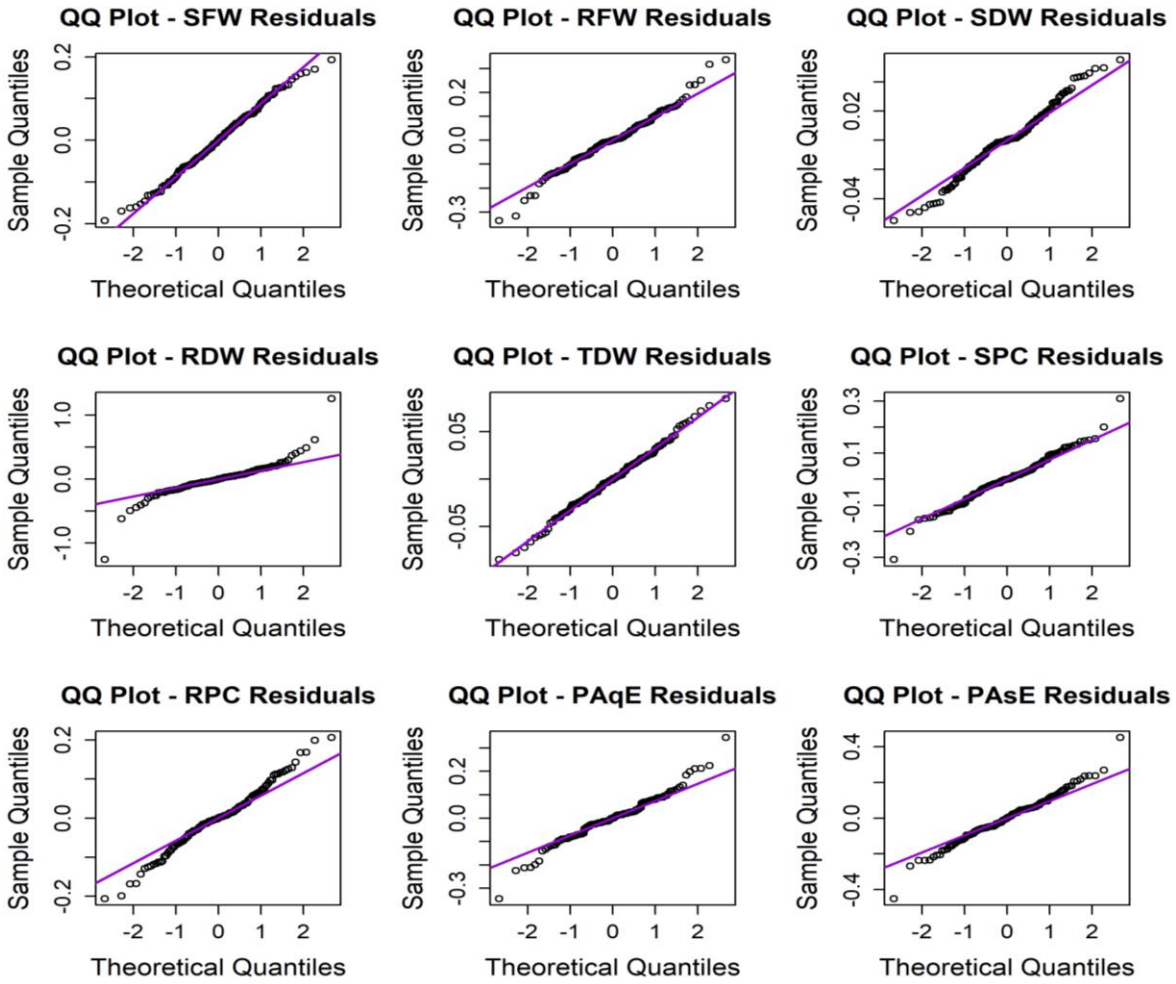

Fig. 1: Quantile-Quantile (QQ) plots of two-way ANOVA residuals for nine PUE traits (traits in sequence) namely, SFW: Shoot fresh weight (g plant^{-1}), RFW: Root fresh weight (g), SDW: Shoot dry weight (g plant^{-1}) [top row], RDW: Root dry weight (g plant^{-1}), TDW: Total dry weight (g plant^{-1}), SPC: Shoot P concentration (mg plant^{-1}), [middle row]RPC: Root P concentration (mg plant^{-1}), PAqE: P acquisition efficiency (mg plant^{-1}), PAsE: P assimilation efficiency (%), [bottom row] across 33 chickpea genotypes evaluated under contrasting P treatments. Points aligned along the purple reference line indicate approximate normality of residuals, thereby validating the ANOVA model assumptions.

their interaction on all evaluated traits. Genotype mean squares were significant ($p < 0.001$) for SFW, RFW, SDW, RDW, TDW, SPC, RPC, PAqE and PAsE indicating substantial genetic variation among the 33 genotypes. Treatment-effects were significant for most traits, with exception of SFW (F = 1.306, $p > 0.05$) and RPC (F =1.359, $p > 0.05$) suggesting that P supply differentially influenced biomass accumulation and P dynamics. Significant genotype × treatment interactions were observed across all traits, demonstrating that genotypic responses to contrasting P regimes were not uniform and confirming the presence of differential PUE among the evaluated genotypes (Table 1). The highly significant genotype, treatment, genotype × treatment effects show that the 33 genotypes differed not only in mean performance, but also in the way they responded to P supply. Similar significant interaction effects have been reported in mungbean (Reddy *et al.*, 2020), lentil (Ramtekey *et al.*, 2021) and wheat (Rajamanickam *et al.*, 2024), indicating the differential genotypic response to P availability is a consistent feature across crop species.

Table 1: Two-way ANOVA results showing mean sum of squares, F values, and model assumption diagnostics for 9 PUE traits evaluated in 33 chickpea genotypes under LP and AP conditions

Trait	Mean sum of squares			F value			Shapiro-Wilk test[$]		Levene's test[#]	
	Genotype	Treatment	Genotype x Treatment	Genotype	Treatment	Genotype x Treatment	Test statistic	p-value	Test statistic	p-value
Df	32	1	32	32	1	32	-	-	-	-
SFW	4.765	0.092	0.252	67.548***	1.306^{ns}	3.569***	0.9931	0.7747	0.0638	0.801
RFW	4.622	2.936	0.242	33.481***	21.27***	1.752*	0.9831	0.1012	1.2113	0.2731
SDW	0.05112	0.02384	0.01267	50.47***	28.54***	12.51***	0.9849	0.1547	0.1217	0.7277
RDW	0.00975	0.09499	0.00262	15.267***	148.697***	4.098***	0.8451	<0.001	0.0071	0.933
TDW	0.09733	0.02365	0.0181	46.051***	11.191**	8.564***	0.9961	0.9776	0.4454	0.5057
SPC	1.373	21.215	0.45	62.21***	960.95***	20.39***	0.9834	0.1092	0.7028	0.4034
RPC	0.17194	0.01573	0.0288	14.85***	1.359^{ns}	2.487**	0.9874	0.2663	2.125	0.1473
PAqE	2.286	22.09	0.545	51.84***	500.89***	12.35***	0.9686	0.0038	0.8605	0.3553
PAsE	72.5	896.4	32.2	23.87***	295.03***	10.6***	0.9828	0.095	2.8634	0.093

^{ns} $p \geq 0.05$; *$p < 0.05$; **$p < 0.01$; and ***$p < 0.001$; ns = non-significant; SFW: Shoot fresh weight (g plant^{-1}), RFW: root fresh weight (g plant^{-1}), SDW: Shoot dry weight (g plant^{-1}), RDW: Root dry weight (g plant^{-1}), TDW: Total dry weight (g plant^{-1}), SPC: Shoot P concentration (mg plant^{-1}), RPC: Root P concentration (mg plant^{-1}), PAqE: P acquisition efficiency (mg plant^{-1}), and PAsE: P assimilation efficiency (%).

[$]Shapiro-Wilk test was used to assess normality of ANOVA residuals, where $p < 0.05$ indicates deviation from normality.

[#]Levene's test was used to assess homogeneity of variances across P treatments, where $p > 0.05$ indicates equal variances

Percentage change in mean values under low P conditions

Under low P conditions, the SPC (-45.6%) was drastically reduced followed by PAqE (-36.1%), SDW (-9.2%), RPC (-4.2%) and SFW (-2.3%). In contrast, PAsE (82.7%) was highest under low P conditions, accompanied by an increase in RDW (62.7%), RFW (19.9%) and TDW (7.1%) (Fig. 2). This pattern indicates that low P stress reduced shoot P accumulation, but stimulated greater biomass allocation to roots, reflecting an adaptive shift toward enhanced nutrient foraging. An increase in root to shoot ratio is a well-documented and characteristic response of plants to P deficiency (Xiao *et al.*, 2024). This response is mechanistically driven by P starvation induced sucrose loading into the phloem, which redirects the shoot derived carbon toward the roots, thereby promoting greater root biomass accumulation relative to shoot biomass (Hammond and White, 2008; Huang and Zhang, 2020). The higher RDW and RFW observed in this study under low P conditions are consistent with this adaptive carbon partitioning strategy, which enhances the root surface area available for P acquisition. At the

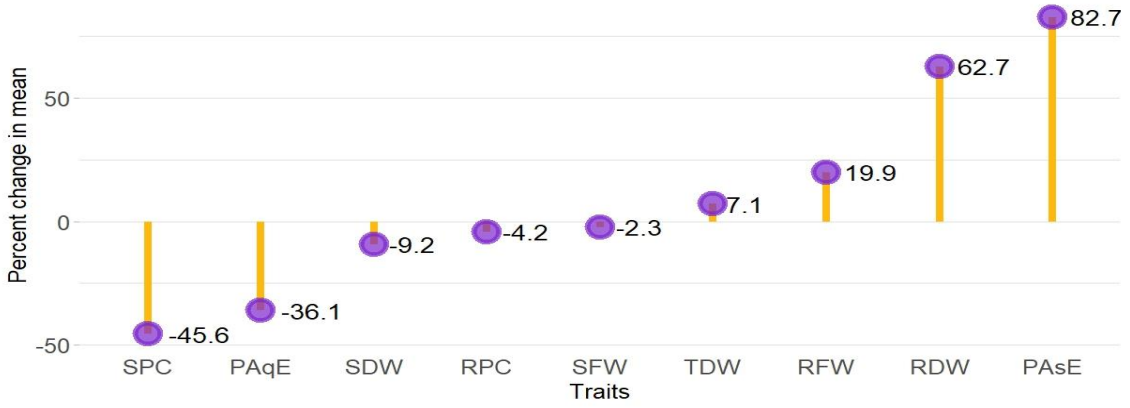

Fig. 2: Lollipop plot depicting the percentage change in mean values under low P conditions. SFW: Shoot fresh weight (g plant^{-1}), RFW: Root fresh weight (g), SDW: Shoot dry weight (g plant^{-1}), RDW: Root dry weight (g plant^{-1}), TDW: Total dry weight (g plant^{-1}), SPC: Shoot P concentration (mg plant^{-1}), RPC: Root P concentration (mg plant^{-1}), PAqE: P acquisition efficiency (mg plant^{-1}), PAsE: P assimilation efficiency (%).

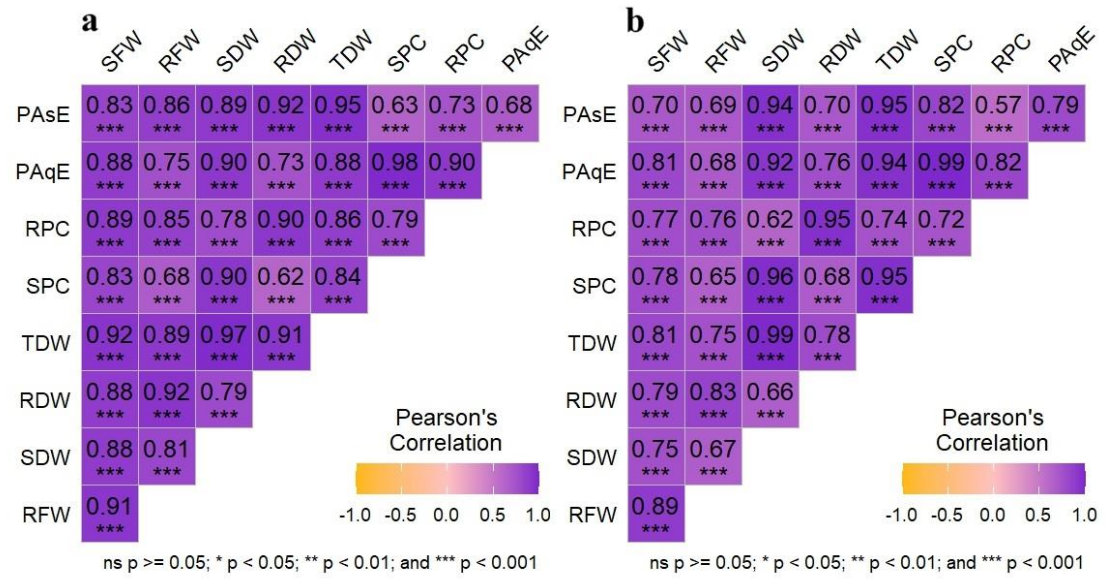

Fig. 3: Pearson correlation heatmap under (a) low P conditions and (b) adequate P conditions across 33 chickpea genotypes. Colour intensity indicates the strength of correlation, with a darker purple representing stronger positive associations. SFW: Shoot fresh weight (g plant^{-1}), RFW: Root fresh weight (g), SDW: Shoot dry weight (g plant^{-1}), RDW: Root dry weight (g plant^{-1}), TDW: Total dry weight (g plant^{-1}), SPC: Shoot P concentration (mg plant^{-1}), RPC: Root P concentration (mg plant^{-1}), PAqE: P acquisition efficiency (mg plant^{-1}), PAsE: P assimilation efficiency (%).

physiological level, this response is partly regulated by hormonal changes, including reduced cytokinin levels that limit shoot growth and altered auxin and ethylene signalling that promote root development and lateral root formation, as demonstrated mainly in model species such as *Arabidopsis* (López-Bucio *et al.*, 2002; Wang *et al.*, 2006). Similar root driven P tolerance has been reported in chickpea (Thudi *et al.*, 2021), soybean (Zhou *et al.*, 2016), maize in semi hydroponics (Linag *et al.*, 2023) and rice in nutrient solution, where low P increased root to shoot ratio and root length in tolerant genotypes (Prathap *et al.*, 2023; Liu *et al.*, 2024). These results support biomass and tissue P as practical indicators of PUE.

Correlation analysis under low P and adequate P conditions

Under low P, Pearson correlation analysis revealed significant correlations ($p < 0.001$) among P accumulation and biomass traits. PAsE was strongly associated with TDW, RDW and SDW, while PAqE showed an especially strong association with SPC and TDW. The biomass traits were also tightly linked with TDW correlating strongly with SDW, RDW, RFW and SFW. Under adequate P, the same overall pattern persisted, but several relationships became even stronger, particularly PAqE with SPC and SDW with TDW. Thus, genotypes with greater shoot and root biomass showed higher P acquisition and better P partitioning. All major correlations were positive and highly significant, indicating coordinated variation among growth, nutrient status and PUE traits in both conditions (Fig. 3).

The positive and highly significant correlations among TDW, SDW, RDW, RFW, and SFW indicated coordinated biomass accumulation across shoot and root components under contrasting P conditions, reflecting integrated growth responses rather than isolated trait adjustments. Similar coordination among dry weight and fresh weight traits under low P was reported in maize seedlings, where RDW was positively associated with SDW and most other traits (Schuster *et al.*, 2024; Hao *et al.*, 2025). The strong PAqE-SPC and PAsE-TDW relationships in present study suggest that efficient genotypes under low P conditions were able to take up P and produce biomass more effectively.

Categorization of genotypes based on PUE index

The highest PUE index score was observed in the efficient check IG 5860 (61.5848), while the lowest

was recorded for RSG 888 (2.7116) (Table 2). Among the 33 genotypes evaluated, five genotypes (IG 5860, Pusa 3022 (BG 3022), PG 0515, Pusa 5023, and Pusa 72) were classified as efficient, 16 as moderately efficient, and 12 as inefficient. Among the inefficient genotypes, RSG 888, GNG 1581, and Pusa Green 112 (BGD 112) exhibited the lowest overall efficiency. Notably, none of the cultivars exhibited a PUE index equal to or higher than IG 5860, emphasizing its potential as a valuable donor for breeding P efficient cultivars.

Table 2: Genotype categorization into Efficient, Moderately Efficient, and inefficient based on PUE index scores

Genotypes	Geno-type No.	SFW	RFW	SDW	RDW	TDW	SPC	RPC	PAqE	PAsE	PUE index score	Category
IG 5860	32	6.356	16.3905	3.6196	10.746	5.1208	1.2863	3.7922	1.6354	12.638	61.5848	E
Pusa 3022 (BG 3022)	10	3.8865	7.1476	4.0127	5.279	4.3358	2.706	3.5145	2.9347	6.308	40.1248	E
PG 0515	22	4.5224	2.4725	2.5813	3.6698	2.8857	2.3653	3.3197	2.6568	3.0853	27.5588	E
Pusa 5023	9	2.8936	3.4907	2.0039	4.1159	2.4516	1.4312	2.9022	1.7645	3.3518	24.4054	E
Pusa 72	6	1.2341	3.2024	1.1089	3.9846	1.6204	0.6609	2.3446	0.9671	2.6761	17.7991	E
Pusa 2085	14	2.0749	2.8219	1.5422	2.5557	1.7677	0.9328	1.5261	1.0777	2.855	17.154	ME
KWR 108	28	1.0539	1.6881	1.8698	1.9234	1.9779	1.05	1.0663	1.1108	3.4836	15.2238	ME
Pusa Chickpea 10216 (BGM 10216)	25	1.5704	1.4185	1.3127	2.6036	1.655	0.5338	1.0453	0.6744	4.0215	14.8352	ME
Pusa Chickpea Manav (BGM 20211)	30	1.2005	1.516	1.4791	1.2941	1.5214	0.7619	0.658	0.7793	2.9751	12.1854	ME
BG 1053	15	1.034	1.0229	0.952	2.1321	1.1797	0.5864	1.2966	0.7308	1.8745	10.809	ME
JG 16	11	1.191	1.018	1.4832	0.8244	1.3215	0.88	0.4829	0.7876	2.1852	10.1738	ME
Pusa 1103	8	0.751	1.115	0.7115	2.2961	0.9933	0.4869	1.5511	0.6796	1.4319	10.0164	ME
BGD 111-1	12	0.9226	0.7885	0.8086	1.2993	0.9266	0.5582	0.8852	0.6431	1.3148	8.1469	ME
BGD 103	17	1.104	0.8909	0.7638	1.1623	0.8525	0.539	0.8098	0.6064	1.1804	7.9091	ME
JG 14	2	1.0056	1.1423	0.4877	1.476	0.6658	0.3758	1.1228	0.5117	0.8543	7.642	ME
RUPALI	19	0.8484	0.7737	0.6749	1.2833	0.8102	0.4219	0.7919	0.5092	1.2694	7.3829	ME
GENESIS 836	20	0.9686	1.1211	0.5655	1.2679	0.7093	0.3346	0.7406	0.4216	1.1747	7.3039	ME
BG 3043	23	0.4789	0.5752	0.5487	1.3845	0.7197	0.2949	0.7346	0.3867	1.3212	6.4444	ME
T-39-1	26	0.5941	0.6291	0.483	1.4167	0.6724	0.2537	0.7344	0.3528	1.2644	6.4006	ME
FG 212	27	0.272	0.1631	0.846	0.7	0.8918	0.4754	0.3883	0.5018	1.5639	5.8023	ME
Pusa 372	21	0.2874	0.254	0.5863	1.1974	0.7154	0.2464	0.4968	0.303	1.6649	5.7516	ME
ICCV 10	1	0.4518	0.5246	0.5247	0.862	0.6058	0.448	0.7267	0.5201	0.6946	5.3583	I
C-235	18	0.3409	0.4365	0.3936	1.2587	0.5657	0.2838	0.896	0.4102	0.7689	5.3543	I
Pusa JG 16	31	0.5945	0.5139	0.5974	0.7309	0.6311	0.3043	0.3676	0.323	1.2146	5.2773	I
JG 11	3	0.5834	0.7623	0.3264	1.0562	0.4683	0.2461	0.7859	0.3556	0.6068	5.191	I
JG 315	24	0.5211	0.2884	0.581	0.6918	0.6258	0.2986	0.351	0.3168	1.2186	4.8931	I
PusaVijay (BGM 10217)	29	0.4853	0.31	0.5712	0.7264	0.609	0.2623	0.3292	0.2809	1.3007	4.875	I
Pusa 362	7	0.4292	0.6511	0.5333	0.499	0.6053	0.2306	0.213	0.2643	1.3672	4.793	I
DCP 92-3	13	0.5898	0.4428	0.5029	0.613	0.538	0.3527	0.4244	0.3795	0.7507	4.5938	I
ILC 10456	33	0.4176	0.5347	0.3985	0.803	0.458	0.3203	0.637	0.3574	0.5366	4.4631	I
Pusa Green 112 (BGD 112)	5	0.3911	0.384	0.3032	0.5437	0.3533	0.1884	0.3336	0.22	0.5585	3.2758	I
GNG 1581	16	0.2832	0.3309	0.2096	0.5509	0.274	0.1575	0.4087	0.2063	0.3587	2.7798	I
RSG 888	4	0.2843	0.3355	0.2005	0.5754	0.2686	0.0992	0.2813	0.1332	0.5336	2.7116	I

SFW: Shoot fresh weight (g plant^{-1}), RFW: Root fresh weight (g), SDW: Shoot dry weight (g plant^{-1}), RDW: Root dry weight (g plant^{-1}), TDW: Total dry weight (g plant^{-1}), SPC: Shoot P concentration (mg plant^{-1}), RPC: Root P concentration (mg plant^{-1}), PAqE: P acquisition efficiency (mg plant^{-1}), PAsE: P assimilation efficiency (%); E = Efficient; ME = Moderately efficient; I = Inefficient.

Principal component analysis (PCA) and cluster analysis

To identify genotypes tolerant to low P stress, PCA was conducted for all traits. The first two principal components (PC), PC1 and PC2 accounted for 86.1 and 7.8% of the total variance, respectively, capturing 94% of variation (Fig. 4). The analysis revealed that SPC and PAqE were closely associated with each other and contributed more strongly along PC2, while SDW, SFW, TDW and RPC were strongly aligned along PC1, reflecting the dominant role of biomass accumulation traits in explaining

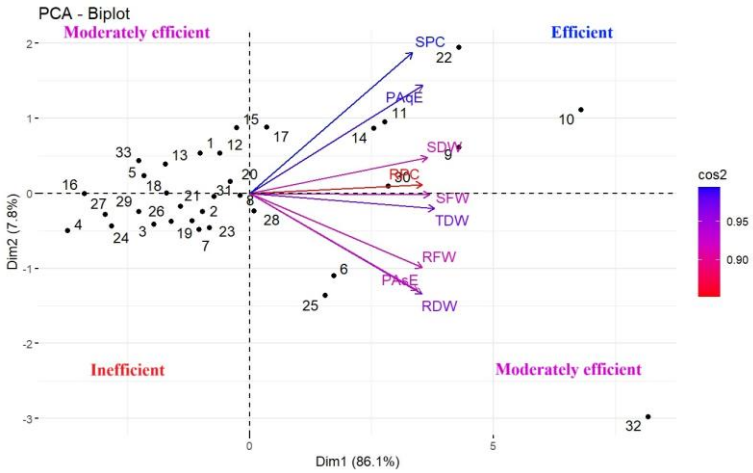

Fig. 4: Principal componnent analysis biplot highlighting PC1 and PC2 axes, with cos2 based representation quality of variables. A high cos2 value indicates that a variable is well represented on the respective PC, while a low value indicates poor representation. SFW: Shoot fresh weight (g plant^{-1}), RFW: Root fresh weight (g), SDW: Shoot dry weight (g plant^{-1}), RDW: Root dry weight (g plant^{-1}), TDW: Total dry weight (g plant^{-1}), SPC: Shoot P concentration (mg plant^{-1}), RPC: Root P concentration (mg plant^{-1}), PAqE: P acquisition efficiency (mg plant^{-1}), PAsE: P assimilation efficiency (%). Genotype numbers are plotted as per their scores on PC1 and PC2, with quadrant positions indicating their classification as efficient, moderately efficient, or inefficient under low P stress.

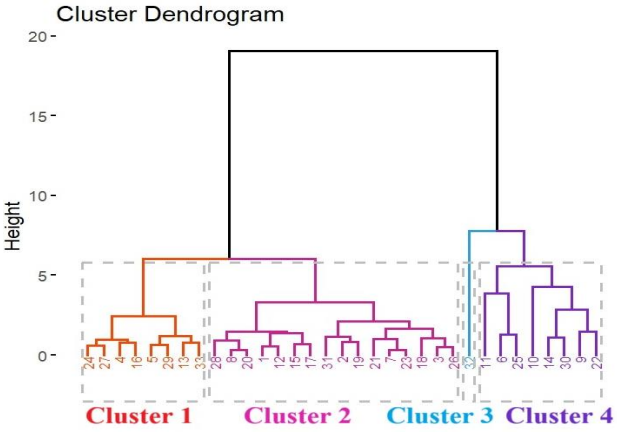

Fig. 5: Cluster dendrogram depicting the grouping of 33 chickpea genotypes into four distinct clusters based on PUE associated trait performance. The height on the Y-axis represents the Euclidean distance between clusters, with greater height indicating greater dissimilarity. Cluster 1 (orange) represents the most P sensitive genotypes, Cluster 2 (pink) comprises moderately performing genotypes, Cluster 3 (sky blue) contains the single highly distinct genotype IG5860, and Cluster 4 (purple) includes the top performing genotypes.

genotypic variation. Genotypes positioned in the upper right quadrant (Quadrant I) of biplot, exhibiting the highest PC1 and PC2 scores, were categorized as efficient. These included Pusa 3022 (BG 3022) (10), PG 0515 (22), Pusa 5023 (9), Pusa Chickpea Manav (BGM 20211) (30), JG 16 (11), Pusa 2085 (14), and BGD 103 (17). Genotypes in the upper left quadrant (Quadrant II) and lower right (Quadrant IV) quadrants, with moderate PC1 and PC2 scores, were moderately efficient. Mean-while, those in the lower left quadrant (Quadrant III), having the lowest PC1 and PC2 scores, were deemed to be inefficient under low P stress. Notably, the efficient check genotype IG 5860 (32) was positioned far from the main cluster, indicating an extreme combination of trait values.

Hierarchical cluster analysis grouped the genotypes into 4 clusters (Fig. 5). Cluster 1 consisted of eight genotypes namely JG 315 (24), FG 212 (27), RSG 888 (4), GNG 1581 (16), Pusa Green 112 (BGD 112) (5), Pusa Vijay (BGM 10217) (29), DCP 92-3 (13), and ILC 10456 (33) all of which were highly sensitive to low P stress and identified as the weakest performers. Cluster 2 comprised 16 genotypes that demonstrated moderate performance under low P conditions. Cluster 3 had a single genotype, IG 5860 (32), which was separated due to its extreme values and unique trait combinations. Cluster 4 included eight genotypes, among which Pusa 3022 (BG 3022) (10), PG 0515 (22), and Pusa 5023 (9) emerged as the top performers, while Pusa Chickpea Manav (BGM 20211) (30), JG 16 (11), Pusa Chickpea 10216 (BGM 10216) (25), and Pusa 72 (6) were also grouped within this cluster.

MGIDI based selection of genotypes

The top five genotypes namely IG 5860, Pusa

3022 (BG 3022), PG 0515, Pusa 5023, and Pusa Chickpea Manav (BGM 20211) were identified as the best performers under low P stress conditions using the MGIDI index, with a selection intensity of 15% (Fig. 6). The genotype with lowest MGIDI score was found to be the closest to the ideotype (Table 3). MGIDI provides a clear and effective approach, eliminating the need for weight coefficients and mitigating issues related to multicollinearity (Olivoto *et al*., 2022).

Comparison of PUE index, PCA and MGIDI for genotype classification and selection

The PUE index was calculated using trait values under both low P and adequate P conditions, identifying genotypes with higher SFW, RFW, SDW, RDW, TDW, SPC, RPC, PAqE, and PAsE as top performers in both stress and non-stress conditions. Based on this, IG5860, Pusa 3022 (BG 3022), PG0515, Pusa 5023, and Pusa 72 were classified as efficient genotypes. Additionally, PCA and MGIDI analyses were conducted to identify the best performing genotypes under low P conditions. PCA highlighted Pusa 3022 (BG 3022), PG0515, Pusa 5023, Pusa Chickpea Manav (BGM 20211), and JG16 as having the highest PC1 and PC2 scores, while IG5860 exhibited the highest positive PC1 scores and lowest negative PC2 scores, reinforcing its status as the most efficient check genotype given that PC1 explained a substantial 86.1% of the variation compared to 7.8% by PC2. MGIDI analysis further confirmed Pusa 3022 (BG 3022), PG 0515, Pusa 5023, and Pusa Chickpea Manav (BGM 20211) as the genotypes closest to the ideotype, with a selection intensity of 15%. Notably, all the three methods PUE index, PCA and MGIDI consistently identified IG 5860, Pusa 3022 (BG 3022), and PG 0515 as top 3 performers, revealing the reliability of these genotypes across different analytical approaches.

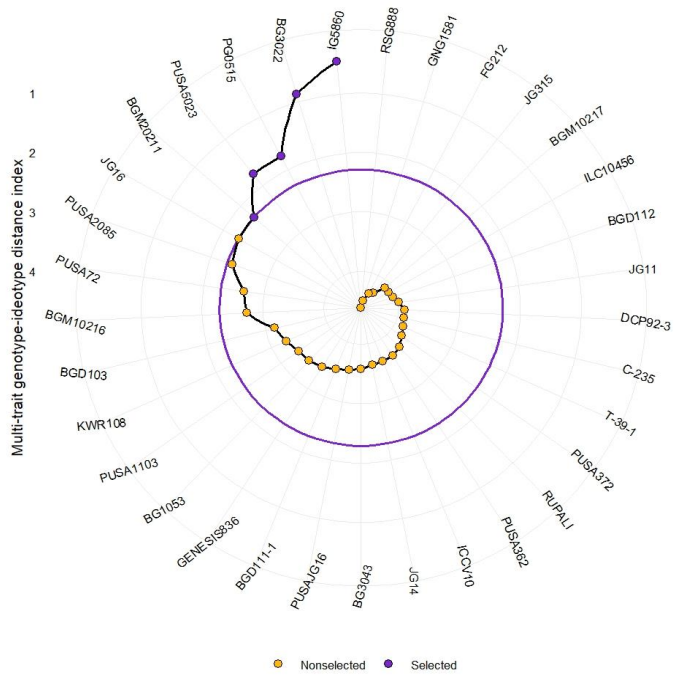

Fig. 6: Genotype ranking in ascending order of the MGIDI score. Genotypes with lower MGIDI score are closest to the ideotype and are shown in the solid purple circles. The purple ring represents the selection cut-point corresponding to a selection intensity of 15%, with selected genotypes falling beyond this cut point.

The PUE index was useful as a first screening tool because it integrated performance across both low and adequate P conditions and separated efficient, moderately efficient and inefficient genotypes. Its main strength is simplicity, but it gives a single summary score and therefore does not show which trait combinations are driving the score. This is where PCA becomes valuable. PCA shows that most of the variation was captured by the first component, meaning that the main PUE pattern in these genotypes was governed by a common trait axis involving biomass and P status. Beyond trait associations, PCA also enabled a direct classification and selection of efficient genotypes through biplot quadrant positioning, providing a visual and multivariate basis for genotype evaluation under low P stress. Such a result is useful for the breeders because it shows that a few linked traits are carrying much of the screening information, rather than many independent traits acting separately. Similar PCA based grouping has been used successfully in mungbean and lentil (Reddy *et al.,* 2020; Ramtekey *et al.,* 2021). The cluster pattern showed clear grouping of genotypes with similar trait profiles. The group containing inferior genotypes may serve as a reference for comparison, whereas the cluster

Table 3: MGIDI scores of the genotypes arranged in ascending order

Genotype number	Genotype name	MGIDI score
32	IG5860	0.449
10	Pusa3022(BG3022)	0.866
22	PG0515	1.75
9	Pusa5023	1.75
30	PusaChickpeaManav(BGM20211)	2.29
11	JG16	2.30
14	Pusa2085	2.38
6	Pusa72	2.67
25	PusaChickpea10216(BGM 10216)	2.74
17	BGD103	3.16
28	KWR108	3.26
8	Pusa1103	3.36
15	BG1053	3.38
20	GENESIS836	3.43
12	BGD111-1	3.50
31	PusaJG16	3.54
23	BG3043	3.59
2	JG14	3.63
1	ICCV10	3.65
7	Pusa362	3.66
19	RUPALI	3.70
21	Pusa372	3.79
26	T-39-1	3.86
18	C-235	3.89
13	DCP92-3	3.90
3	JG11	3.99
5	PusaGreen112(BGD112)	4.06
33	ILC10456	4.09
29	PusaVijay(BGM10217)	4.10
24	JG315	4.30
27	FG212	4.34
16	GNG1581	4.49
4	RSG888	4.61

with superior performance can be considered as a source of promising material for further improvement. IG 5860 was separated from the remaining genotypes, indicating a distinct trait combination of potential interest in future selection work.

MGIDI added another layer of interpretation by providing a more robust framework for genotype selection, as it ranks genotypes by their distance from an ideotype rather than by any single trait, and it incorporates factor analysis to handle correlated traits and reduce multi-collinearity. This makes the index easy to interpret, less dependent on arbitrary weighting schemes, and more suitable for multi trait breeding decisions (Olivoto *et al.*, 2022; Olivoto and Nardino, 2020). The effectiveness of MGIDI for genotype selection has been demonstrated in other crops, including maize and rice, where it successfully identified superior genotypes across the multiple correlated traits (Shilpa *et al.*, 2026; Paramasivam *et al.*, 2025). The convergence among the PUE Index, PCA and MGIDI reflects a coherent trait architecture underlying PUE. Because the key traits were positively associated, genotypes with greater biomass also tended to show superior P acquisition and P assimilation, leading the three methods to favour the same high performing genotypes, especially IG 5860, PUSA 3022 and PG 0515. At the same time, the methods are not identical. The PUE index is best for a broad phenotypic summary, PCA is best for understanding trait structure and redundancy, and MGIDI is best for final breeding prioritization because it captures overall multitrait desirability. Therefore, the agreement among these approaches strengthens confidence in the identified genotypes and shows that the methods are complementary rather than interchangeable.

From a breeding perspective, the present study indicate the early-stage hydroponic screening can effectively distinguish chickpea genotypes with useful PUE related phenotypes. The top performing genotypes identified here may be considered promising parental material for further field evaluation. This is important because the seedling stage serves as an initial screening step rather than a final measure of field performance. However, it must be acknowledged that hydroponic conditions do not fully replicate the complexity of soil environments, where factors such as P fixation, microbial activity and root soil interactions collectively influence genotype performance. The selected efficient genotypes should therefore be advanced to multi location field trials under P deficient soil conditions to validate whether their early-stage advantage is maintained at the yield level. In parallel, molecular

characterization through QTL mapping and candidate gene analysis can provide insights into the genetic basis of superior PUE and support future marker assisted breeding efforts.

Conclusions: The findings of this study highlight the diverse responses of chickpea genotypes to varying P levels. Enhanced root growth under low P conditions signified a prioritized allocation of resources for efficient nutrient absorption. As expected, PAqE was lower, while PAsE was higher under low P conditions. ANOVA revealed significant variation due the genotypes, treatments and their interaction. The PUE index effectively classified genotypes based on the robust first order statistics. PCA proved valuable in identifying key traits associated with low P adaptability and selecting efficient genotypes. MGIDI provided a clear visual approach for pinpointing top-performing genotypes. The consistency in identifying the best performing genotypes among all the 3 methods reinforces the reliability of the selection process. However, field validation across seasons and environments is needed to confirm whether genotypes with superior seedling establishment also maintain yield performance and superior PUE under practical conditions consistently. Notably, none of the tested cultivars outperformed the efficient check genotype IG5860, indicating significant potential for breeding high PUE cultivars. IG5860 could serve as a valuable parent in developing mapping populations. Additionally, the most efficient and inefficient genotypes identified across all 3 methods can be utilized for allele mining and SNP detection of candidate loci associated with PUE.

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