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Effects of phosphorus availabilities on growth and yield of foxtail millet: Insights from high-throughput phenotyping platforms

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Main conclusion: Foxtail millet performance under low phosphorus (P) is determined by growth potential, with tiller number as a key indicator. Yield is influenced by P dilution rather than total P concentration.

Abstract

Foxtail millet, renowned for its high nutrient content and drought resilience, faces limited breeding investment despite being cultivated in vulnerable agri-systems. Low phosphorus (P) levels affect approximately 50% of global agricultural soils, and particularly impact regions

like Sub-Saharan Africa and Southeast Asia, the latter where foxtail millet is extensively grown. This study explores the effects of low P (<5 ppm; Hedley Fractionation Method; Cross and Schlesinger, 1995) on foxtail millet plant growth and yield-related traits, utilizing high-throughput platforms (HTP) with a selected subset of genotypes (n=10) from the core collection of ICRISAT Genebank. Results uncover substantial variation in plant growth and agronomical traits at both treatment and genotype levels. Under low P conditions, genotypic variation is noted, with a 6-fold difference in tiller count, 2.4-fold in grain yield, 2.7-fold in 3D-leaf area, and 2.3-fold in root surface area. A significant relationship was found between grain yield under low P and high P conditions ($R^2 = 0.65$; $P < 0.01$). This suggests that genetic yield potential (vigor) under high P conditions strongly influences grain yield and tiller numbers under low P conditions. Residual grain yield under low P conditions, not explained by high P conditions, had a strong positive association with tiller numbers ($R^2 = 0.70$; $P < 0.01$) and showed a significant negative association with total P concentration ($R^2 = 0.54$; $P < 0.05$). Conversely, under high P conditions, grain yield (GY_LF) from Lysi-Field exhibited significant positive correlations with phosphorus use efficiency (PUE) ($r = 0.94$; $P < 0.001$) and total biomass ($r = 0.84$; $P < 0.01$). These findings underscore the critical role of P availability in influencing grain yield and related traits. Under low P conditions, performance is primarily driven by growth potential, with tiller number serving as a reliable marker of this potential. The significant genotypic variation observed highlights the importance of selecting for growth-related traits in P limited environments. Additionally, P dilution, rather than total P concentration, appears to play a key role in determining yield under low P. Optimizing P management strategies and breeding for improved growth potential may significantly enhance crop performance in regions facing P limitation.

Key words: Foxtail millet, Grain P content, High throughput phenotyping (HTP) platforms, Low soil Phosphorus, Phosphorus use efficiency, Resource poor soil.

Introduction:

Phosphorus (P), essential alongside nitrogen (N) and potassium (K), is critical for plant growth (Roch et al. 2020). Despite being primarily sourced from inorganic phosphate (Pi), its limited

soil availability often necessitates using phosphorus fertilizers (Roch et al. 2019). Concerns over depleting rock phosphate reserves and environmental impacts highlight the need for sustainable management (Cordell et al. 2009; Sattari et al. 2012; Baker et al. 2015; Ceasar et al. 2017). Globally, about 50% of agricultural soils, especially in Sub-Saharan Africa and Southeast Asia, face phosphorus limitations. In India, almost 98% of districts require phosphorus fertilizers due to varying deficiency levels (Tiwar et al. 2001; Hasan, 1996), underscoring the need to address P deficiency for improved productivity.

P deficiency negatively impacts the growth and yield of various crop plants, including rice (*Oryza sativa*) (Wissuwa and Ae, 2001a), maize (*Zea mays*) (Plenet et al. 2000), wheat (*Triticum aestivum*) (Lazaro et al. 2010), sorghum (*Sorghum bicolor*) (Camacho et. al 2002), common bean (*Phaseolus vulgaris*) (Bonser et al. 1996), soybean (*Glycine max*) (Mahamood et al. 2009), foxtail millet (*Setaria italica*) (Ceasar et al., 2014, 2020) and other millets (Maharajan et al., 2019). P deficiency affects a significant portion of global agricultural land, (Navea et al. 2023) raising concerns about potential food scarcity (Childers et al. 2011). Consequently, farmers resort to phosphorus fertilizer application to optimize soil fertility and enhance crop yield (Maharajan et al. 2017). However, prudent P management is essential to ensure a continuous supply of P to sustain soil fertility and prevent eutrophication and water pollution (Maharajan et al. 2021).

Low P levels in the soil profile have been observed to lead to poor seedling emergence (Valluru et al. 2010), representing a significant constraint for achieving higher millet yield (Rebafka et al, 1993). Phosphorus Use Efficiency (PUE) is a ratio that quantifies the efficiency with which a plant utilizes phosphorus for growth and development and is calculated as the square of the total plant biomass divided by the total P content in the plant, which is derived from the weighted sum of P concentrations in the leaf, stem, and grain, each weighted by their respective dry weights (Hayes et al 2022 and Gourley et al 1992). The inefficiency in P utilization, characterized by a low PUE in modern cultivars, poses a significant challenge in cropping systems heavily reliant on phosphate fertilizer inputs (Dixon et al 2020). Despite external inputs, P deficiency persists, necessitating urgent efforts to improve PUE for sustainable agriculture (Vinod et al. 2015; Ceasar et al. 2020). In this context, breeding efforts for PUE focus on enhancing adaptation to P starvation.

Foxtail millet (*Setaria italica*), ranking as the second most cultivated millet crop globally, holds significance for both food and forage purposes (Jaiswal et al. 2019). This C4 self-pollinated cereal has a rich cultivation history dating back to 5000–6000 BC along the Yellow River in China. Foxtail millet is celebrated for its agronomic advantages, cost-effectiveness, stress resilience, efficient water utilization, and nutritional value. Its primary production hubs are situated in China and India (Lin et al. 2024). In Africa, foxtail millet is cultivated in upland regions across East Africa, Cameroon, and southern Africa (Brink, 2006). With its relatively small diploid genome of 510 Mb, foxtail millet serves as an ideal C4 model for genetic studies. This includes investigating the molecular, genetic, and physiological mechanisms underlying the C4 photosynthetic pathway, such as its efficiency in carbon fixation, adaptation to high temperature conditions, and water-use efficiency. These traits make foxtail millet particularly valuable for research aimed at enhancing crop productivity and resilience (Jaiswal et al. 2019; Ceasar et al. 2017; 2020).

Among millets, foxtail millet stands out as an excellent source of protein (12.3g/100g), dietary fibers (14g/100g), minerals (3g/100g), and ß-carotene (126-191µg/100g), while processing limited bioavailable carbohydrate content (60.9g/100g) (Ballolli et al. 2014). Despite these nutritional advantages, there is a noticeable gap in comprehensive studies exploring the responses of diverse foxtail millet cultivars to limited phosphorus conditions. A few studies have investigated aspects of plant growth, development, and the molecular expression of the PHT1 transporter family under phosphorus limitations (Ceasar et al. 2014; 2020; 2017; Roch et al. 2020; Ahmad et al. 2018). A systematic study aimed at characterizing foxtail millet genotypes for plant growth and development, water use efficiency, and agronomical trait values under a limited P regime, utilizing relevant phenotyping methodology, was notably absent. Our hypothesis is that under limited P condition, overall plant growth and development are critical factors in determining the grain yield of foxtail millet. To examine this hypothesis, we undertook a comprehensive investigation involving 10 foxtail millet genotypes from the core collection of ICRISAT Genebank. This investigation explored responses to both phosphorus sufficiency (high P) and starvation (low P) using diverse phenotyping platforms, namely Lysi-Field, LeasyScan, and hydroponics. Our specific objectives were i) to identify genotypic variations in plant canopy growth, root growth, phenology and agronomic traits under different phosphorus regimes (low P and high P) ii) to analyse functional trait associations under low P and high P conditions and propose potential driving factors or key component traits for foxtail millet breeding programs, with a specific emphasis on low P adaptation.

Materials and methods

Plant materials: Ten foxtail millet genotypes were selected from the core collection based on the previous study (Krishnamoorthy et al. (2016). The primary objective was to investigate and comprehend the extent of plant growth and agronomical traits variation across diverse P regimes, employing various phenotyping platforms. Details on experimental overview including list of traits assessed across different phenotyping platforms are available in Table 1. In the initial Lysimeter trial, ISe710 was utilized. However, in subsequent LeasyScan and Hydroponics experiments, CV Maxima cultivar was chosen to replace ISe710 due to the scientific interest in evaluating the cultivar and space constraints in these setups.

Water use and agronomical traits assessment at Lysi-Field facility under different P regimes (Low and High P)

The Lysimetric facility is located at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India (17°30′N; 78°16′E; altitude 549 m). It provides an experimental setup to assess key crop agronomic features, track the crop's ability to convert water into biomass (grams of dry mass per unit of water transpired), and measure water use patterns throughout the cropping season (Vadez et al. 2016). Plants were grown in PVC plumbing pipe lysimeters with a diameter of 20 cm and a length of 1.2 m, positioned outdoors under a rain-out shelter. The procedures for preparing soil, filling, spacing arrangement, and plant cultivation followed the methods outlined by Vadez et al. (2008, 2016). The soil utilized in this study from ICRISAT field exhibited low P level (2.11 ppm; available P) analysed through Hedley Fractionation Method (Cross and Schlesinger, 1995). The methodology for cultivating and testing plants in lysimeters adhered to the protocol established by Vadez et al. (2013). Seeds were sown in each PVC cylinder, and later, the plants were thinned to four per cylinder two weeks after sowing. Subsequently, the number was further reduced to two plants per cylinder at 3 weeks after sowing. Six replications were designated for the high P treatment, and another six replications for the low P treatment. Following the final thinning, high-P cylinders received 5g Di-ammonium phosphate (DAP) per cylinder and 2g potash (K) per cylinder, while low-phosphorus cylinders received 2g K per cylinder and 2g urea per cylinder to compensate for the nitrogen provided by DAP in high-P cylinders (Kadirimangalam et al. 2022). At 28 days after sowing (DAS), polythene beads were applied to cover the surface of the soil in the cylinders, preventing direct evaporation (more details in Vadez et al. 2011).

Starting from the 5th week, cylinder weighing was carried out on a weekly basis with flowering time visually recorded. Tiller numbers were manually scored at the time of harvest. At the end of the experiment, the plant samples of leaf, stem, and panicles were dried in a hot air oven at 72°C for about 3 days. Individual biomass components, such as leaf dry weight, stem dry weight, and panicle dry weight, were measured using a KERN 3600 g precision balance (Kern & Sohn GmbH, Balingen, Germany). Grain yield was obtained by threshing panicles. Thousand grain numbers were counted by seed counter machine (Data Count S60 seed Counter, Data technologies, Israel (details in https://data-technologies.com/product/seed-counter-s60/)) and the thousand grain weights were recorded using a weighing scale (KERN 360-3N, Kern &Sohn GmbH, Balingen, Germany). Plant transpiration was assessed based on consecutive cylinder weight differences and water additions. Total transpiration was determined as the sum of weekly plant transpiration. Transpiration efficiency (TE; grams of biomass per kilogram of water transpired; g/kg^{-1}) was calculated as the ratio of total dry biomass to the unit of water transpired. Finally, Harvest Index (HI) was computed as the ratio of total grain yield to the total biomass. For additional details on the methodology and data collection, please refer to Vadez et al. (2011, 2013, 2015, 2022), Tharanya et al. (2018), and Sivasakthi et al. (2019). The dried samples of leaf, stem and grains were ground, weighed and subjected to total P estimation through nitric acid pressure digestion (Heinrichs et al. 1986), followed by measurement using an inductively coupled plasma optical emission spectrometer (ICP-OES) (Thermo Scientific iCap 6000 Series, Thermo Fisher Scientific, Bremen, Germany). This method allowed for the determination of leaf P (Leaf P; mg g^{-1}), stem P (Stem P; mg g^{-1}), and grain P (Grain P; mg g^{-1}) concentration. Total P concentration (mg g^{-1}) was determined as the sum of P concentrations in leaves, stems, and grains, weighted by their relative contributions to the total plant biomass. Phosphorus Use Efficiency (PUE; $g^2\ mg^{-1}$) was calculated as the square of the total plant biomass divided by the total P content in the plant, which is derived from the weighted sum of P concentrations in the leaf, stem, and grain, each weighted by their respective dry weights (Gourley et al 1992). (Irfan et al. 2020). The percent reduction in traits under low P conditions compared to high P conditions was calculated using the formula

$$\text{Percent Reduction in Trait} = (\text{Trait}_{HP} - \text{Trait}_{LP}) / (\text{Trait}_{HP}) \times 100$$

where Trait_{HP} = Value of the trait under high P conditions

Trait_{LP} = Value of the trait under low P conditions

Canopy development related traits assessed at LeasyScan under different P regimes (low and high P)

LeasyScan, a high-throughput phenotyping platform, was designed to effectively monitor crop canopy-related parameters during the vegetative phase with exceptional throughput and accuracy. For a detailed understanding of LeasyScan technology and its setup, please refer to the works of Vadez et al. (2015), Sivasakthi et al. (2018, 2019), Tharanya et al. (2018), and Kar et al. (2020). Ten seeds sown in individual 10-inch pots during November 2022 post-rainy season. The soil used in this experiment displayed low P level (2.11 ppm), sourced from the ICRISAT field, which was also the origin of the soil used in the Lysi-field experiment. Each genotype and treatment combination involved eight replications, with each replication consisting of two pots, and after the final thinning, two plants were retained per pot. The treatments with low P (1g of urea and 1g of potash per pot) and high phosphorus (2.5g of DAP and 1g of potash per pot) were applied (Kadirimangalam et al. 2022). Throughout the experiment, plants were maintained under well-watered conditions. Continuous measurements of canopy size-related parameters, including 3D-leaf area, projected leaf area, plant height and digital biomass (Estimate of biomass based on observed plant dimensions - height and volumes), were taken from 15 to 40 days after sowing (DAS), with the final harvest conducted at 40 DAS. The daily temperature and humidity fluctuated between 11/35.8 °C and 17.2/93.2% on average during the crop growth period, as recorded by the attached weather station (Model: WxPRO™; Campbell Scientific Ltd., Shepshed, UK).

Hydroponic facility for plant shoot and root morphological traits under different P regime (low and high P)

To evaluate plant growth, especially root-related traits under high and low P conditions, plants were cultivated in a greenhouse under natural daylight fluctuations, with an average day/night temperature of around 28/22°C and relative humidity ranging from 70% to 90%. Seeds were initially sown in sand, and when the plants reached the 3rd leaf stage, they were transferred to trays with nutrient solution (modified Hoagland solution; macronutrients: $MgSO_4$ (2.05 mM), K_2SO_4 (1.25 mM), $CaCl_2*2H_2O$ (3.3 mM), Fe-EDTA (0.04 mM), urea (5 mM) and micronutrients: H_3BO_3 (4 mM), $MnSO_4$ (6.6 mM), $ZnSO_4$ (1.55 mM), $CuSO_4$ (1.55 mM), $CoSO_4$ (0.12 mM), Na_2MoO_4 (0.12 mM)). Subsequently, the plants were grown in hydroponic solutions within trays measuring 40cm x 20cm (length and width), utilizing the modified

Hoagland solution in accordance with the protocol outlined in Tharanya et al. 2018, and Sivasakthi et al. 2020. However, concerning KH_2PO_4, the high P treatment involved a nutrient solution with 300 µM KH_2PO_4, while the low P treatment received 10 µM KH_2PO_4 (Ceasar et al. 2020). The pH of the nutrient solution was maintained between 6.0 and 6.3, with continuous aeration to facilitate root nutrient absorption. The nutrient solution was replenished every 3 days. At 45 DAS, the plants cultivated through hydroponics underwent phenotypic assessment for morphological characteristics, including root length, crown root numbers and leaf area. Leaf area was measured utilizing a leaf area meter (LI-3100C area meter, LI-COR BioSciences, USA). The root surface area was determined by scanning the roots with a Shimadzu scanner and analyzing the scans with Winrhizo software (Winrhizo, Regent Ltd). Additionally, plant samples comprising leaves, stems, and roots were dried at 60 °C in an oven for a minimum of 72 hours, and their dry weights were measured using a KERN 3600 g precision balance (Kern & Sohn GmbH, Balingen, Germany).

Data analysis

The datasets collected from LeasyScan, hydroponics, and Lysimetric systems were statistically analyzed. One-way ANOVA was used to assess differences among genotypes, while two-way ANOVA evaluated the effects of genotypes, treatments, and their interactions. The Tukey–Kramer test was subsequently applied to identify significant variations between genotypes or treatments. All analyses were performed using the statistical software package CoStat version 6.204 (Cohort Software, Monterey, CA, USA). Residual yields can be effectively used to assess key adaptation traits under low P conditions. In the absence of genotype-by-treatment interaction (GxTrt) for yield components, the performance of genotypes under low P conditions reflects both their inherent grain yield potential and residual yield variation. This residual component includes the genotypes' adaptation to low P and an error factor, capturing the part of yield variation under low P that is not explained by grain yield potential (Beggi et al. 2015; Vadez et al. 2007; Bidinger et al. 1987). In this study, residual yields were calculated by taking the difference between the predicted yields (based on a linear regression model comparing low P to high P yields) and the observed yields under low P.

Graphical representations such as box plots, bar graphs, and simple linear regressions were created using Microsoft Excel 2017 (Microsoft Office 365, Microsoft Corp., Redmond, WA, USA). To evaluate correlations among selected phenotypic traits, a simple Pearson correlation

252 analysis was carried out with R software (version 2.11.1) using the 'metan' library.
253 Additionally, Principal Component Analysis (PCA) was conducted with R software (version
254 2.11.1) using the 'factoextra' library.

255

256 **Results**

257 **Treatment and genotypic variation due to varying P conditions**

258 **Plant growth, water use and agronomical traits**

259 The study focused on evaluating various traits of foxtail millet genotypes using multiple
260 phenotyping platforms, including Lysi-field, LeasyScan, and hydroponics facilities under low
261 P and high P conditions. Using two-way analysis of variance, significant variations in genotype
262 and treatment were identified for most traits under both low and high P conditions (Table 2).
263 In the one-way analysis, a range of plant traits, including growth, water use, and agronomical
264 features, exhibited significant genotypic differences under both low and high P conditions
265 (Table 3). Under high P conditions, the majority of genotypes exhibited enhanced plant growth
266 and agronomic parameters compared to low P conditions (Table 2).

267 Grain yield from the Lysi-Field experiment, ranged from 4.86 g to 50.41 g, with an average of
268 24.26 g under high P condition. Under low P conditions, it ranged from 1.17 g to 27.95 g, with
269 an average of 14.12 g (Fig. 1), indicating a 42% decline compared to high P conditions. This
270 decline underscores the sensitivity of grain production to low P availability. The genotypic
271 differences in yield across both P conditions are illustrated in Fig. 1b and detailed in Table 3.
272 Subsequently, biomass accumulation also varied across the treatments with high P having
273 higher biomass than the than the low P conditions (Table 2). TE exhibited a significant
274 reduction under low P conditions, with a mean of 2.01 g biomass per kg water under high P
275 conditions compared to 1.10 g biomass per kg water under low P conditions, representing a
276 50% reduction. This substantial decline highlights the critical role of phosphorus availability
277 in influencing water-use (Table 2).

278 Tiller counts under high P conditions ranged from 3.17 to 32.67, with a mean of 17.5.
279 Conversely, under low P conditions, tiller counts ranged from 2.17 to 11.50, with a mean of
280 6.37, representing a 64% reduction compared to the high P treatment (Suppl. Fig. S1a).
281 Furthermore, genotypic variability in tiller counts under both low and high P conditions is
282 illustrated in Suppl. Fig. S1b and detailed in Table 3.

In the LeasyScan facility, the 3D leaf area under high P conditions ranged from 6000 mm² to 50565 mm², with a mean of 23356 mm² Conversely, under low P conditions, the 3D leaf area ranged from 2500 mm² to 21000 mm², with a mean of 10595 mm², representing a 50% reduction compared to the high P treatment (Fig. 2a & Suppl Fig. S2). In hydroponic experiments, root surface area under high P conditions varied from 163 cm² to 699 cm², with a mean of 419 cm². In contrast, under low P conditions, root surface area ranged from 94 cm² to 575 cm², with a mean of 302 cm², indicating a 28% reduction compared to the high P treatment (Fig. 2a & Suppl. Fig. S3). Notably, the reduction in root surface area was considerably smaller than the reduction in 3D leaf area, which may be due to the plant's prioritization of root growth to enhance P acquisition under P nutrient limitation. Genotypic variability in 3D leaf area and root surface area under both low and high P conditions were provided in Table 3 and Suppl. Fig S2 & S3.

P concentration and PUE in different plant organs

The distribution of P content exhibited significant variability among plant organs, with the highest concentration found in the grain, followed by the leaf and stem (Table 3).Notably, grain P concentration ranged from 2.1 mg g^{-1} to 4.2 mg g^{-1} (mean 3.17 mg g^{-1}) under high P conditions, and from 1.9 mg g^{-1} to 3.4 mg g^{-1} (mean 2.55 mg g^{-1}) under low P conditions, indicating a 24% reduction compared to high P conditions (Table 3). Similarly, leaf P concentration exhibited substantial variation, ranging from 1.27 mg g^{-1} to 3.2 mg g^{-1} (mean 2.26 mg g^{-1}) under high P, and from 0.86 mg g^{-1} to 2.35 mg g^{-1} (mean 1.46 mg g^{-1}) under low P, resulting in a 35% reduction (Table 3). Additionally, stem P concentration demonstrated significant variation, ranging from 0.65 mg g^{-1} to 2.13 mg g^{-1} (mean 1.41 mg g^{-1}) under high P, and from 0.23 mg g^{-1} to 1.25 mg g^{-1} (mean 0.47 mg g^{-1}) under low P, resulting in a 66% reduction (Table 3). Additionally, genotypic variability in grain, leaf, and stem P content under low and high P conditions is shown in Suppl. Fig. S4& S5.

Total P concentration ranged from 1.72 mg g^{-1} to 3.6 mg g^{-1} (mean 2.45 mg g^{-1}) under high P conditions and from 1.14 mg g^{-1} to 2.39 mg g^{-1} (mean 1.66 mg g^{-1}) under low P conditions, reflecting a 35% reduction compared to high P conditions (Suppl. Fig. S6A and Table 2). Genotypic variability in total P concentration under both low and P conditions is provided in in Table 3 and Suppl. Fig. S6B

Similarly, phosphorus use efficiency (PUE) ranged from 6.08 g^2 mg^{-1} to 53.55 g^2 mg^{-1} (mean 18.88 g^2 mg^{-1}) under low P conditions and from 9.04 g^2 mg^{-1} to 35.14 g^2 mg^{-1} (mean 19.38 g^2

315 mg^{-1}) under high P conditions, representing a 2.58% reduction in low P compared to high P
316 conditions (Table 2). Genotypic variability in PUE under both low and high P conditions is
317 shown in Table 3 and Suppl. Fig. S7.

318 **Functional trait associations**

319 Grain yield under both low and high P conditions demonstrated a significant association (R^2 =
320 0.65; Fig. 3), suggesting a certain level of consistency in performance across different P levels.
321 This indicates that grain yield under low P was in a large part influenced by the yield potential
322 under high P conditions, although other factors may also contribute to yield variations. To
323 explore the factors contributing to yield variation under low P conditions, the residuals of GY
324 under low P, which were not explained by GY under high P, were calculated. These residuals
325 revealed a strong relationship with tiller numbers under both low P (R^2 = 0.70; Fig. 4) and high
326 P (R^2 = 0.66; Fig. 4), indicating that tiller production plays a key role in determining yield,
327 especially in P limited environments. This suggests that increasing tiller numbers could help
328 improve yield in conditions where P is limited.

329 Additionally, a regression analysis was performed to examine the relationship between biomass
330 and total P concentration. Under low P conditions, a significant negative relationship was
331 observed (R^2 = 0.71, $p < 0.05$; Fig. 5), indicating that genotypes maintaining growth under P-
332 limited conditions are those that effectively dilute P. In contrast, genotypes unable to dilute P
333 are more likely to experience biomass limitations. No significant relationship was found under
334 high P conditions (Fig. 5), suggesting that, when P is sufficient, biomass accumulation is less
335 dependent on total P concentration.

336 **Discussion**

337 **Growth potential as the key driver of performance under low P conditions**

338 The imposition of low P deficiency significantly affected various plant traits, including tillers,
339 leaf area, root surface area, agronomic characteristics (notably grain yield), and P concentration
340 in different plant tissues. This deficiency led to an overall decrease in plant growth and grain
341 yield, with reductions in plant growth and development traits ranging from 30% to 50%
342 compared to high P conditions (see Fig. 1). These findings align with prior studies, showing
343 similar trends observed in various crops like sorghum (Leiser et al., 2012), maize (Parentoni et
344 al., 2010), common bean (Beebe et al., 2008), and foxtail millet (Ceasar et al., 2020) under low
345 P conditions.

The current study highlights a notable variability in the number of tillers and grain yields among tested foxtail millet genotypes under low P conditions. Zhao et al. (2023) reported similar reductions in P accumulation, photosynthetic function, and biomass in wheat under low P conditions. Rajamanickam et al. (2024) also observed significant genotypic variability in root traits and their association with P utilization efficiency in wheat seedlings under low P conditions. These findings emphasize the critical role of growth potential in plant performance under low P conditions.

Our findings align with previous studies by Beggi et al. (2015) and Gemenet et al. (2015), who investigated low P adaptation in pearl millet. Beggi et al. (2015) reported a significant positive correlation ($r = 0.69$; $P < 0.01$) between grain yield under low and high P conditions and used residual yields as a proxy for assessing low P adaptation in pearl millet genotypes. Consistent with Beggi et al. (2015), we observed a significant reduction in transpiration efficiency (TE) under low P conditions, similar to their findings in pearl millet. However, while their study indicated that this decrease was less pronounced in genotypes adapted to low P (as shown by higher grain yields), our results suggest a stronger physiological response to P deficiency, with a more pronounced reduction in TE.

Genetic variability in plant growth and agronomic traits under low P conditions is essential for the success of breeding programs, as it enables the identification and selection of traits that improve crop performance in nutrient-limited environments. The effectiveness of a breeding program depends on the availability of significant genetic variability for the targeted traits and the use of efficient selection methods to increase the frequency of desirable genes or gene combinations (Gemenet et al., 2016). In this study, significant genotypic variation was observed in plant growth and agronomic traits among the foxtail millet genotypes, with more than a two-fold difference under low P treatments. These findings are consistent with previous research indicating greater genotypic variation in P uptake compared to PUE traits in crops such as wheat, maize, rice, sorghum, and foxtail millet (Jones et al., 1989; Wissuwa et al., 1998; Parentoni et al., 2010; Leiser et al., 2014; Ceasar et al., 2020). The considerable variation observed underscores the importance of breeding programs focusing on key traits like tiller development and PUE, which are crucial for improving crop performance under P-deficient conditions. Specifically, genotypes ISe 480 and ISe 710 exhibited enhanced tiller counts, PUE, and grain yield under low P stress, highlighting the value of selecting for these traits to boost crop resilience and productivity in phosphorus-limited soils.

In the present study, a 24% reduction in grain P concentration under low P conditions indicates that this variable is relatively less impacted by phosphorus deficiency. This suggests that the observed increase in grain yield under low P conditions is likely due to the plant's enhanced ability to extract phosphorus from the soil. In contrast, more substantial changes were observed in P concentrations across other plant organs. Specifically, stem P concentration showed a significant decline, reflecting the limited role of stems in biomass accumulation under phosphorus-deficient conditions. Conversely, leaf P concentration experienced a relatively smaller reduction, likely due to the essential role of leaves in photosynthesis and biomass production. These results highlight a strategic redistribution of phosphorus within the plant, prioritizing critical organs like leaves to sustain growth and yield under low P availability. This observation aligns with findings by Veneklaas et al. (2012), which emphasize that phosphorus allocation among plant organs is closely linked to crop growth and suggest that optimizing this distribution can improve overall phosphorus-use efficiency.

Plants adapt to low P conditions by allocating biomass to roots, increasing the root-to-shoot ratio, and adjusting root morphological and physiological traits to enhance P uptake efficiency (Iqbal et al., 2020 and Lambers et al.,2015). Insights into the physiological and molecular mechanisms of plant adaptation to P deficiency, including changes in root architecture and P acquisition strategies, have been provided by Vance et al. (2003). Additionally, genetic variability in common bean for phosphorus uptake and use efficiency, highlighting the importance of root traits and P allocation under low P conditions, was explored by Ramaekers et al. (2010).

Tiller Number: A key trait for low P adaptation

The current study observed substantial genotypic variation in tiller numbers among foxtail millet genotypes under low P conditions. Specifically, there was a 6-fold difference in tiller count among the tested genotypes. This variation underscores the importance of tiller number as a key trait for assessing growth potential under low P conditions.

A strong correlation was observed between grain yield under low P and high P conditions ($R^2 = 0.65$; $P < 0.01$), indicating that genetic yield potential (vigor) in high P environments significantly influences grain yield and tiller numbers under low P conditions. This suggests that genotypes with higher tiller numbers tend to perform well in both high and low P conditions, making tiller number a reliable indicator of growth potential. These results are

consistent with Bhatta et al. (2021), who highlighted tiller number as a critical trait for improving crop performance in phosphorus-deficient environments. Their study emphasizes the importance of evaluating genotypes based on tiller number, along with shoot and root biomass, to enhance yield stability and optimize productivity under phosphorus-limited conditions.

Residual grain yield under low P conditions, not explained by high P conditions, had a strong positive association with tiller numbers ($R^2 = 0.70$; $P < 0.01$). This suggests that tiller number contributes significantly to yield under phosphorus-limited conditions, even after accounting for the overall vigor observed under high P conditions. These results align with previous studies that indicate alterations in growth, biomass, and yield as key indicators of adaptation to phosphorus deficiency, as reported in various cereals, including oat (Żebrowska et al., 2017), rice (He et al., 2005; Wissuwa et al., 2020), maize (Mollier et al., 1999), sorghum (Yoneyama et al 2007), and foxtail millet (Ceasar et al., 2020).

The observed genotypic differences in tiller development highlights its role in enabling plants to cope with low P stress while maintaining yield. For example, genotypes ISe 480 and ISe 710 exhibited higher tiller counts, improved PUE, and increased grain yield in the Lysi-Field under low P conditions compared to high P conditions. These findings highlight the value of selecting for traits like tiller number to enhance crop resilience in -limited environments.

Supporting evidence from other studies further underscores the significance of tiller number in crop performance. A genome-wide association study (GWAS) by Ren et al. (2021) identified multiple quantitative trait loci (QTLs) associated with effective tiller number (ETN) in rice, revealing the genetic basis of this trait and its influence on grain yield. Similarly, Cui et al. (2004) mapped QTLs for tiller number in rice and demonstrated strong correlations between tiller number, plant height, and heading date, underscoring its critical role in determining final grain yield. Additionally, Chen et al. (2012) showed that overexpression of specific genes in rice resulted in increased tiller numbers, further highlighting the role of genetic regulation in this trait.

P dilution and its impact on yield in low P environments

The current study reveals a strategic reallocation of P in foxtail millet under low P conditions, highlighting significant differences in P uptake and utilization efficiency among genotypes. Grain P concentration exhibited the least reduction (24%) compared to high P conditions, while leaf and stem P concentrations decreased by 37% and 68%, respectively. These results are

consistent with those of Ceasar et al. (2020), who observed a reduction in total shoot P concentration under P-deficient conditions.

The relatively small reduction in grain P concentration suggests that foxtail millet maintains P allocation to reproductive structures, likely prioritizing reproductive success under nutrient stress. This trait is particularly important for ensuring yield stability in P-deficient soils. By contrast, the substantial reduction in stem P concentration suggests that stems, being less critical for immediate growth and productivity, serve as a lower priority reservoir for P under stress. Leaves, which are crucial for photosynthesis and biomass accumulation, experienced a lower reduction than observed in stems, reflecting their higher priority in P allocation under low P conditions.

These findings highlight the physiological adaptations of foxtail millet to low P conditions. The observed changes in P allocation suggest that under P deficiency, plants employ mechanisms to optimize P use by prioritizing allocation to organs essential for photosynthesis and reproduction, while reducing allocation to non-essential biomass components. This strategic redistribution of P within the plant underscores the importance of P dilution in determining yield under low P conditions.

The study also found that residual grain yield under low P conditions, not explained by high P conditions, had a significant negative association with total P concentration ($R^2 = 0.54$; $P < 0.05$). This indicates that lower total P concentration, or P dilution, is associated with higher grain yield under low P conditions. Conversely, under high P conditions, grain yield (GY_LF) from Lysi-Field exhibited significant positive correlations with PUE ($r = 0.94$; $P < 0.001$) and total biomass ($r = 0.84$; $P < 0.01$).

Additional studies support the role of P dilution in crop performance. For instance, Zamuner et al. (2016) established a critical P dilution curve for potato, demonstrating that P dilution is a robust diagnostic tool for assessing crop P status and improving P fertilizer management. Similarly, Kong et al. (2024) validated the use of the P nutrition index in potato, showing a significant relationship between PNI and relative tuber yield. Rose et al. (2013) highlighted the importance of P remobilization efficiency in maintaining grain P concentration under low P supply.

Conclusion

This study underscores the critical role of P availability in shaping plant growth and yield-related traits in foxtail millet, particularly under low P conditions. Plant performance in these environments is primarily influenced by growth potential, with tiller number serving as a reliable marker of this potential. The significant genotypic variation observed highlights the importance of selecting growth-related traits to improve crop resilience and productivity in phosphorus-limited environments. The findings reveal substantial variation in plant growth and agronomic traits, such as tiller count, grain yield, leaf area, and root surface area, among foxtail millet genotypes under low P conditions. This variation emphasizes the necessity for breeding programs to prioritize traits that enhance growth potential, including tiller development and PUE, to optimize crop performance in nutrient-deficient soils.

Moreover, the study highlights the strategic redistribution of P within the plant under low P conditions, where critical organs like leaves maintain higher P concentrations to support growth and yield. This strategic P allocation suggests that P dilution, rather than total P concentration, plays a key role in determining yield under low P conditions. The observed negative association between total P concentration and residual grain yield under low P conditions further supports this finding.

In conclusion, optimizing P management strategies and breeding for improved growth potential are essential for enhancing crop performance in regions facing P limitation. By selecting for traits that enhance growth potential and understanding the mechanisms of P allocation and dilution, breeding programs can develop foxtail millet varieties that are better adapted to low P environments, ensuring yield stability and food security in vulnerable agri-systems.

Author's contribution

The experiment was conceptualized by JK, AB, and MT. The phenotyping experiments were conducted by MT, KS, SC, KV, DSG, AA, and SK, with the experimental design overseen by RB. Nutrient analysis was carried out by SL and MAD. Data analysis and summarization were undertaken by MT, KS, and KSG. Support in data interpretation was provided by AB, SAC, SC, and JK. The manuscript was drafted by MT and KS, and it underwent review by JK, SC, MAD, SL, AB, and SAC. The manuscript was read and approved by all authors, and their consent was given for the final version.

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Data availability

The data supporting the conclusions of this study can be obtained from the corresponding author, upon request.

Declarations

Conflict of interest: The authors declare that they have no competing interests.

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TABLE CAPTIONS

Table 1: Details of genotypes, treatments, and replications, along with a list of phenotyped traits obtained from various phenotyping platforms (Lysi-Field, HTP-LeasyScan, and hydroponics).

Table 2: Two-way ANOVA results for Plant Growth, Water Use, Phenology, and Agronomical Traits Across Various Phenotyping Platforms. The table includes Mean Sum of Squares for treatment (T), genotypes (G), and T x G interactions, along with corresponding P values and significance levels. The use of different alphabets in the Tukey-Kramer test with trait mean values denote significant differences between low and high P treatments at least significant difference of 0.05. *, ** and *** signify significant differences at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively, while “ns” denote non-significant differences.

Table 3: One-way ANOVA Results for Plant Growth, Water Use, Phenology, and Agronomical Traits Across Various Phenotyping Platforms. The table includes Mean Sum of Squares for genotypes (G) along with corresponding P values and significance levels. The use of different alphabets in the Tukey-Kramer test with trait mean values indicates differences between genotypes at least significant difference of 0.05. *, ** and *** signify significant differences at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively, while "ns" denote non-significant differences.

FIGURE CAPTIONS

Fig. 1: a) Boxplot depicting the variation in grain yield under low and high phosphorus (P) treatments, measured using a Lysimeter. The blue boxplot represents the high P treatment, and the orange boxplot represents the low P treatment. Statistically significant differences between treatments at $P < 0.05$ are indicated by different letters on the boxplots. b) Bar graph showing genotypic variation in grain yield under low and high P treatments, assessed using a Lysimeter. Blue bars represent the high P treatment, and orange bars represent the low P treatment. Statistically significant differences among genotypes ($P < 0.05$) are indicated by distinct upper-case letters for low P treatment and lower-case letters for high P treatment, while bars with the same letters denote no significant differences.

Fig. 2: a) Boxplot illustrating the variation in percentage reduction relative to the high P treatment [((high P – low P)/high P) * 100]. The orange boxplot represents root surface area (cm^2), measured using a hydroponics facility, while the green boxplot represents leaf area (mm^2), measured using the HTP-LeasyScan facility. The cross symbol inside each boxplot indicates the mean percentage reduction values.

Fig. 2: b) Boxplot showing the variation in percentage reduction relative to the high P treatment [((high P – low P)/high P) * 100]. The pink boxplot represents grain P content, the green boxplot represents leaf P content, and the orange boxplot represents stem P content. The cross symbol inside each boxplot indicates the mean percentage reduction values.

Fig. 3: Regression analysis showing the relationship between grain yield under low P treatment and grain yield under high P treatment, measured at the Lysi-field facility. The figure includes the slopes, R^2, and r values of the regressions. R^2 and r values marked with an asterisk (**) indicate significant differences at $P < 0.01$.

Fig. 4: Regression analysis showing the relationship between residual grain yield under low P conditions (unexplained by high P treatment) and tiller numbers from the Lysi-field, under both low and high phosphorus treatment conditions. In the scatterplots, blue dots and a red trend line represent the high P treatment, while orange dots and a red trend line represent the low P treatment. The figure includes the slopes and R^2 values of the regressions. R^2 values marked with an asterisk (**) indicate significant differences at $P < 0.01$.

Fig. 5: Regression analysis showing the relationship between total biomass under low and high P treatment conditions and total P concentration from the Lysi-field facility. In the scatterplots, blue dots and a red trend line represent the high P treatment, while orange dots and a red trend line represent the low P treatment. The figure includes the slopes and R^2 values of the regressions. R^2 values marked with an asterisk (**) indicate significant differences at $P < 0.01$, while R^2 values labeled as "ns" indicate no significant difference.

SUPPLEMENTARY INFORMATION

Supplementary Fig. S1 a) Boxplot showing the variation in tiller numbers under low and high phosphorus treatments, measured using a Lysimeter. The blue boxplot represents the high phosphorus treatment, and the orange boxplot represents the low phosphorus treatment. Different letters on the boxplots indicate statistically significant differences between treatments at the 0.05 level of least significant difference. b) Bar graph illustrating genotypic variation in tiller numbers under low and high phosphorus (P) treatments, assessed using a Lysimeter. The high P treatment is represented by blue bars, and the low P treatment by orange bars. Bars marked with distinct lower-case letters (for high P treatment) and upper-case letters (for low P treatment) indicate statistically significant differences ($p < 0.05$), while bars with the same letters show no significant differences.

Supplementary Fig. S2 Bar graph showing genotypic variation in 3D-leaf area under low and high phosphorus (P) treatments, assessed using a Lysimeter. The high P treatment is represented by blue bars, and the low P treatment by orange bars. Bars labeled with distinct lower-case letters (for high P treatment) and upper-case letters (for low P treatment) indicate statistically significant differences ($p < 0.05$), while bars with the same letters show no significant differences.

Supplementary Fig. S3 Bar graph showing genotypic variation in root surface area under low and high phosphorus (P) treatments, assessed using a Lysimeter. The high P treatment is represented by blue bars, and the low P treatment by orange bars. Bars labeled with distinct lower-case letters (for high P treatment) and upper-case letters (for low P treatment) indicate statistically significant differences ($p < 0.05$), while bars with the same letters show no significant differences.

Supplementary Fig. S4 The stacked bar graph illustrates the distribution of phosphorus concentration in different plant tissues (leaf, stem, and grain) under high phosphorus treatments. Phosphorus concentration in the leaf is represented by green bars, in the stem by blue bars, and in the grain by orange bars. Different lowercase letters on the bars indicate statistically significant differences in mean phosphorus content among genotypes within each tissue (leaf, stem, or grain) at $P < 0.05$. Bars with the same letter indicate no significant difference.

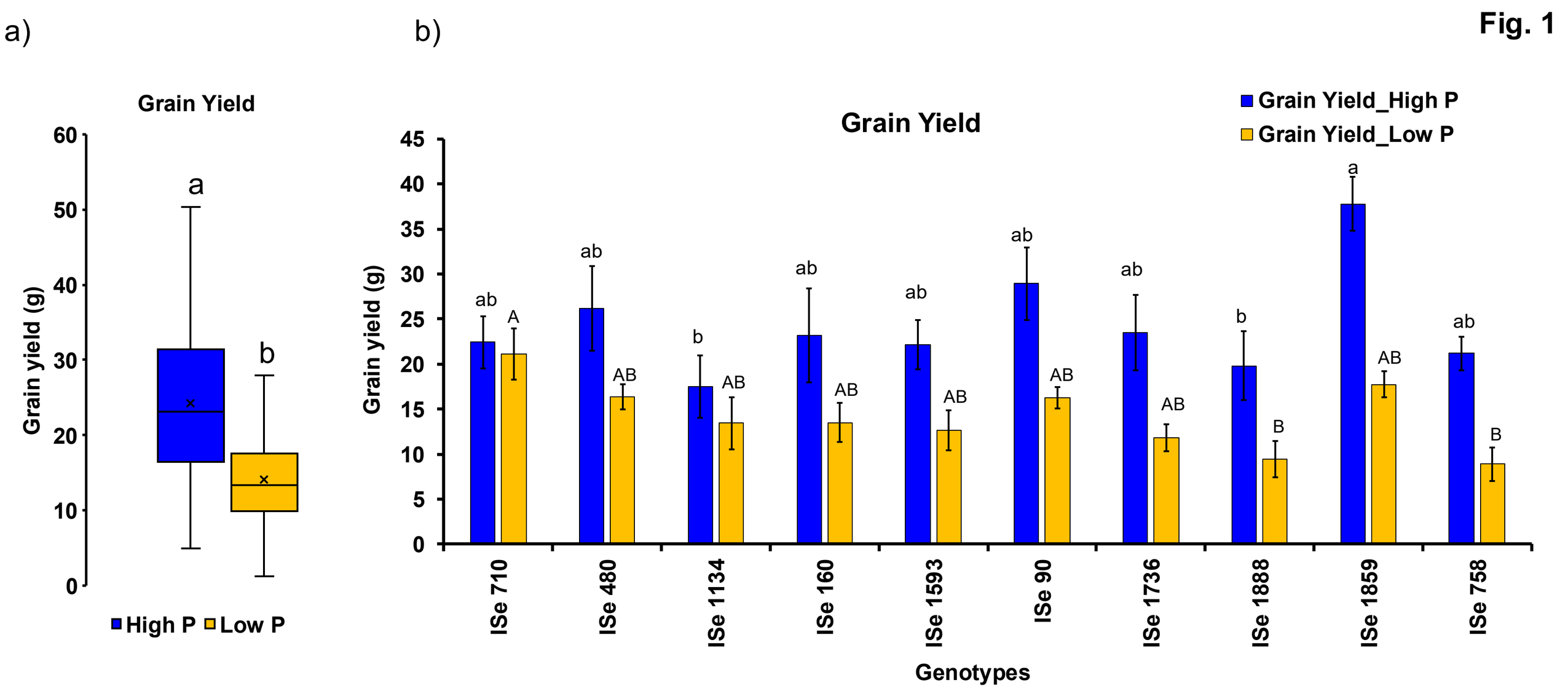
Supplementary Fig. S5 The stacked bar graph illustrates the distribution of phosphorus concentration in different plant tissues (leaf, stem, and grain) under low phosphorus treatments. Phosphorus concentration in the leaf is represented by green bars, in the stem by blue bars, and in the grain by orange bars. Different uppercase letters on the bars indicate statistically significant differences in mean phosphorus content among genotypes within each tissue (leaf, stem, or grain) at $P < 0.05$. Bars with the same letter indicate no significant difference.

Supplementary Fig. S6 a) Boxplot showing the variation in total phosphorus (P) concentration (mg per gram) under low and high P treatments. The blue boxplot represents the high P treatment, and the orange boxplot represents the low P treatment. Statistically significant differences between treatments at $P < 0.05$ are indicated by different letters on the boxplots. b) Bar graph illustrating genotypic variation in total P concentration under low and high P treatments. Blue bars represent the high P treatment, and orange bars represent the low P treatment. Statistically significant differences among genotypes ($P < 0.05$) are indicated by distinct upper-case letters for low P treatment and lower-case letters for high P treatment, while bars with the same letters show no significant differences.

Supplementary Fig. S7 The bar graph shows genotypic variation in phosphorus use efficiency under both low and high phosphorus (P) treatments. High P treatment is represented by blue bars, while low P treatment is shown by orange bars. Distinct lowercase letters (for high P

936 treatment) and uppercase letters (for low P treatment) on the bars indicate statistically
937 significant differences ($P < 0.05$). Bars with the same letter signify no significant differences.

Fig. 1


a)
Grain Yield
Grain yield (g)
60
50
40
30
20
10
0
a
b
High P
Low P
b)
Grain Yield
Grain Yield_High P
Grain Yield_Low P
Grain yield (g)
45
40
35
30
25
20
15
10
5
0
ab
A
ab
AB
b
AB
ab
AB
ab
AB
ab
AB
ab
AB
b
B
a
AB
ab
B
ISe 710
ISe 480
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ISe 1593
ISe 90
ISe 1736
ISe 1888
ISe 1859
ISe 758
Genotypes

Fig. 2a

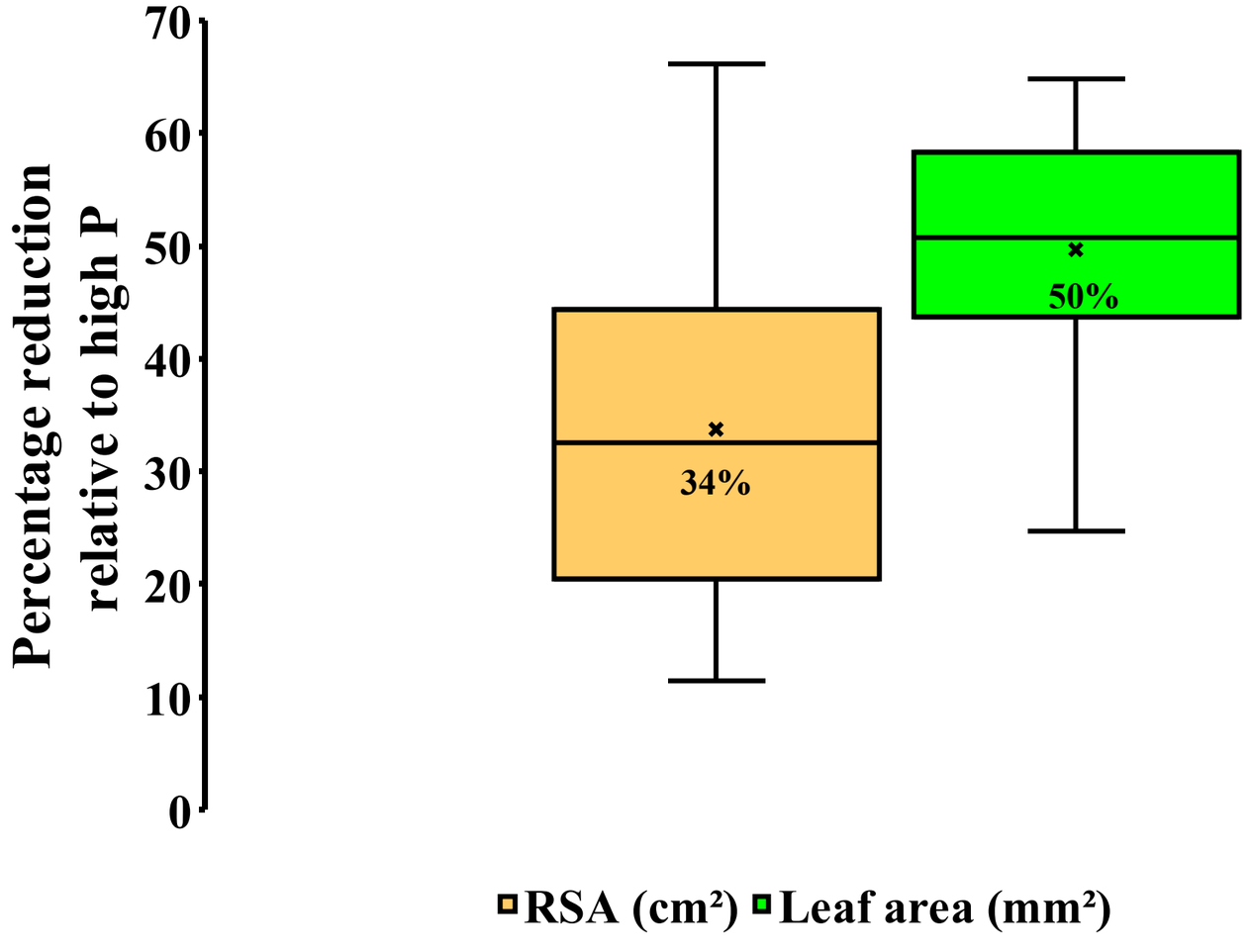

Percentage reduction relative to high P
70
60
50
40
30
20
10
0
34%
50%
RSA (cm²)
Leaf area (mm²)

Fig. 2b

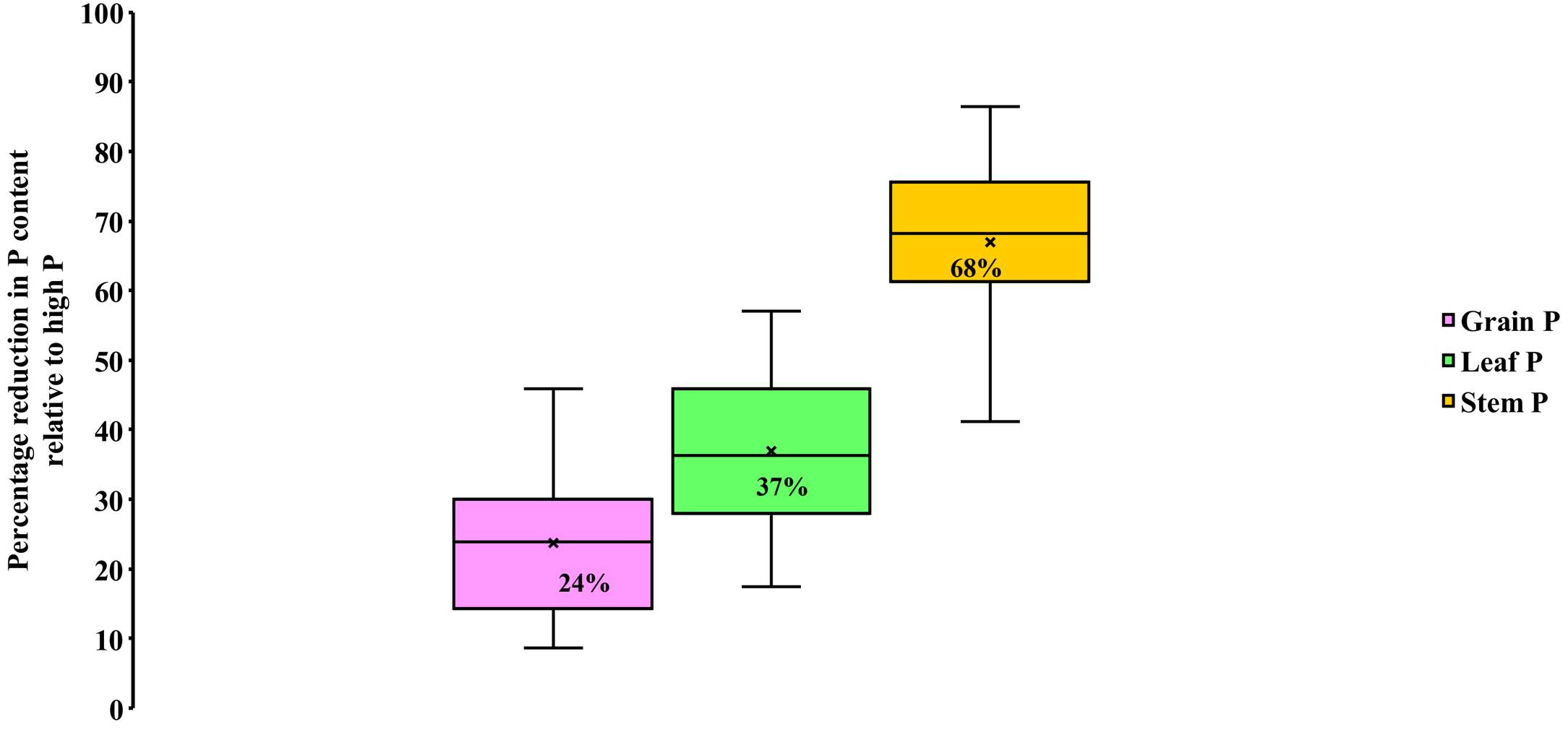

Percentage reduction in P content relative to high P
100
90
80
70
60
50
40
30
20
10
0
24%
37%
68%
Grain P
Leaf P
Stem P

Fig. 3

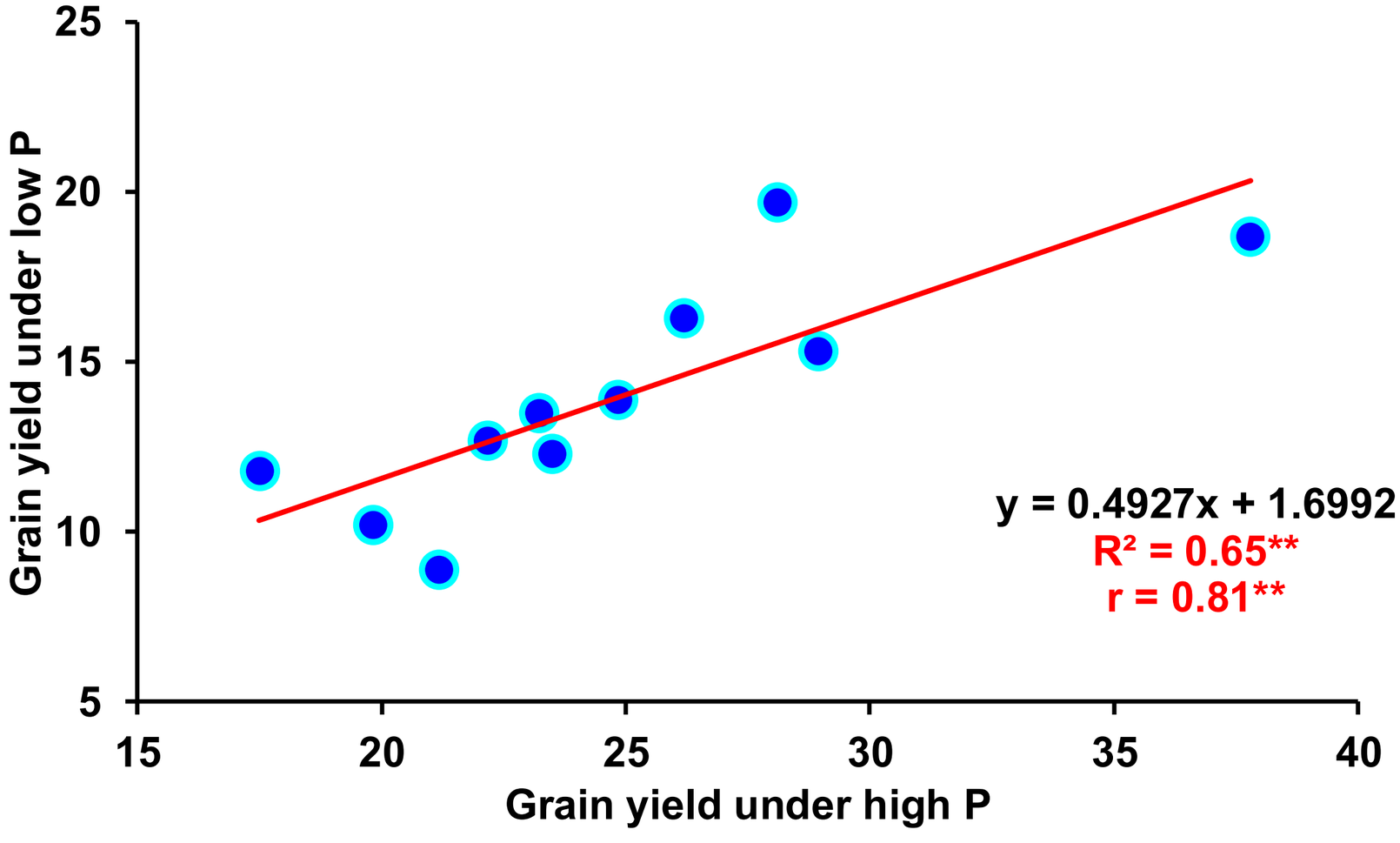

Grain yield under low P
25
20
15
10
5
15
20
25
30
35
40
Grain yield under high P
y = 0.4927x + 1.6992
R² = 0.65**
r = 0.81**

Fig. 4

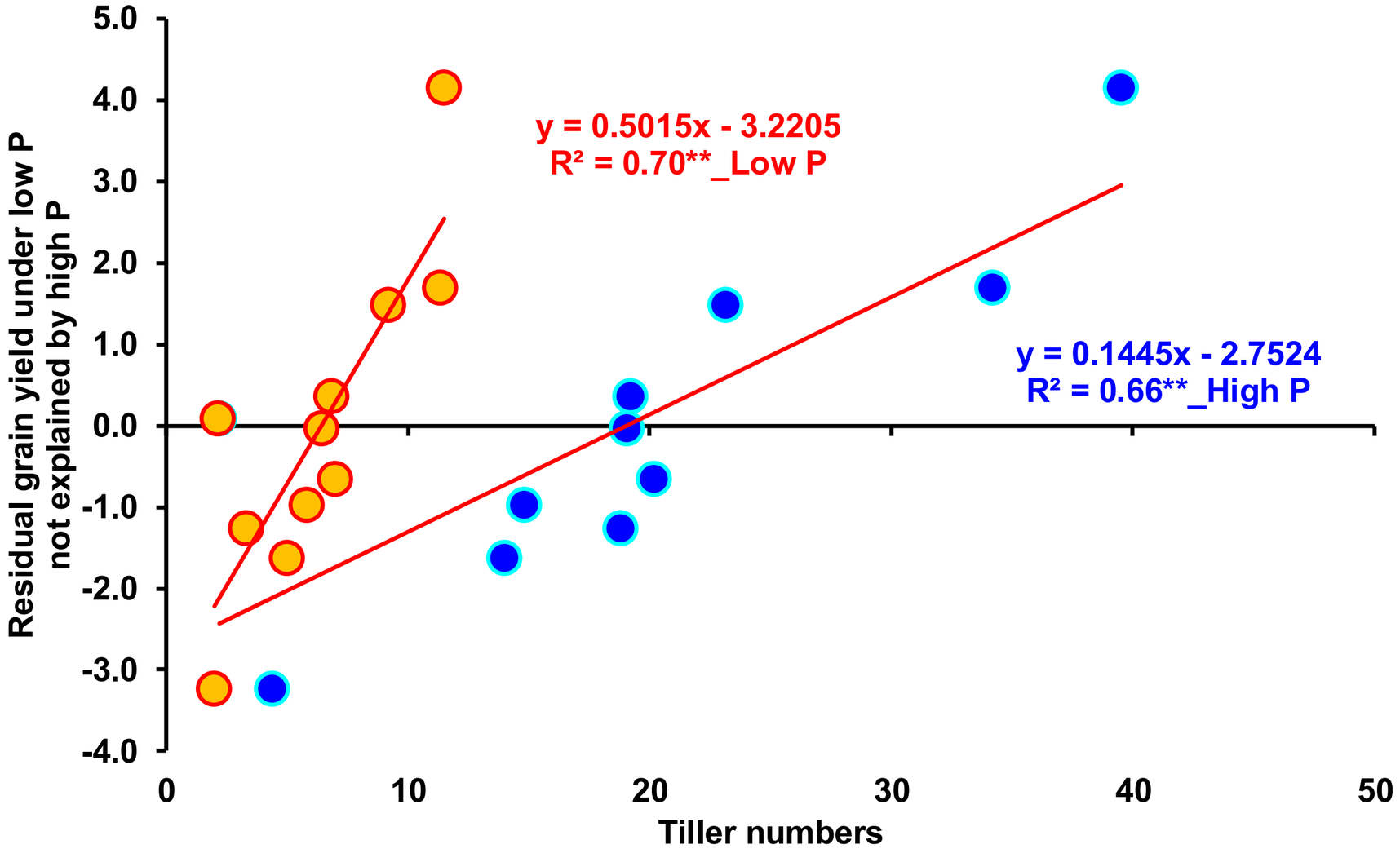
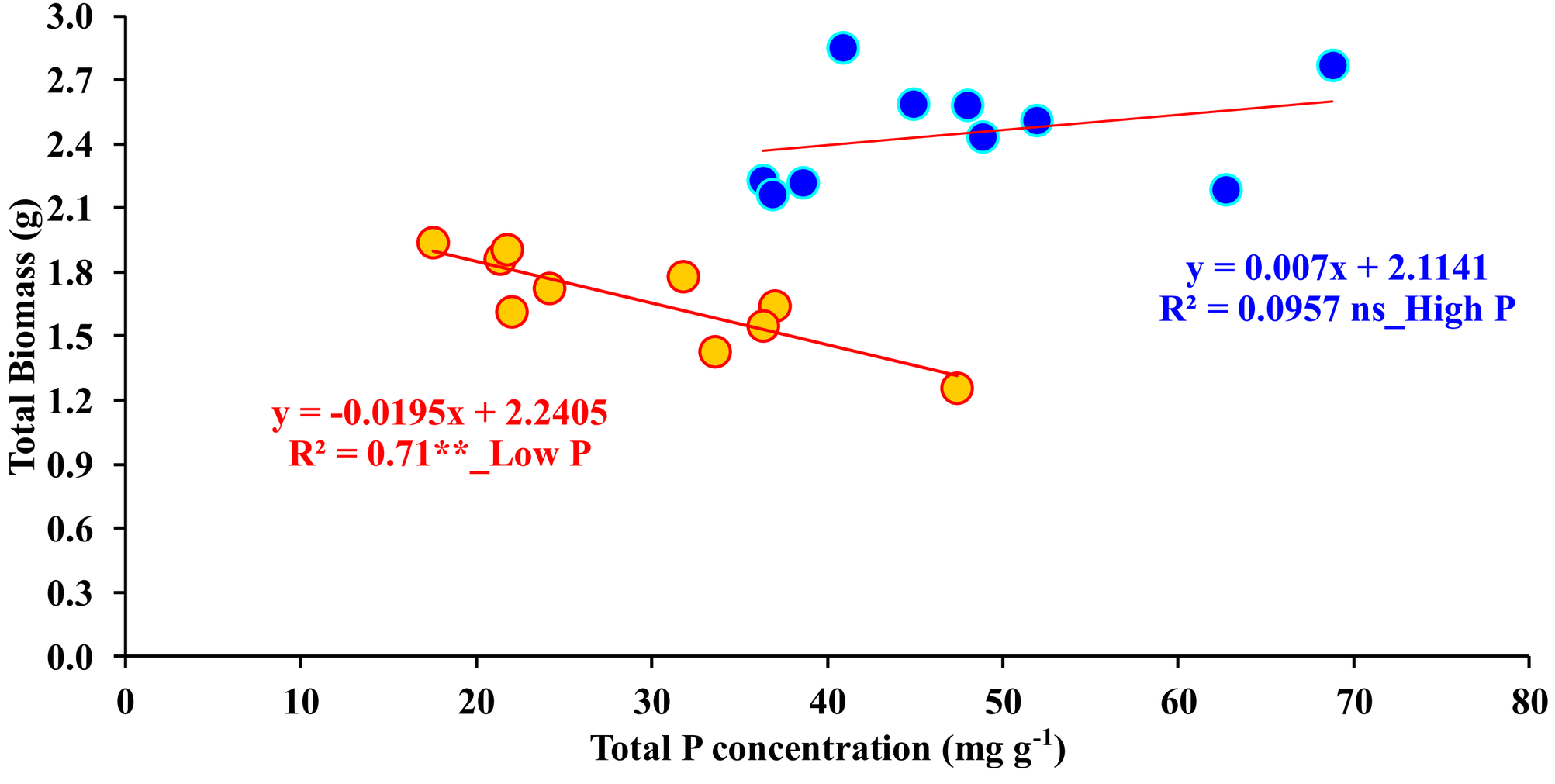
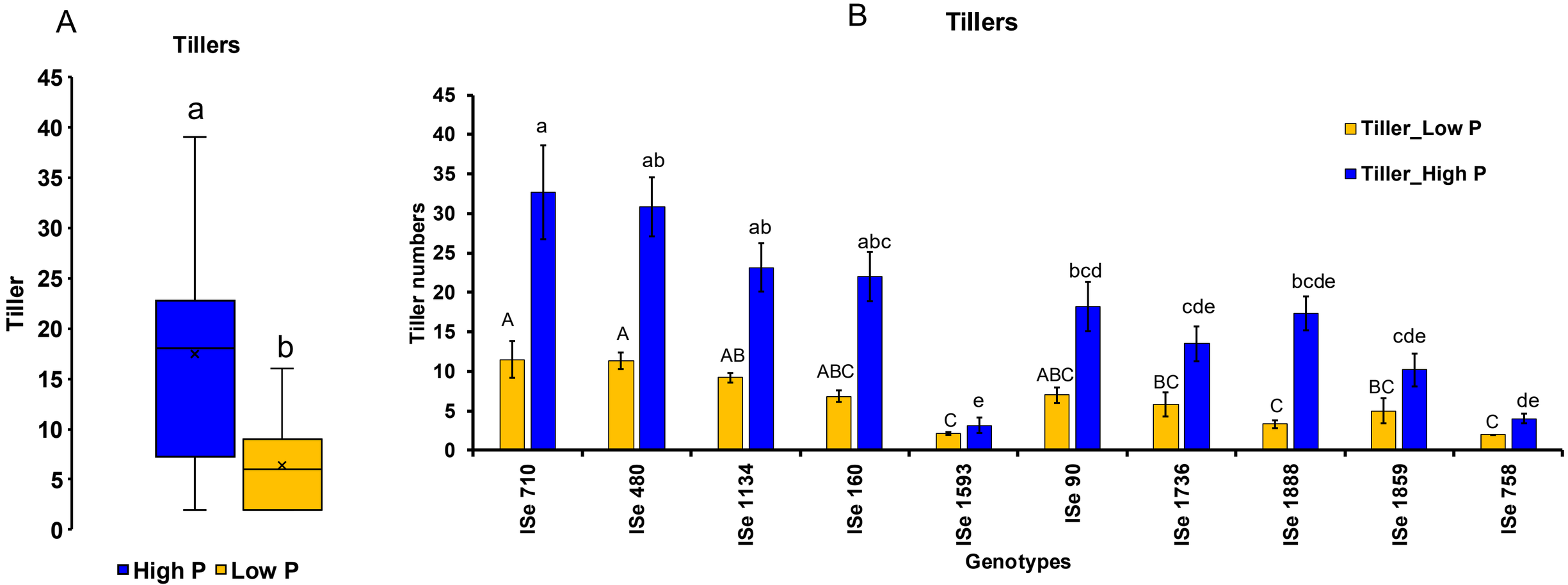

y = 0.5015x - 3.2205
R² = 0.70**_Low P
y = 0.1445x - 2.7524
R² = 0.66**_High P
Residual grain yield under low P not explained by high P
Tiller numbers
5.0
4.0
3.0
2.0
1.0
0.0
-1.0
-2.0
-3.0
-4.0
0
10
20
30
40
50

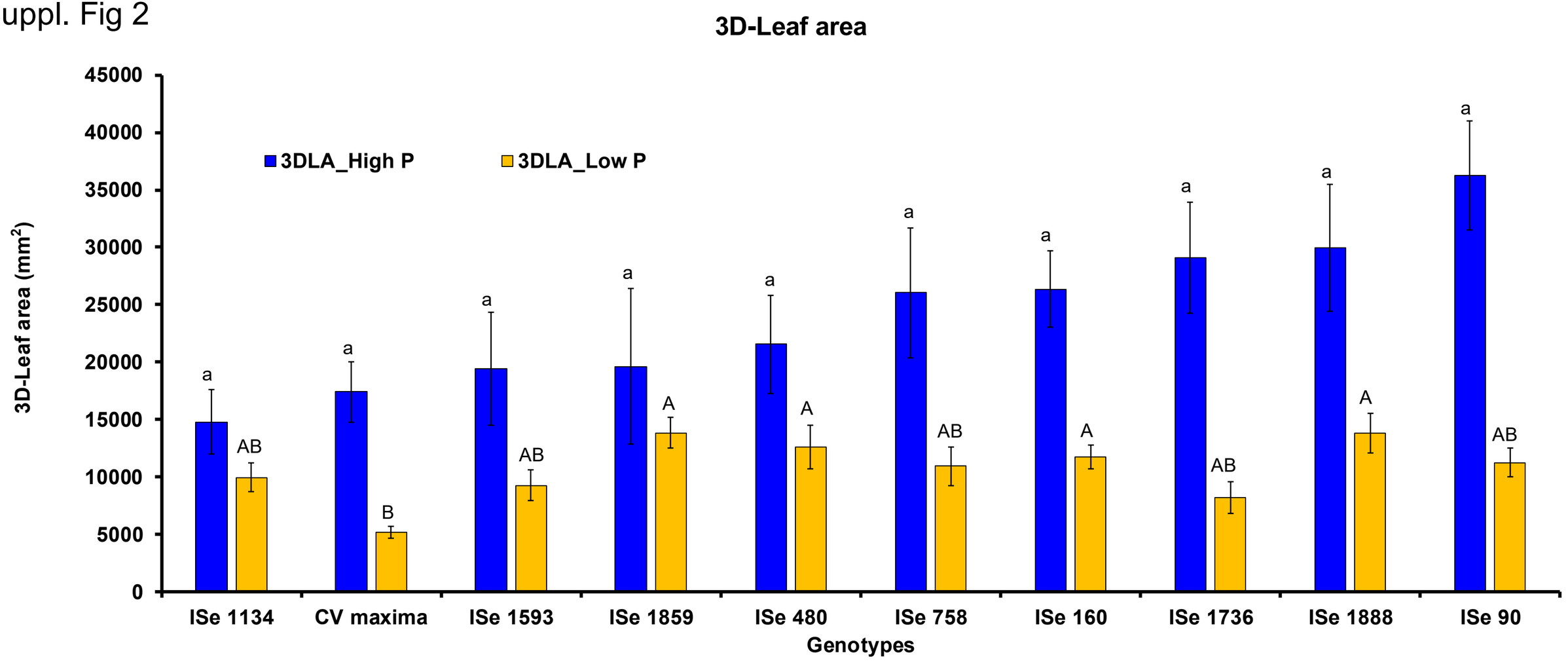
Fig. 5


3.0
2.7
2.4
2.1
1.8
1.5
1.2
0.9
0.6
0.3
0.0
Total Biomass (g)
0
10
20
30
40
50
60
70
80
Total P concentration (mg g-1)
y = 0.007x + 2.1141
R² = 0.0957 ns_High P
y = -0.0195x + 2.2405
R² = 0.71**_Low P

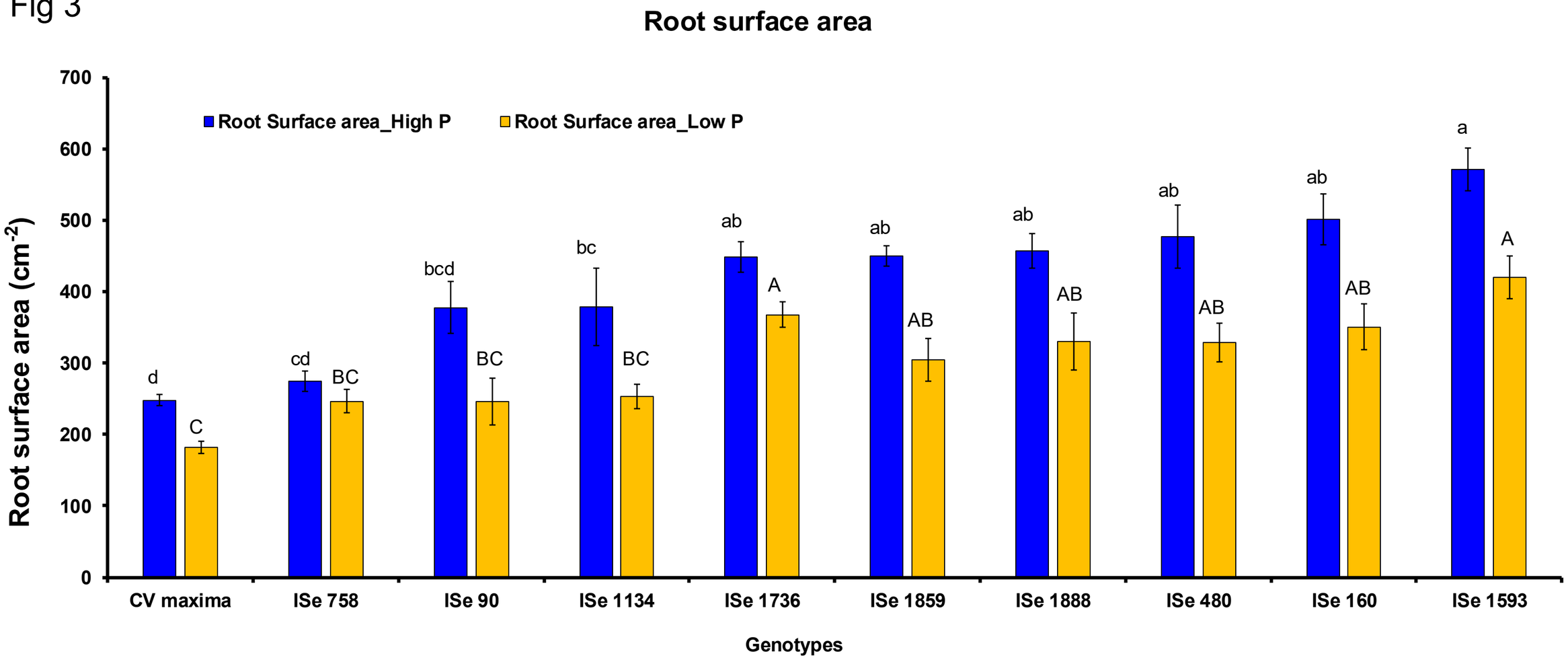
Suppl. Fig 1


A
Tillers
Tiller
45
40
35
30
25
20
15
10
5
0
a
b
High P
Low P
B
Tillers
Tiller numbers
Tiller_Low P
Tiller_High P
A
a
A
ab
AB
ab
ABC
abc
C
e
ABC
bcd
BC
cde
C
bcde
BC
cde
C
de
ISe 710
ISe 480
ISe 1134
ISe 160
ISe 1593
ISe 90
ISe 1736
ISe 1888
ISe 1859
ISe 758
Genotypes

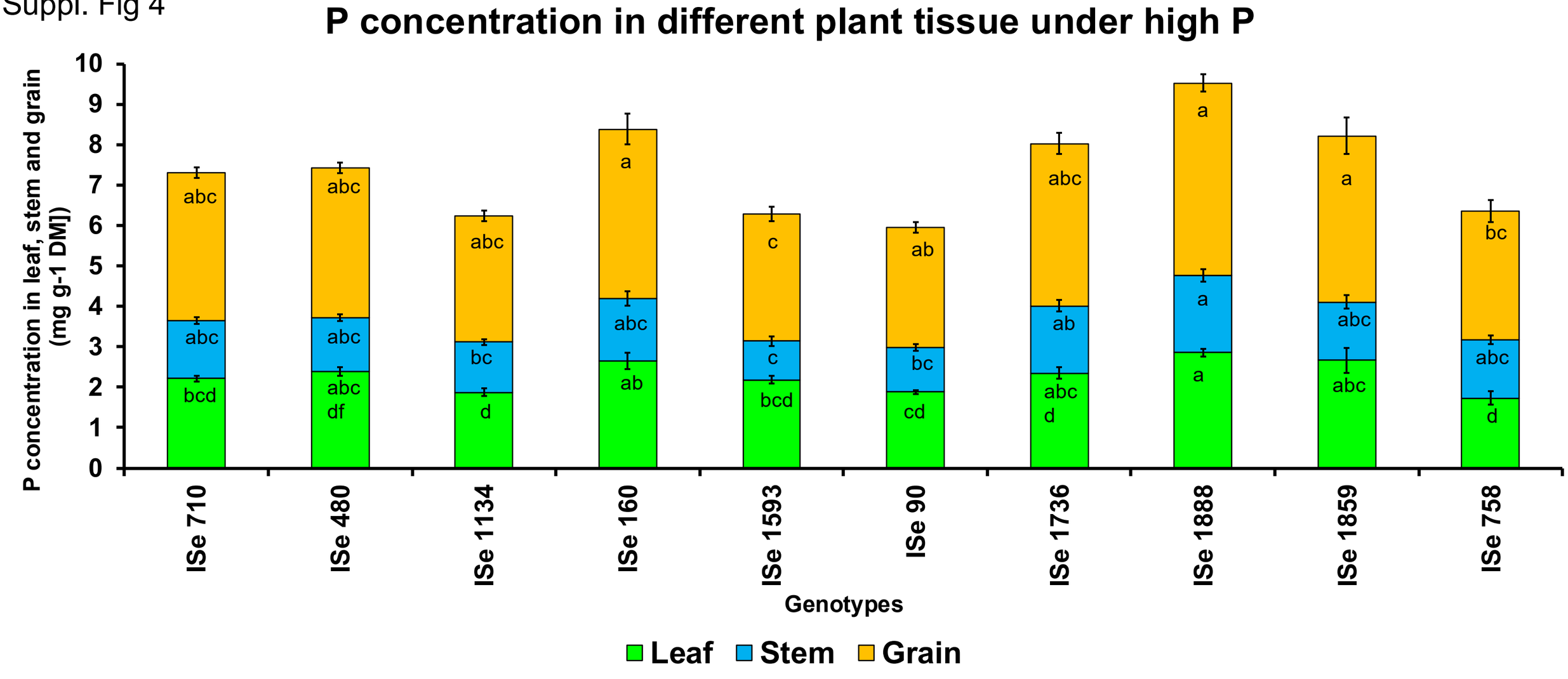
Suppl. Fig 2


3D-Leaf area
3DLA_High P
3DLA_Low P
3D-Leaf area (mm2)
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
a
AB
a
B
a
AB
a
A
a
A
a
AB
a
A
a
AB
a
A
a
AB
ISe 1134
CV maxima
ISe 1593
ISe 1859
ISe 480
ISe 758
ISe 160
ISe 1736
ISe 1888
ISe 90
Genotypes

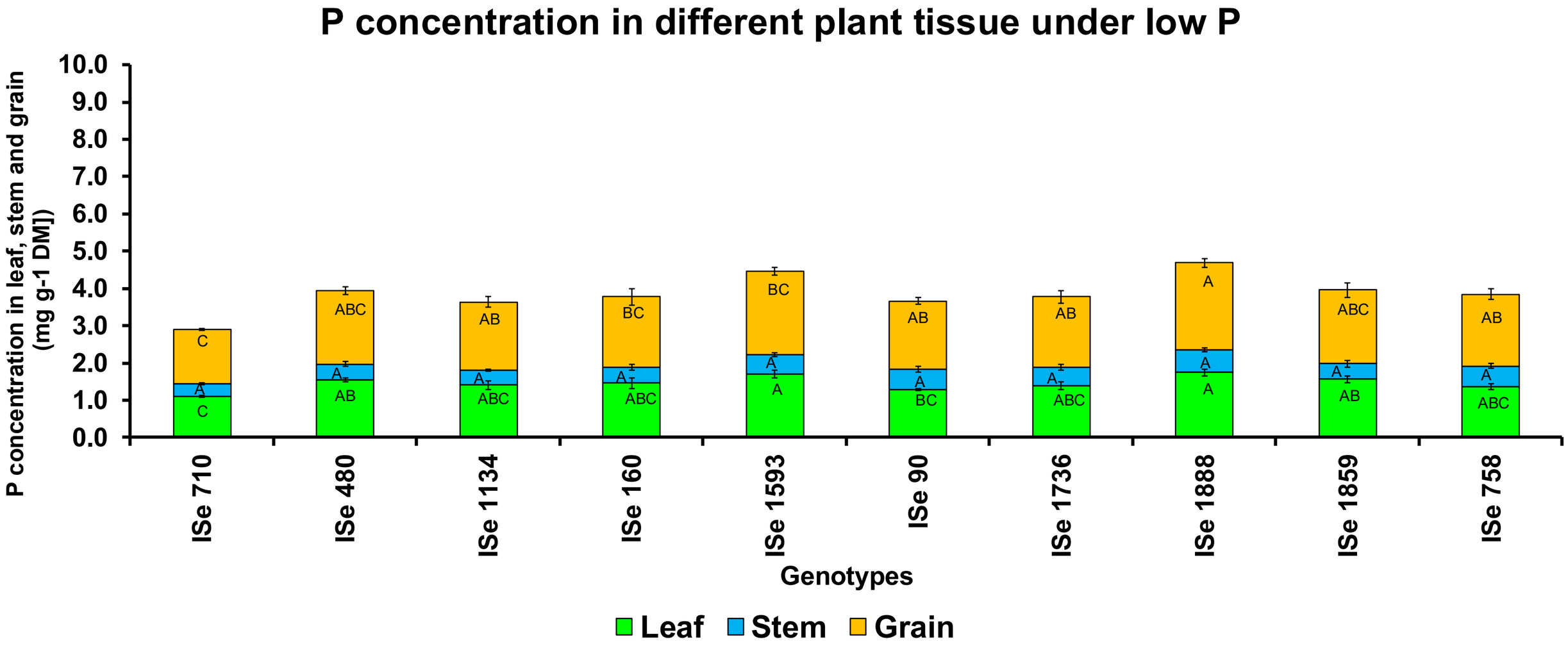
Suppl. Fig 3


Root surface area
Root Surface area_High P
Root Surface area_Low P
Root surface area (cm-2)
700
600
500
400
300
200
100
0
d
C
cd
BC
bcd
BC
bc
BC
ab
A
ab
AB
ab
AB
ab
AB
ab
A
CV maxima
ISe 758
ISe 90
ISe 1134
ISe 1736
ISe 1859
ISe 1888
ISe 480
ISe 160
ISe 1593
Genotypes

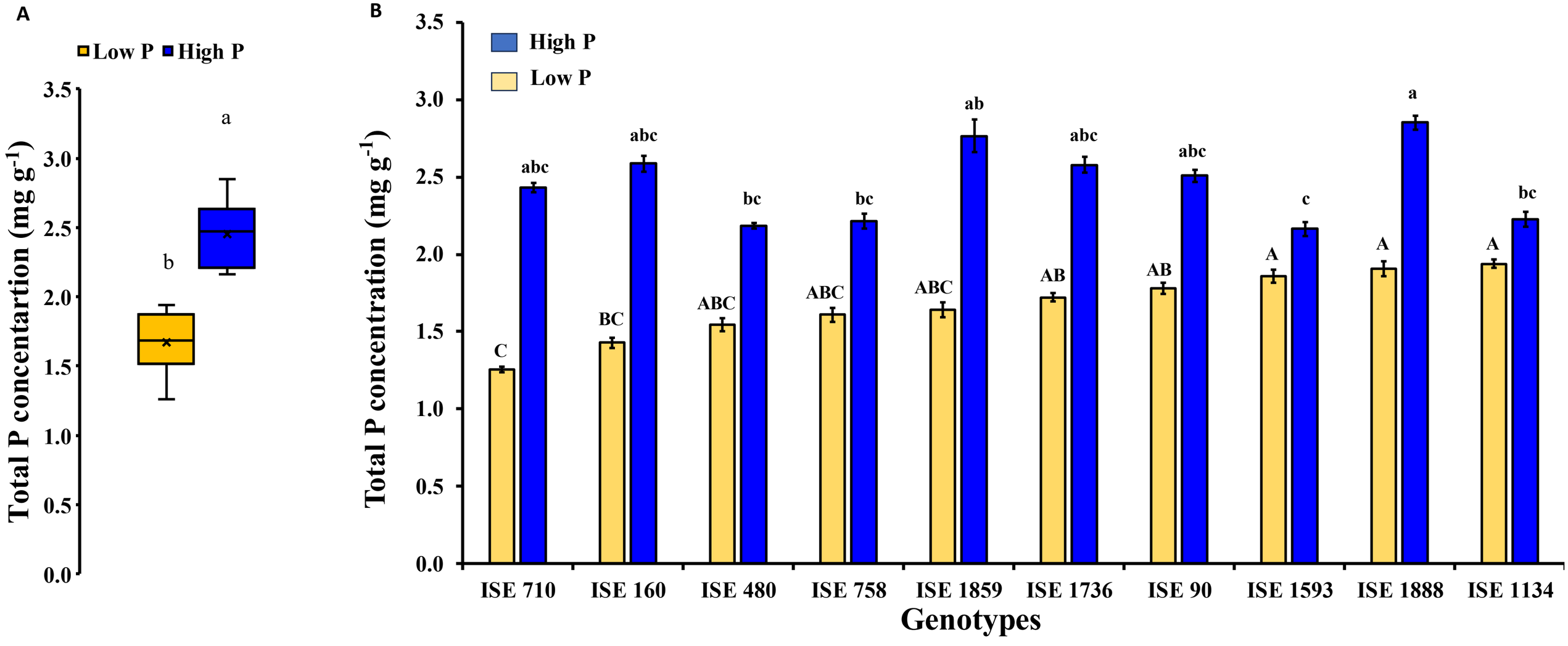
Suppl. Fig 4


P concentration in different plant tissue under high P
P concentration in leaf, stem and grain (mg g-1 DM])
0
1
2
3
4
5
6
7
8
9
10
abc
abc
bcd
abc
abc
abc
df
abc
bc
d
a
abc
ab
c
c
bcd
ab
bc
cd
abc
ab
abc
d
a
a
a
abc
a
bc
abc
d
ISe 710
ISe 480
ISe 1134
ISe 160
ISe 1593
ISe 90
ISe 1736
ISe 1888
ISe 1859
ISe 758
Genotypes
Leaf
Stem
Grain

Suppl. Fig 5


P concentration in different plant tissue under low P
P concentration in leaf, stem and grain (mg g-1 DM])
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
C
A
C
ABC
A
AB
AB
A
ABC
BC
A
ABC
BC
A
A
BC
AB
A
BC
AB
A
ABC
A
A
A
A
ABC
A
AB
AB
A
ABC
ISe 710
ISe 480
ISe 1134
ISe 160
ISe 1593
ISe 90
ISe 1736
ISe 1888
ISe 1859
ISe 758
Genotypes
Leaf
Stem
Grain

Suppl. Fig 6


A
Low P
High P
Total P concentartion (mg g-1)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
a
b
B
High P
Low P
Total P concentration (mg g-1)
C
BC
ABC
ABC
ABC
AB
AB
A
A
A
abc
abc
bc
bc
ab
abc
abc
c
a
bc
ISE 710
ISE 160
ISE 480
ISE 758
ISE 1859
ISE 1736
ISE 90
ISE 1593
ISE 1888
ISE 1134
Genotypes

Suppl Fig. 7

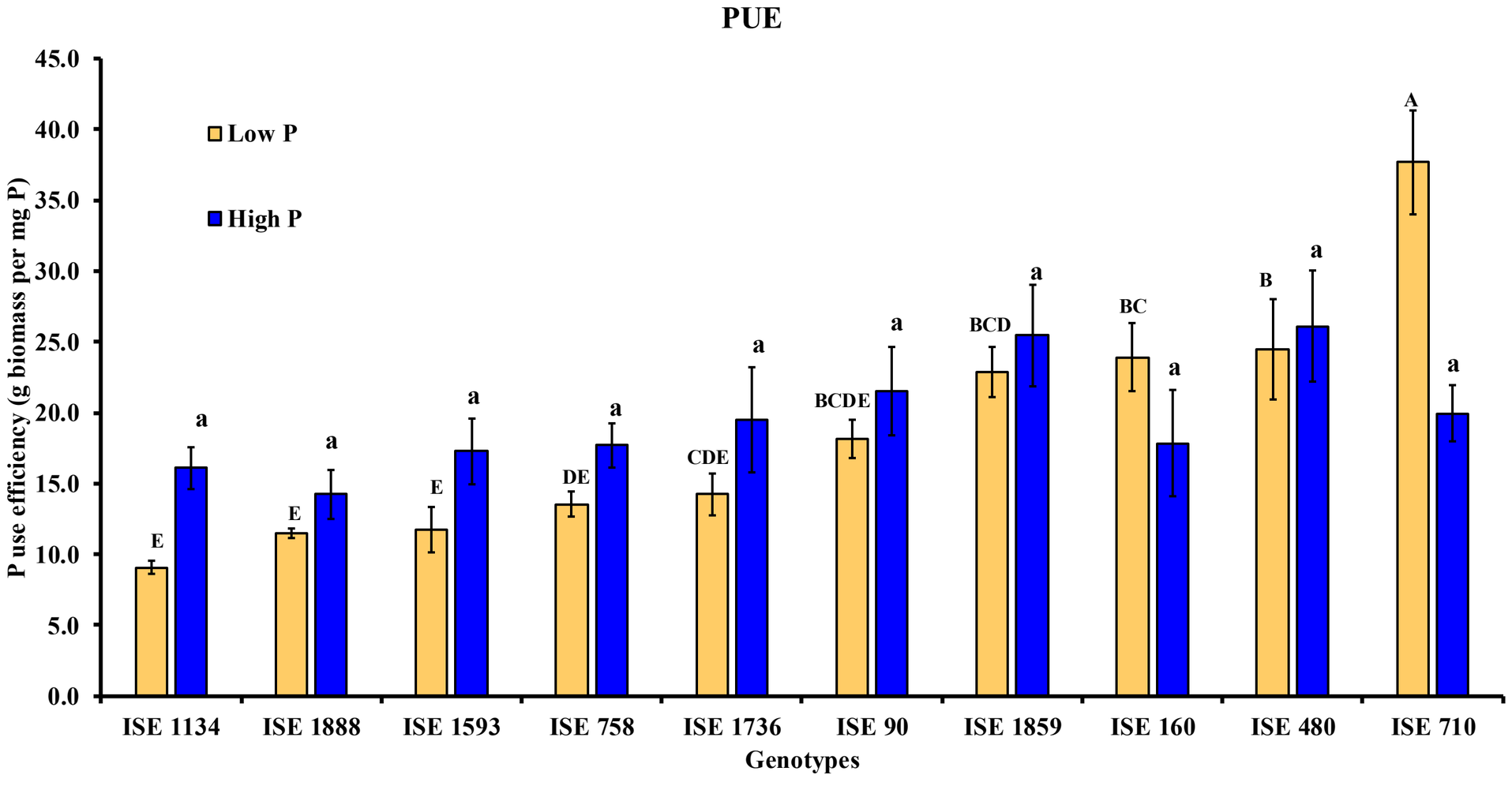

PUE
Low P
High P
P use efficiency (g biomass per mg P)
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
E
a
E
a
E
a
DE
a
CDE
a
BCDE
a
BCD
a
BC
a
B
a
A
a
ISE 1134
ISE 1888
ISE 1593
ISE 758
ISE 1736
ISE 90
ISE 1859
ISE 160
ISE 480
ISE 710
Genotypes

Table 1: Details on genotypes, treatments, and replications, along with a list of phenotyped traits obtained from various phenotyping platforms (Lysi-Field, HTP-LeasyScan, and Hydroponics).

Sl.No	Phenotyping Platform	Trait description	Unit	Trait code	Trait category	No. of genotypes	No. of treatments	Replication per treatment	Method employed for trait measurement
1	Lysi-Field (LF)	Tiller Numbers	count	TLR-LF	Growth	10	2 (Low and high P)	6	Manual counting
2	Lysi-Field (LF)	Leaf dry weight	g	LDW	Biomass	10	2 (Low and high P)	6	Weighing
3	Lysi-Field (LF)	Stem dry weight	g	StDW	Biomass	10	2 (Low and high P)	6	Weighing
4	Lysi-Field (LF)	Panicle dry weight	g	PnDW	Biomass	10	2 (Low and high P)	6	Weighing
5	Lysi-Field (LF)	Total biomass	g	TBM	Biomass	10	2 (Low and high P)	6	Weighing
6	Lysi-Field (LF)	Days to flowering	count	DFL	Phenology	10	2 (Low and high P)	6	Visual scoring based on days after sowing
7	Lysi-Field (LF)	Grain yield	g	GY	Agronomy	10	2 (Low and high P)	6	Weighing
8	Lysi-Field (LF)	Harvest Index	%	HI	Agronomy	10	2 (Low and high P)	6	Weighing
9	Lysi-Field (LF)	1000-Grain weight	g	ThGW	Agronomy	10	2 (Low and high P)	6	Mechanical counting
10	Lysi-Field (LF)	Total transpiration	kg	Tot-T	Water use	10	2 (Low and high P)	6	Weighing
11	Lysi-Field (LF)	Transpiration efficiency	gkg^{-1}	TE	Water use	10	2 (Low and high P)	6	Weighing
12	Lysi-Field (LF)	Phosphorus concentration in leaf	mg g^{-1}	Leaf P	Nutrient	10	2 (Low and high P)	6	Chemical

13	Lysi-Field (LF)	Phosphorus concentration in stem	mg g^{-1}	Stem P	Plant nutrient uptake	10	2 (Low and high P)	6	Chemical
14	Lysi-Field (LF)	Phosphorus concentration in grain	mg g^{-1}	Grain p	Plant nutrient uptake	10	2 (Low and high P)	6	Chemical
15	Lysi-Field (LF)	Total phosphorus concentration	mg g^{-1} dry	Tot-P conc	Plant nutrient uptake	10	2 (Low and high P)	6	Chemical
16	Lysi-Field (LF)	Phosphorus use efficiency	g^2mg^{-1}	PUE	Plant nutrient use efficiency	10	2 (Low and high P)	6	Chemical
17	LeasyScan (LS)	Digital biomass	mm^{-3}	DBM	Biomass	10	2 (Low and high P)	8	3D imaging
18	LeasyScan (LS)	Plant height	mm	PH	Growth	10	2 (Low and high P)	8	3D imaging
19	LeasyScan (LS)	3D-Leaf area	mm^{-2}	3DLA	Biomass	10	2 (Low and high P)	8	3D imaging
20	LeasyScan (LS)	Pojected leaf area	mm^{-2}	Proj.LA	Biomass	10	2 (Low and high P)	8	3D imaging
21	Hydroponics (Hydro)	Root length	cm	RL	Growth	10	2 (Low and high P)	8	Manual measurement with a ruler
22	Hydroponics (Hydro)	Crown Root Numbers	count	Crown root No.	Growth	10	2 (Low and high P)	8	Manual counting
23	Hydroponics (Hydro)	Shoot dry weight	g	ShDW	Biomass	10	2 (Low and high P)	8	Weighing
24	Hydroponics (Hydro)	Root dry weight	g	RDW	Biomass	10	2 (Low and high P)	8	Weighing
25	Hydroponics (Hydro)	Root: Shoot ratio		RDW/ShDW	Biomass	10	2 (Low and high P)	8	Weighing
26	Hydroponics (Hydro)	Root Surface area	cm^2	RSA	Biomass	10	2 (Low and high P)	8	Digital imaging

27	Hydroponics (Hydro)	Leaf area	cm^2	LA	Biomass	10	2 (Low and high P)	8	Area quantification
28	Hydroponics (Hydro)	Tiller Numbers	count	TLR-LS	Growth	10	2 (Low and high P)	8	Manual counting

Table 2: Two-way ANOVA results for Plant Growth, Water Use, Phenology, and Agronomical Traits Across Various Phenotyping Platforms. The table includes Mean Sum of Squares for treatment (T), genotypes (G), and T x G interactions, along with corresponding P values and significance levels. The use of different alphabets in the Tukey-Kramer test with trait mean values denote significant differences between low and high P treatments at least significant difference of 0.05. *, ** and *** signify significant differences at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively, while "ns" denote non-significant differences.

Sl.No	Phenotyping platform	Trait code	Treatment (T)	P value	Genotype (G)	P value	T x G Int.	P value	Error	High P Mean	Low P Mean	LSD (0.05)
1	Lysi-Field (LF)	TLR-LF	3646.52	***	536.06	***	145.37	***	32.68	17.5a	6.37b	2.088
2		LDW	387.735	***	26.463	***	4.51	ns	3.631	8.91a	5.32b	0.696
3		StDW	852.929	***	216.746	***	33.86	*	13.504	15.0a	9.74b	1.342
4		DFL	542.417	***	125.715	***	6.22	ns	5.784	47.0a	42.7b	0.878
4		PnDW	4949.259	***	304.701	***	57.14	ns	60.117	29.0a	16.2b	2.832
6		TBM	13749.789	***	1201.165	***	140.46	ns	144.963	52.6a	31.3b	4.398
7		GY	3063.63	***	203.64	***	74.84	ns	53.45	24.2a	14.1b	2.671
8		HI	14.416	ns	316.766	***	138.79	**	52.713	45.6a	44.7a	2.652
9		ThGW	0.815	***	0.508	***	0.01	ns	0.0348	2.78a	2.63b	0.07
10		Tot-T	6.312	ns	47.554	***	5.51	ns	11.9	23.2a	22.7b	1.26
11		TE	24.707	***	1.311	***	0.16	ns	0.181	2.01a	1.10b	0.155
12		Leaf P	18.82	***	0.73	***	0.283	***	0.089	2.27a	1.46b	0.114
13		Stem P	24.41	***	0.247	***	0.209	***	0.058	1.41a	0.47b	0.093
14		Grain p	10.77	***	0.503	***	0.403	***	0.089	3.17a	2.55b	0.114

15		Tot-P	17.15	***	0.322	***	0.302	***	0.063	2.45a	1.66b	0.096
16		PUE	20.26	ns	359.54	***	161.59	***	32.91	19.38a	18.88a	2.19
17		DBM	9.815	***	2.597	*	2.09	ns	1.09	7158113 a	2600247 b	1018793
18		PH	175664	***	11828	***	3562	ns	2711	302 a	239 b	16.059
19		3DLA	7.693	***	2.635	**	1.673	*	84406052	23356 a	10595 b	2834
20	HTP-LeasyScan	Proj.LA	2.159	***	90734768	**	56400038	ns	30118890	13233 a	6512 b	1693
21		RL	9402	***	890	***	155	*	63.961	38.502 a	23.51 b	2.58
22		Crown Root No	900	***	96.9	***	7.714	ns	5.662	-	-	0.795
23		ShDW	2.014	***	0.336	***	0.044	**	0.014	0.563 a	0.333 b	0.039
24		RDW	0.196	***	0.024	***	7.916	ns	9.97	0.403 a	0.337 b	0.009
25		RDW/ShDW	6.235	***	2.937	***	0.092	ns	0.266	1.319a	0.873b	0.188
26		RSA	617844	***	114092	***	9380	ns	6134	419 a	302 b	24.62
27	Hydroponics	LA	8453	***	4970	***	754	***	128	50.464 a	35.582 b	4.404

Table 3: One-way ANOVA Results for Plant Growth, Water Use, Phenology, and Agronomical Traits Across Various Phenotyping Platforms. The table includes Mean Sum of Squares for genotypes (G) along with corresponding P values and significance levels. The use of different alphabets in the Tukey-Kramer test with trait mean values indicates differences between genotypes at least significant difference of 0.05. *, ** and *** signify significant differences at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively, while "ns" denote non-significant differences.

S.NO	Phenotyping Platform	Trait code	Trt	G_MS	P value	Error	ISE 710 (LF) & CV Maxima (LS &Hydro)	ISE 480	ISE 1134	ISE 160	ISE 1593	ISE 90	ISE 1736	ISE 1888	ISE 1859	ISE 758	LSD (0.05)
1	Lysi-Field (LF)	Leaf P	High P	0.79	***	0.13	2.22 abc	2.38 abc	1.88 c	2.65 ab	2.18 abc	1.88 cd	2.35 abc	2.85 a	2.66 a	1.73 c	0.47
			Low P	0.22	***	0.05	1.12 c	1.55 ab	1.42 abc	1.46 abc	1.71 a	1.28 bc	1.40 abc	1.75 a	1.57 ab	1.38 abc	0.28
2		Stem p	High P	0.39	***	0.09	1.43 abc	1.33 abc	1.24 bc	1.54 abc	0.96 c	1.09 bc	1.66 ab	1.90 a	1.44 abc	1.44 abc	0.39
			Low P	0.04	ns	0.03	0.34 a	0.43 a	0.41 a	0.43 a	0.53 a	0.56 a	0.50 a	0.60 a	0.42 a	0.56 a	0.21
3		Grain P	High P	0.54	***	0.12	3.22 abc	2.98 abc	3.09 abc	3.47 a	2.61 c	3.35 ab	3.25 abc	3.52 a	3.50 a	2.75 bc	0.44
			Low P	0.34	***	0.06	2.09 c	2.45 abc	2.71 ab	2.33 bc	2.40 bc	2.72 ab	2.65 ab	2.91 a	2.55 abc	2.74 ab	0.32
4		Tot-P	High P	3.41	***	0.08	2.43 abc	2.18 bc	2.22 bc	2.56 abc	2.16 c	2.51 abc	2.58 abc	2.85 a	2.76 ab	2.21 bc	0.35
			Low P	0.28	***	0.05	1.26 c	1.54 abc	1.94 a	1.43 bc	1.86 a	1.78 ab	1.72 ab	1.91 a	1.64 abc	1.61 abc	0.28
5		PUE	High P	79.02	ns	40.18	19.95 a	26.10 a	16.08 a	17.81 a	17.27 a	21.55 a	19.49 a	14.23 a	25.45 a	17.70 a	8.08
			Low P	442.15	***	26.38	37.67 a	24.50 b	9.05 e	23.92 bc	11.74 e	18.17 bcde	14.22 cde	11.47 e	22.87 bcd	13.54 de	6.52
6		DFL	High P	73.2	***	5.96	44.0 abc	48.5 a	37.0 d	47.3 ab	39.8 cd	41.2 cd	42.7 bc	39.8 cd	44.0 abc	42.5 c	2.83
			Low P	59.72	***	5.6	50.3 ab	50.7 ab	42.6 d	51.8 a	44.0 d	45.3 cd	44.3 cd	45.2 cd	48.8 abc	46.5 bcd	3.01
7		StDW	High P	162.97	***	23.58	16.0 abcd	24.9 a	11.2 cd	17.9 abc	7.49 d	13.3 bcd	15.5 bcd	13.0 bcd	20.7 ab	10.0 cd	5.63
			Low P	87.21	***	3.22	15.8 a	13.2 ab	4.58 d	13.1 ab	4.79 d	9.80 bc	7.74 cd	7.22 cd	12.7 ab	7.60 cd	2.28
8		LDW	High P	14.72	*	5.95	10.2 ab	11.6 a	7.65 ab	8.72 ab	6.40 b	9.69 ab	9.07 ab	8.10 ab	10.3 ab	7.45 ab	2.83
			Low P	15.89	***	1.27	8.39 a	6.44 ab	2.32 d	5.42 bc	3.94 cd	5.79 bc	4.57 bcd	4.10 cd	6.54 ab	5.55 bc	1.43
9		PnDW	High P	268.35	*	100.88	29.9 ab	35.1 ab	20.1 b	29.5 ab	25.4 ab	33.9 ab	27.2 ab	23.9 ab	42.5 a	23.0 b	11.65
			Low P	97.66	***	18.53	22.2 a	20.0 ab	12.4 bc	18.6 ab	14.2 abc	18.4 abc	13.4 bc	12.4 bc	19.6 ab	10.3 c	5.47
10		GY	High P	195.53	*	82.86	22.4 ab	26.2 ab	17.5 b	23.2 ab	22.2 ab	28.94 ab	23.5 ab	19.8 b	37.8 a	21.2 ab	10.55
			Low P	85.73	**	23.44	21.1 a	16.3 ab	13.4 ab	13.5 ab	12.6 ab	16.23 ab	11.8 ab	9.44 b	17.7 ab	8.88 b	6.15

11		TBM	High P	854.17	**	248.17	56.2 abc	69.7 ab	38.9 c	56.2 abc	39.3 c	56.9 abc	51.7 abc	45.0 abc	72.0 a	40.5 bc	18.27
			Low P	491.69	***	39.65	46.4 a	39.7 ab	19.3 e	37.2 abc	22.8 de	34.0 bcd	25.7 cde	23.7 de	38.8 ab	23.4 de	8
12		ThGW	High P	0.27	***	0.03	3.02 abc	2.70 bcd	2.48 d	2.70 bcd	2.81 abcd	2.83 abcd	2.55 d	3.10 a	3.05 ab	2.68 cd	0.21
			Low P	0.26	***	0.04	2.95 a	2.48 bc	2.29 c	2.54 abc	2.66 abc	2.69 abc	2.44 c	2.83 ab	2.89 a	2.44 c	0.27
13		TLR-LF	High P	612.07	***	56.72	32.7 a	30.8 ab	23.2 abc	22.0 abc	3.17 e	18.2 bcd	13.5 cde	17.3 bcde	10.2 cde	4.00 de	8.73
			Low P	70.92	***	8.15	11.5 a	11.3 a	9.20 ab	6.84 abc	2.17 c	7.00 abc	5.84 bc	3.34 c	5.00 bc	2.00 c	3.62
14		Tot-T	High P	24.31	ns	17.93	24.9 a	24.9 a	21.4 a	22.8 a	21.5 a	22.8 a	22.0 a	21.3 a	27.4 a	22.2 a	4.91
			Low P	28.66	***	5.75	27.2 a	23.7 abc	20.4 c	21.0 bc	22.3 bc	23.0 abc	21.3 bc	21.8 bc	25.5 ab	20.7 c	3.05
15		TE	High P	0.91	**	0.3	2.01 ab	2.59 a	1.55 ab	2.20 ab	1.54 b	2.29 ab	2.05 ab	1.8 ab	2.53 ab	1.62 ab	0.64
			Low P	0.57	***	0.06	1.48 a	1.42 a	0.72 d	1.49 a	0.78 d	1.25 abc	0.94 bcd	0.82 cd	1.29 ab	0.85 bcd	0.31
16		HI	High P	239.86	***	55	39.8 bc	36.7 c	43.0 abc	39.3 bc	55.7 a	50.3 abc	45.5 abc	42.7 abc	50.8 abc	52.0 ab	8.6
			Low P	210.59	***	50.38	39.7 b	41.3 ab	55.2 a	39.3 b	54.0 a	47.7 ab	46.0 ab	43.3 ab	45.3 ab	37.0 b	9.02
17	LeasyScan (LS)	DBM	High P	3.893	ns	2.21	6401059 ab	5875710 ab	4406207 b	8166179 ab	5727012 ab	12385575 a	8156354 ab	9001382 ab	5507217 ab	8414657 ab	5410949
			Low P	4.636	**	1.537	1529939 b	2735205 ab	2870798 ab	2895631 ab	1979291 ab	2976411 ab	1661432 b	3707089 a	3297371 ab	2645904 ab	1232316
18		PH	High P	5676	ns	3559	340 a	269 a	292 a	301 a	301 a	352 a	283 a	302 a	275 a	322 a	68.662
			Low P	9635	***	2000	272 ab	208 bcd	279 a	242 abcd	206 cd	271 abc	191 d	268 abc	235 abcd	226 abcd	44.453
19		3DLA	High P	3.433	*	1.631	17395 a	21539 a	14801 a	26347 a	19414 a	36228 a	29057 a	29921 a	19628 a	26052 a	14702
			Low P	68697120	***	18466113	5180 b	12622 a	9964 ab	11712 a	9246 ab	11243 ab	8188 ab	13794 a	13836 a	10930 ab	4271
20		Proj.LA	High P	1.147	*	56487665	9766 a	12276 a	8682 a	14855 a	10171 a	20152 a	16325 a	17660 a	10940 a	15455 a	8650
			Low P	26746652	**	8042707	3082 b	7843 a	6131 ab	7332 a	5625 ab	7171 ab	4961 ab	7581 a	8759 a	6978 ab	2819
21		TLR-LS	High P	16.858	ns	11.481	9.67 a	10.5 a	8.38 a	11.43 a	11:00 AM	13.15 a	9.17 a	9.63 a	12.5 a	11.88 a	3.913
			Low P	1.631	ns	2.817	5.2 a	5.1 a	5.4 a	5.5 a	4.7 a	5.125 a	4.6 a	5.75 a	5.6 a	5.8 a	1.668
22	Hydroponics (Hydro)	RL	High P	667	***	73.633	23.09 d	40.84 b	39.32 bc	56.48 a	46.94 ab	34.69 bcd	44.06 ab	35.56 bcd	38.12 bcd	27.03 cd	9.894
			Low P	334	***	56.476	12.3 c	27.77 ab	19.63 bc	26.42 ab	33.35 a	23.76 ab	28.18 ab	21.72 abc	23.32 ab	18.56 bc	7.47
23		Crown Root No	High P	63.38	***	5.169	8.43 e	17.84 a	10.34 de	13 bcd	15.84 ab	14.5 abc	14.43 abcd	17.72 a	13.72 abcd	11.13 cde	2.625
			Low P	38.867	***	6.023	5.8 c	10.23 ab	5.5 c	8.8 abc	11.63 a	9.75 ab	9.5 ab	11 ab	9.4 ab	7.4 bc	2.609
24		ShDW	High P	0.263	***	0.013	0.35 cd	0.55 b	0.52 bc	0.84 a	0.83 a	0.61 b	0.59 b	0.59 b	0.61 b	0.17 d	0.144

			Low P	0.096	***	0.016	0.2 bc	0.32 abc	0.38 ab	0.4 a	0.48 a	0.38 ab	0.33 abc	0.36 ab	0.39 a	0.12 c	0.134
25		RDW	High P	0.014	***	0.001	0.36 cd	0.43 ab	0.4 bcd	0.44 ab	0.48 a	0.4 bc	0.42 bc	0.43 ab	0.39 bcd	0.34 d	0.039
			Low P	0.011	***	8.448	0.29 e	0.36 bc	0.32 cde	0.37 ab	0.41 a	0.34 bcde	0.34 bcd	0.36 b	0.33 bcde	0.31 de	0.027
26		RDW/ShDW	High P	1.142	***	0.036	1.14b	0.8bc	0.81bc	0.57c	0.64c	0.66c	0.73c	0.74bc	0.66c	2.13a	0.315
			Low P	2.055	***	0.415	1.63 ab	1.51 b	1.05 b	1.19 b	0.88 b	0.93 b	1.09 b	1.3 b	1.08 b	2.57 a	0.685
27		RSA	High P	87957	***	6689	248 d	503 ab	380 bc	520 ab	559 a	378 bcd	449 ab	457 ab	450 ab	275 cd	94.2
			Low P	37374	***	5667	182 c	328 ab	254 bc	348 ab	400 a	251 bc	368 a	321 ab	305 ab	246 bc	74.876
28		LA	High P	5860	***	150	4.04 d	55.96 bc	18.06 d	101 a	70.08 b	65.23 bc	43.97 c	54.8 bc	61.16 bc	19.04 d	14.165
			Low P	1321	***	98.223	2.52 e	38.38 bc	11.13 de	44 ab	60.77 a	32.19 bcd	30.71 bcd	36.72 bc	44.92 ab	15.29 cde	16.33
29		TLR-Hydro	High P	2.468	**	0.704	2.45 a	1.71 a	2.78 a	1.28 a	-	-	2.24 a	1a	-	1a	2.41
			Low P	1.464	**	0.324	1.62 ab	1.25 ab	2.56 a	1.34 ab	1.34 ab	-	1.62 ab	1b	-	1 ab	1.64