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Integrated iron foliar application and phosphorus fertilization enhance yield and nutrient dynamics of mung bean in calcareous soils

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Abstract

Background Mung bean production in calcareous soils is often constrained by low nutrient availability, particularly iron (Fe) and phosphorus (P), which limit plant growth and yield. This study aimed to evaluate the effects of Fe foliar application and P fertilization on growth, yield, and nutrient dynamics of mung bean under calcareous soil conditions.

Results A two-year field experiment (2022–2023) was conducted using a randomized complete block design with Fe foliar application (0, 0.2, 0.4, and 0.6%) and P fertilization rates (0, 30, 60 and 90 kg ha^{-1}). Iron and phosphorus applications significantly influenced yield components, physiological traits and nutrient accumulation. Foliar application of 0.4% Fe resulted in the highest thousand seed weight (39.42 g), biological yield (3132 kg ha^{-1}), seed yield (790 kg ha^{-1}) and crop growth rate (13.58 g m^{-2} day^{-1}), representing increases of 12.21%, 12.54%, 23.44% and 44.47%, respectively over the control treatment. Similarly, phosphorus application at 60 kg ha^{-1} significantly enhanced thousand seed weight (39.83 g), biological yield (3156 kg ha^{-1}) and seed yield (796 kg ha^{-1}), chlorophyll content (3.12 mg g^{-1}) and crop growth rate (13.48 g m^{-2} day^{-1}) with increase of 14.49%, 14.06%, 32.89% and 26.22%, respectively compared with the unfertilized control. Seed and straw N (3.58% and 1.27%) and Fe concentrations (36.2 mg kg^{-1} and 315 mg kg^{-1}) were highest under 0.6% Fe, while in case of phosphorus, the plots with 90 kg P ha^{-1} produced the maximum seed and straw N (3.97% and 1.06%). The highest seed and straw P concentration (0.31% and 0.16%) was observed at 0% Fe, while application of 90 kg P ha^{-1} also enhanced seed and straw P content (0.38% and 0.20%). Agronomic phosphorus efficiency peaked at 30 kg P ha^{-1} (4.26 kg kg^{-1}) and 0.4% Fe (4.29 kg kg^{-1}), while total N uptake (41.51 kg ha^{-1}), seed carbohydrate content (62.79%) and soil P (7.02 mg kg^{-1}) were highest with application of 90 kg P ha^{-1}. The foliar spray of 0.6% Fe resulted in higher total N uptake (40.45 kg ha^{-1}), seed carbohydrate content (61.19%) and soil P (6.79 mg kg^{-1}). Economic analysis revealed that the combined application of 0.4% Fe and 60 kg P ha^{-1} produced the highest value cost ratio.

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Conclusion The combined application of 0.4% Fe and 60 kg P ha^{-1} is recommended for farmers to enhance mung bean yield and profitability under calcareous soil conditions, where Fe and P availability is often limited, whereas 0.6% Fe with 90 kg P ha^{-1} is more effective for enhancing nutrient accumulation. This nutrient management strategy can be effectively adopted under field conditions to improve productivity and nutrient use efficiency.

Keywords Chlorophyll content, Carbohydrates, Nitrogen content, Crop growth rate, Seed yield

Introduction

Mung bean serves as a crucial source of dietary protein, playing a significant role in ensuring nutritional security for the rapidly growing population of South Asia [1, 2]. Belonging to the Fabaceae family, mung bean is cultivated as an essential summer pulse crop in several Asian countries, including Pakistan, due to its excellent flavor, high market value, superior digestibility, and ability to biologically fix nitrogen. The seed of mung bean is rich in essential nutrients, including proteins, fiber, ash, and fats [3–5]. Inoculation with arbuscular mycorrhizal fungi and *Rhizobium* spp. has been shown to improve mung bean adaptation to low phosphorus conditions, enhancing phosphorus uptake from the soil. This symbiotic relationship, therefore, holds the potential to sustain and improve mung bean productivity [6]. Beyond its nutritional benefits, mung bean is an economically vital short duration cash crop that first well into wheat-rice cropping systems, offering quick returns to smaller farmers in Pakistan. It can be grown in marginal, rainfed conditions and requires relatively low inputs, making it a climate resilient and profitable option for resource poor households [7, 8]. The increasing global demand for plant-based proteins further elevates its market value, highlighting its dual contribution to food security and rural livelihoods [9, 10].

Various strategies are employed to address micronutrient deficiencies in crops, including dietary diversification, food supplements, food fortification and biofortification [11, 12]. The two primary approaches to improving the nutritional quality of food crops are genetic and agronomic biofortification. However, agronomic biofortification is considered a more cost-effective and immediate method for increasing micronutrient content in the edible portions of crops compared to genetic biofortification [13, 14]. Agronomic biofortification is considered a rapid and practical strategy for improving micronutrient concentration because it can be directly implemented through fertilizer management without the lengthy breeding process required in genetic biofortification [15]. Unlike genetic biofortification, agronomic approaches can be readily adopted and adjusted according to soil and environmental conditions [16]. Moreover, agronomic biofortification provides immediate enhancement in grain Fe and Zn concentrations, crop productivity and nutrient bioavailability, particularly under micronutrient-deficient soils [13]. It is also recognized as one of the most efficient strategies for mitigating mineral deficiencies in human diets. Iron (Fe) and zinc, in particular, are essential micronutrients that pose a challenge for biofortification due to their low bioavailability, requiring substantial amounts to be effective [17, 18]. It is possible to boost micronutrient concentrations without a corresponding loss in yield. Agronomic biofortification techniques such as seed priming, seed coating, and soil or foliar fertilization have proven effective in increasing the micronutrient content of grains [19, 20]. Foliar application of Fe has been shown to address deficiencies and enhance yields [21]. Additionally, the combined application of phosphorus and Fe can have synergistic effects, improving pod number and biomass in legumes [22, 23].

Iron is essential for the activation of various key enzymes and acts as a co-factor in enzymes such as cytochrome oxidase, catalase, peroxidase and nitrogenase [24, 25]. At the biochemical level, Fe and P are tightly interconnected in legumes that Fe is a constituent of nitrogenase and leghemoglobin, while P drives the ATP-dependent energy transfer required for nitrogen fixation in nodules; however, these two nutrients also exhibit antagonistic interactions as their homeostasis is coordinately regulated and imbalances in one can impair the utilization of the other [21, 22]. However, in calcareous soils, Fe availability is limited due to its precipitation into inorganic forms, resulting in reduced chlorophyll synthesis, impaired enzyme function and stunted growth [20]. In calcareous soils, Fe availability is critically limited because of high pH (7.5–8.5) lowers Fe solubility by several orders of magnitude and elevated bicarbonate concentrations buffer rhizosphere pH and inhibit root ferric reductase activity, preventing Fe acquisition [26]. Similarly, P availability is governed by sorption and precipitation reactions with calcium, which form insoluble Ca-P compounds, with approximately 80% of applied P rapidly fixed and lost from the available pool [27]. These combined constraints severely impair mung bean growth and yield on calcareous soil. Iron contributes to chlorophyll production, nodule development and enzymatic activities related to nitrogen fixation; however, high soil pH restricts its availability, thereby hindering growth and reducing yield [21, 22]. Split applications of Fe can improve mung bean yield, grain Fe concentration and economic returns. Nevertheless, excessive Fe application can be toxic and interfere with cellular processes [28, 29]. Phosphorus plays a key role in root growth, timely crop

maturity and nodulation, which in turn enhances total dry matter production, seed yield and protein content [30]. Phosphorus deficiency significantly limits mung bean growth and yield by impairing root development and grain formation. Adequate phosphorus availability is essential for root proliferation, nodule formation, and nitrogen fixation [31]. It also supports pod development, energy production, nitrogen absorption, carbohydrate metabolism and reproductive growth [30]. Several studies confirm that balanced Fe-P rates can enhance individual nutrient uptake, the interactive effect of these nutrients on their final bioavailability in the human diet particular the influence of phytic acid, which constitutes approximately of total seed phosphorus which remains largely underexplored [32]. According to [33], combined Fe and P biofortification in mung bean grown in calcareous soils is limited, despite the widespread prevalence of such soils in South Asia. This study was conducted to evaluate the individual and combined effects of iron foliar application and phosphorus fertilization on growth, yield, nutrient uptake and soil nutrient dynamics of mung bean under calcareous soil conditions. We hypothesized that the integrated application of Fe and P would synergistically improve nutrient availability, enhance physiological performance and increase mung bean productivity compared with their sole application or untreated control.

Materials and methods

Experimental site

A field trial was conducted at the Agronomy Research Farm of the University of Agriculture Peshawar, Pakistan, over two consecutive years (2022 and 2023) during the summer season. The objective of the study was to evaluate the effects of phosphorus and Fe foliar applications on mung bean yield, quality, agronomic phosphorus efficiency, and total nitrogen uptake. The experimental site is situated at 34.01° N latitude and 71.35° E longitude, at an elevation of 350 m above sea level. The climate data for the experimental site is provided in Fig. 1. During the experimental period, summer temperatures ranged from 35.1 °C to 40.5 °C, with an average minimum temperature spanning from 22.8 °C to 25.9 °C. Precipitation varied considerably across the summer months, with the highest rainfall recorded in August (57 mm), while June experienced the lowest rainfall (8 mm). Relative humidity ranged from 42% to 70% throughout the experimental period. The soil at the site was classified as clay loam with a lime content of 6.7%, indicative of calcareous conditions. The total nitrogen content in the soil was 0.053%, and its electrical conductivity measured 0.34 dS m^{-1}, reflecting slightly alkaline conditions (pH 7.5). Organic matter content was 0.61% and phosphorus availability was recorded at 3.28 mg kg^{-1}. Iron concentration availability was recorded at 5.7 mg kg^{-1}.

Research design and treatments

The experiment was conducted using a randomized complete block design (RCBD) with three replications. Each experimental plot measured 3 m by 2.4 m and consisted of six rows. Although uniform agronomic practices and experimental conditions were maintained throughout the study, minor variations in environmental factors such as temperature, humidity and soil heterogeneity may have influenced nutrient availability and crop response under field conditions. However, the randomized complete block design and replication were used to minimize the effects of such variability on the experimental outcomes. The mung bean cultivar Ramzan was sown using a single-row hand drill, with a row spacing of 40 cm and a plant-to-plant spacing of 10 cm, at a seeding rate of 25 kg ha^{-1}. The Ramzan variety, with parentage VC1482C × NM92, was provided by the Agronomy Research Farm at the University of Agriculture Peshawar. This variety exhibits improved traits compared to standard varieties, including larger seed size, reduced plant height, early maturity, stronger stems, higher harvest index, and a higher yield potential [34]. A recommended nitrogen dose of 25 kg ha^{-1} was applied at sowing from urea as the nitrogen source. Thinning was performed 20 days after sowing to maintain the proper plant-to-plant spacing. The crop was harvested in two pickings when the pods had turned brown, after which they were sun-dried and threshed. The study assessed four phosphorus levels (0, 30, 60, and 90 kg ha^{-1}) and four Fe levels (0% [water spray as a control], 0.2%, 0.4%, and 0.6%). Single superphosphate was used as the phosphorus source, while ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) served as the Fe source. The selected Fe foliar concentrations and phosphorus application rates were adopted based on previous published studies demonstrating their effectiveness in enhancing nutrient availability, physiological traits, and productivity of mung bean and other legumes under alkaline and calcareous soil conditions. These levels were selected to evaluate the optimum range of Fe and P supplementation without causing nutrient toxicity or antagonistic effects. Phosphorus was applied at sowing, and Fe was applied through foliar sprays 30 days after sowing. The Fe foliar spray was applied 30 days after sowing to coincide with the active vegetative growth stage of mung bean, during which nutrient demand and physiological activity are high. Application at this stage improves Fe absorption and utilization because at this stage root nodule were active for nitrogen fixation, particularly under calcareous soil conditions where Fe availability is limited due to fixation and reduced solubility. For the Fe spray application, the required amount of water for one plot was determined using a water sprayer and the appropriate concentration of $FeSO_4 \cdot 7H_2O$ solution was prepared for each treatment. Iron foliar sprays were applied

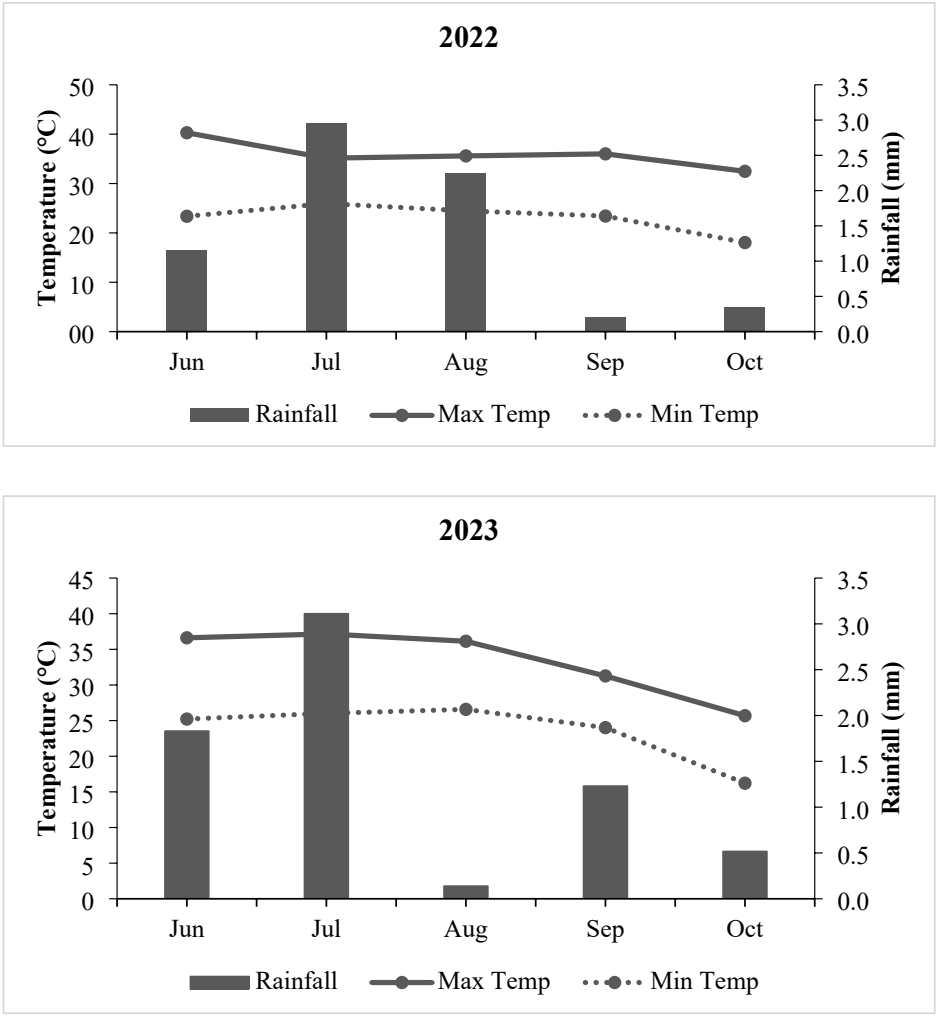


Fig. 1 Weather data for the experimental location during the mung bean growing seasons of 2022 and 2023

at concentrations of 10, 20 and 30 g of $FeSO_4{\cdot}7H_2O$ per liter of water (0.2%, 0.4% and 0.6% iron, respectively) one month after sowing applied before evening under calm and dry weather conditions to ensure uniform spray coverage and minimize evaporation losses. The foliar spray volume used in the experiment was equivalent to approximately 1389 L ha^{-1}, through which $FeSO_4{\cdot}7H_2O$ was applied at the rates of 13.89, 27.78 and 41.67 kg ha^{-1} corresponding to 10, 20 and 30 g L^{-1} of water, respectively.

Measurements

Seed and yield measurements

The thousand-seed weight was determined by counting and weighing one thousand seeds from each plot seed lot using an electronic balance. The weight was recorded, and the results were averaged across replications. For yield measurements, the middle four rows of each experimental unit were harvested when the pods had turned brown, using a sickle. The harvested material was bundled and left to dry under direct sunlight for one week. After drying, each bundle was weighed to determine the biological yield, followed by threshing to separate the seeds. Both biological and seed yields were then changed to kg ha^{-1} for standardization.

Total chlorophyll content (mg g^{-1})

Chlorophyll content was determined according to the method of Machlachlan and Zalik [35]. Fresh leaf samples (0.5 g) were collected from mung bean plants and homogenized in 10 mL of 80% acetone using a mortar and pestle under low-light conditions to minimize pigment degradation. The homogenate was centrifuged at 3000 rpm for 10 min and the supernatant was collected. The residue was re-extracted repeatedly with 80% acetone until complete pigment extraction was achieved and the residue became colorless. The collected extracts were pooled, and the final volume was adjusted to 25 mL with 80% acetone. Absorbance of the extract was measured

using a spectrophotometer at wavelengths of 663 and 645 nm against 80% acetone as a blank. Total chlorophyll contents were calculated using the following equations.

$$\text{Chlorophyll content } \left(\text{mg g}^{-1}\right) = \frac{[20.2\ (\text{A645}) + 8.02\ (\text{A663})]}{1000 \times \text{W}}$$

Crop growth rate (g m^{-2} day^{-1})

To measure the crop growth rate (CGR), four plants were randomly selected from each plot 50 days after sowing (DAS). Fresh plant samples were initially weighed and then oven-dried at 70 °C for 72 h in the laboratory. After drying, the samples were reweighed using a precision scale. The first sampling occurred at 30 DAS before the Fe spray and the second at 50 DAS. The dry weight of the plants was expressed on a per square meter basis. CGR was calculated using the formula proposed by Hunt [36].

Plant nutrient analysis

Seed and straw N content (%)

Samples of grain and straw were oven-dried at 100 °C for approximately 24 h to express nutrients concentration in plant tissue. After drying, the samples were finely ground using a grinding machine and passed through a 0.2 mm sieve. A 2 g sample was placed in a test tube, to which 20 mL of concentrated H_2SO_4 was added. The mixture was then distilled, followed by the addition of 50 mL distilled water. For titration, 0.2 N H_2SO_4 was used as the titrant solution, and 70 mL of 32% NaOH was added. Nitrogen content was determined using the Kjeldahl apparatus [37].

Seed and straw iron content (mg kg^{-1})

Iron concentration was determined using Atomic Absorption Spectrophotometry (AAS) following the procedure described by Singhal and Singh [38]. Briefly, 0.5 g of oven-dried and finely ground sample was transferred into a digestion flask, and 10 mL of concentrated nitric acid (HNO_3) was added for predigestion overnight. Subsequently, 4 mL of perchloric acid ($HClO_4$) was added, and the mixture was heated gradually on a hot plate until a clear digest was obtained. After cooling, the digest was filtered through Whatman No. 42 filter paper and diluted to a final volume of 100 mL with distilled water. Iron concentration in the digest was measured using an Atomic Absorption Spectrophotometer equipped with a hollow cathode lamp and calibrated with standard Fe solutions. The Fe concentration in plant samples was calculated from the standard calibration curve and expressed as mg kg^{-1} dry weight using the following equation:

$$\text{Fe}(\text{mg kg}^{-1}) = \frac{C \times V}{W}$$

where C is the Fe concentration obtained from the AAS (mg L^{-1}), V is the final volume of the digest (L), and W is the sample weight (kg).

Seed and straw P content (%)

Total phosphorus was determined using the ascorbic acid-molybdenum blue method [39]. Samples were digested with HNO_3-H_2O_2 to convert phosphorus into orthophosphate form, and the digest was diluted to a known volume. A 1 mL aliquot was transferred into a 25 mL volumetric flask followed by the addition of 5 mL distilled water and 2 mL combined color reagent containing ammonium molybdate, ascorbic acid, sulfuric acid, and antimony potassium tartrate. The volume was adjusted to 25 mL with distilled water, mixed thoroughly, and allowed to stand for 15 min for color development. Absorbance was measured at 880 nm using a PerkinElmer Lambda-35 spectrophotometer. Phosphorus concentration was quantified using a KH_2PO_4 standard calibration curve prepared from known standard concentrations. Phosphorus content was calculated as:

$$Phosphorus\ content\ (\%) = \frac{C\ x\ 0.025\ x\ D}{W\ x\ 10}$$

Where C = phosphorus concentration from the standard curve, D = dilution factor (total digest volume/aliquot volume) and W = sample weight (g).

Carbohydrates content (%)

Total carbohydrates were determined following the Anthrone method described by Sadasivam and Manickam [40]. Briefly, 0.5 g of powdered seed sample was homogenized with 10 mL of 80% ethanol and centrifuged at 3000 x g for 15 min to separate the insoluble residue. The precipitate was re-extracted with 2 mL of 80% ethanol and centrifuged again, after which the supernatants were pooled and evaporated under a stream of hot air. The dried residue was subsequently reconstituted in 5 mL distilled water. For carbohydrate estimation, aliquots of standard solutions and test samples were prepared in separate test tubes, with distilled water used as a blank. The sample extract was diluted to 100 mL, and 1 mL of the diluted extract was used for analysis. The test tubes were placed in an ice-water bath for 10 min, followed by the addition of 4 mL anthrone reagent. The reaction mixture was then heated in a boiling water bath for 15 min and cooled to room temperature. Absorbance was recorded at 640 nm using a spectrophotometer, and total carbohydrate concentration was calculated from the standard calibration curve.

Agronomic phosphorus efficiency (kg kg^{-1}) and total N uptake (kg ha^{-1})

Agronomic phosphorus efficiency (APE) was calculated using the procedure outlined by Alam et al. [41].

$$APE = \frac{Yield\ in\ fertilized\ plots\ \left(kg\ ha^{-1}\right) - Yield\ in\ control\ plot\ \left(kg\ ha^{-1}\right)}{Amount\ of\ P\ applied\ in\ the\ plot\ \left(kg\ ha^{-1}\right)}$$

Total nitrogen uptake was calculated based on nutrient concentration and biomass yield by multiplying the biological yield by the whole plant nitrogen concentration and divided by 100:

$$N\ uptake\ \left(kg\ ha^{-1}\right) = \frac{Biological\ yield\ \left(kg\ ha^{-1}\right)\ x\ Plant\ N\ (\%)}{100}$$

P content in post-harvest soil sample

Available soil phosphorus was extracted using the ammonium bicarbonate-diethylenetriaminepentaacetic acid (AB-DTPA) method Soltanpour and Schwab (1977) [42]. Air dried soil (10 g) was placed in a 125 mL conical flask, 20 mL of AB-DTPA solution was added and the mixture was shaken on a reciprocal shaker for exactly 15 min. The suspension was immediately filtrate through Whatman N0. 42 filter paper. A 1 mL aliquot of the clear filtrate was pipetted into a 25 mL volumetric flask and 4 mL of a composite colour-developing reagent (containing ammonium molybdate, ascorbic acid, sulfuric acid and antimony potassium tartrate) was added. The volume was made up to 25 mL with distilled water, the flask was shaken and the blue colour was allowed to develop for 15 min. Absorbance was measured at 880 nm using a UV-Vis spectrophotometer (PerkinELmer Lamba-25). The phosphorus concentration in the measured solution read from a standard curve prepared with KH_2PO_4. Soil available phosphorus was calculated as:

$$P\ \left(mg\ kg^{-1}\right) = \frac{C\ x\ V_{ext}}{W}\ x\ \frac{V_{final}}{V_{aliquot}}$$

Where:

C = P concentration from standard curve.
V_{ext}= volume of extracting solution.
W = mass of air-dried soil.
V_{final} = final volume of the coloured solution.
$V_{aliquot}$ = volume of filtrate taken.

Statistical analysis

Data was analyzed using a two-way analysis of variance (ANOVA) appropriate for in a randomized complete block design with factorial arrangements of treatments. ANOVA was used to determine the main effect of Fe and P and their interaction effects. When ANOVA indicated a significant difference among treatments, means were compared using the Least Significant Difference (LSD) test at a significance level of $P \leq 0.05$ Jan et al. [43]. Simple linear regression analysis, correlation analysis, and principal component analysis were conducted using Origin Pro 2025 (Origin Lab Corporation, Northampton, MA, USA) and R programming language (version 4.5.1; R Core Team, 2025) to explore relationships among agronomic, physiological and nutrient traits. The Shapiro-Wilk test showed that some variables were normally distributed ($p > 0.05$), while others exhibited minor but statistically significant deviations from normality ($p < 0.05$). Q-Q plots confirmed that these deviations were small and the data were deemed appropriate for parametric analysis. Levene's test indicated homogeneous variances for most traits, including Phosphorus, thousand seeds weight, biological yield, grain yield, seed N content, straw N content, seed Fe content, seed P content and total N uptake ($p > 0.05$), while total chlorophyll content, crop growth rate, straw Fe content, straw P content, carbohydrates and soil P were heterogeneous ($p < 0.05$). Log transformation improved variance stability for crop growth rate and soil P, but total chlorophyll content, straw Fe content, straw P content and carbohydrates remained heterogeneous. These results suggested that variance was largely stable across treatments.

Results

Seed weight and yield

Iron foliar spray and phosphorus fertilization significantly influenced thousand seeds weight, biological yield and seed yield of mung bean during both years. All the interactions were non-significant for thousand seed weight, seed yield and biological yield except the Fe x P interaction was fount significant for biological yield. Across Fe treatments, the application of 0.4% Fe produced the highest seed weight with values of 37.92 g in 2022 and 40.91 g in 2023, whereas the lowest values were recorded in control (0% Fe) with 34 g and 36.25 g during 2022 and 2023, respectively (Fig. 2A). Similarly, biological yield increased with Fe application and reached maximum of 3038 kg ha^{-1} in 2022 and 3227 kg ha^{-1} in 2023 under 0.4% Fe (Fig. 2B). The same trend was observed for seed yield, where 0.4% Fe produced the highest seed yield (711 kg ha^{-1} in 2022 and 808 kg ha^{-1} in 2023) compared with the control treatment which resulted in 620 kg ha^{-1} and 659 kg ha^{-1} in 2022 and 2023, respectively (Fig. 2C). Although the application of 0.6% Fe improved yield components compared with the control, its performance was slightly lower than that of 0.4% Fe. Phosphorus rates also significantly affected yield attributes. The highest thousand seed weight was obtained with 60 kg ha^{-1}, producing 38.50 g in 2022 and 41.17 g in 2023, while the lowest values were recorded in the control with 33.67 g and 35.92 g during 2022 and 2023, respectively. Likewise,

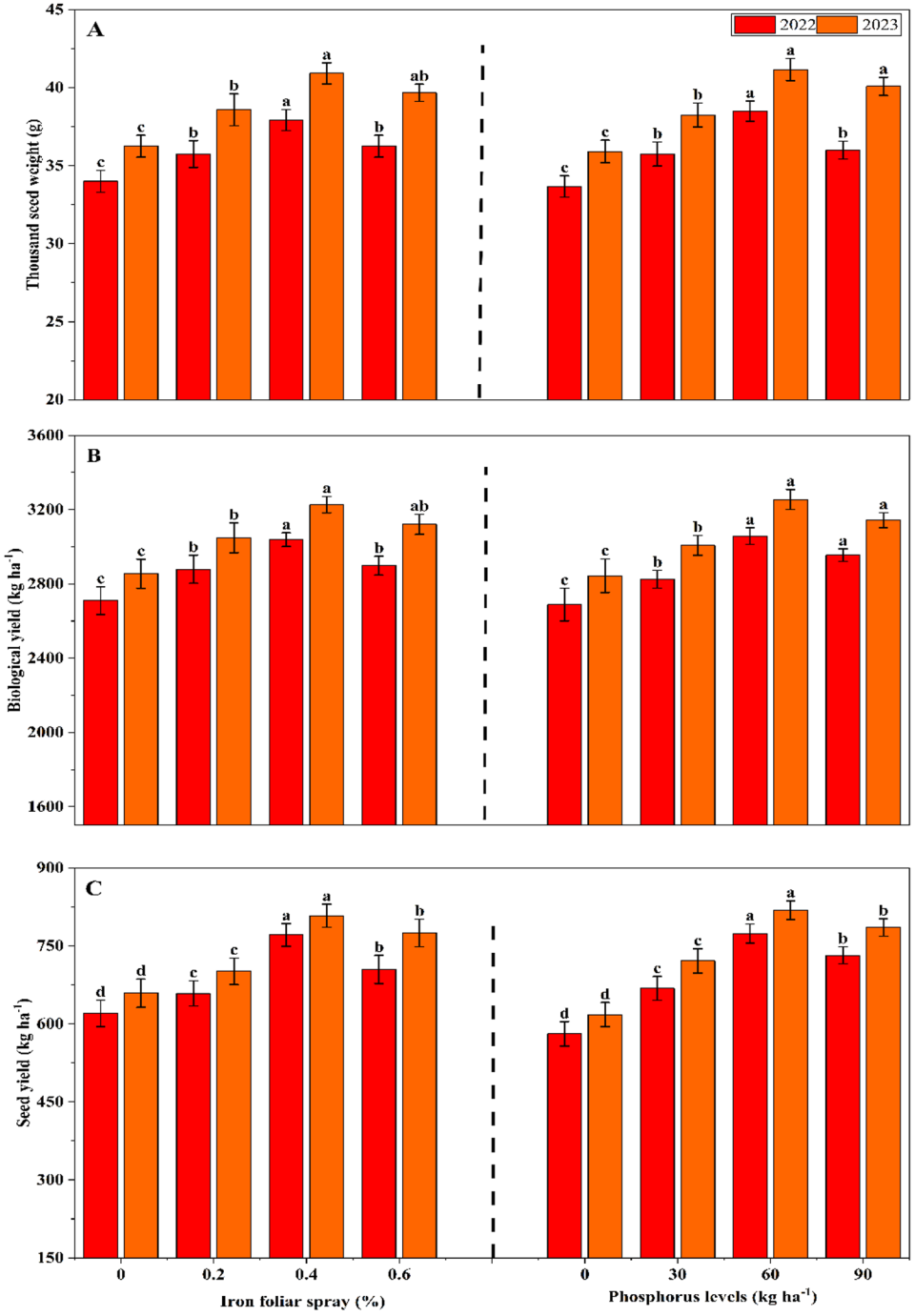


Fig. 2 Effects of iron foliar sprays and phosphorus levels on (**A**) thousand seed weight (g), (**B**) biological yield (kg ha^{-1}) and (**C**) seed yield (kg ha^{-1}) of mung bean during 2022 and 2023 years. Vertical bars represent standard error ± mean. Means followed by different letters indicate differences among treatments at LSD ≤ 0.05 according to the LSD test

biological yield increased from 2689 kg ha^{-1} in 2022 and 2845 kg ha^{-1} in 2023 under the control treatment to 3058 kg ha^{-1} and 3255 kg ha^{-1} with 60 kg P ha^{-1}. Seed yield followed a similar trend, where the highest values (774 kg ha^{-1} in 2022 and 819 kg ha^{-1} in 2023) were recorded at 60 kg P ha^{-1}, whereas the lowest seed yield was obtained in the control treatment.

Total chlorophyll (mg g^{-1}) and crop growth rate (g m^{-2} day^{-1})

Iron foliar spray and P rates significantly affected total chlorophyll content and crop growth rate of mung bean during both years. The interactions between Fe x P, Year x Fe and Year x Fe x P were found significant for total chlorophyll except the Year x P interaction, whereas for crop

growth rate all the interactions were non-significant. Among Fe foliar spray treatments, 0.6% Fe produced the highest total chlorophyll content, recording 3.36 mg g^{-1} in 2022 and 3.61 mg g^{-1} in 2023, followed closely by 0.4% Fe with 2.93 mg g^{-1} and 3.50 mg g^{-1} in the respective years. In contrast, the lowest chlorophyll content was observed in the control with 2.01 mg g^{-1} in 2022 and 2.15 mg g^{-1} in 2023 (Fig. 3A). The highest CGR was recorded at 0.4% Fe, reaching 13.24 g m^{-2} day^{-1} in 2022 and 13.92 g m^{-2} day^{-1} in 2023 which was statistically similar with 0.6% Fe (13.05 g m^{-2} day^{-1} and 13.77 g m^{-2} day^{-1} in 2022 and 2023, respectively). The lowest CGR was recorded in the control treatment (9.18 and 9.63 g m^{-2} day^{-1}) as shown in Fig. 3B. Among P rates, the application of 60 kg P ha^{-1} produced the highest total chlorophyll content (3.05 mg g^{-1} in 2022 and 3.19 mg g^{-1} in 2023), followed by 90 kg P ha^{-1} with 2.73 and 3.08 mg g^{-1}, respectively. The lowest total chlorophyll content was observed in the control treatment. Similarly, CGR was significantly improved with P application, where 60 kg P ha^{-1} produced the highest CGR (13.13 and 13.83 g m^{-2} day^{-1} in 2022 and 2023, respectively), whereas the lowest CGR was recorded in the control.

Seed and straw N content (%)

Seed and straw nitrogen (N) content was significantly impacted by Fe and P levels across both years. Seed N content increased significantly with Fe and P levels in both years (Fig. 4A). The interaction between Fe x P was found to be significant for seed and straw N content, while all other interactions were non-significant. The highest seed N content was recorded at 0.6% Fe spray (3.52% in 2022 and 3.64% in 2023), while control treatment showed the lowest seed N content (3.04% in 2022 and 3.33% in 2023). The plots with 90 kg P ha^{-1} produced the maximum seed N content (3.89% in 2022 and 4.05% in 2023), followed by 60 (3.51% in 2022 and 3.64% in 2023) and 30 kg P ha^{-1} (2.98% in 2022 and 3.07% in 2023). The control treatment showed the lowest seed N content (2.64% in 2022 and 2.97% in 2023). Across treatments, seed N content was generally higher in 2023 than in 2022. Straw N content exhibited similar trends (Fig. 4B). Increasing Fe concentration significantly enhanced straw N with maximum values obtained at 0.6% Fe spray (1.22% in 2022 and 1.32% in 2023), while the lowest values were recorded in the control (0.42% in 2022 and 0.48% in 2023). P rates also resulted in a significant and gradual increase in straw N content, reaching the highest levels at 90 kg P ha^{-1} (1.01% in 2022 and 1.12% in 2023),whereas the lowest values were observed in control treatments (0.50% in 2022 and 0.54% in 2023).

Seed and straw Fe content (mg kg^{-1})

Seed Fe content was significantly affected by Fe foliar spray and P levels in both years (Fig. 5A). All interactions were found non-significant for seed and straw Fe content except the Fe x P interaction which significantly affected seed straw content. Increasing Fe spray concentration resulted in a consistent and significant increase in Fe content with the highest Fe content recorded at 0.6% Fe spray (35 mg kg^{-1} in 2022 and 37.4 mg kg^{-1} in 2023) followed by 0.4% (32.3 mg kg^{-1} in 2022 and 34.7 mg kg^{-1} in 2023) and 0.2% (30.4 mg kg^{-1} in 2022 and 33.9 mg kg^{-1} in 2023), while the control treatments exhibited the lowest Fe concentration (27.8 mg kg^{-1} in 2022 and 30.3 mg kg^{-1} in 2023). P application also exerted a significant effect on seed Fe content in both years with increasing P levels leading to gradual reduction in seed Fe content. The highest seed Fe content was recorded in the control plots (32.3 mg kg^{-1} in 2022 and 34.6 mg kg^{-1} in 2023), whereas the minimum values observed at 90 kg P ha^{-1} (30 mg kg^{-1} in 2022 and 32.9 mg kg^{-1} in 2023). Straw Fe content was significantly affected by Fe application in both years. The highest Fe content was recorded under 0.6% spray (309 mg kg^{-1} in 2022 and 322 mg kg^{-1} in 2023), while the lowest values were observed in the control (254 mg kg^{-1} in 2022 and 258 mg kg^{-1} in 2023). P rates had a non-significant effect on straw Fe content during the first year, however a significant response was observed in the second year (Fig. 5B). In the second year, straw Fe content tended to decline with increasing P levels, the highest Fe content was recorded 30 kg P ha^{-1} (304 mg kg^{-1}), while lowest values were recorded at control plots (283 mg kg^{-1}). Across both years, maximum straw Fe content was obtained with 0.6% Fe spray, whereas the control treatment recorded the lowest values.

Seed and straw phosphorus content (%)

Seed P concentration of mung bean was significantly influenced by P fertilization in both years. Fe foliar application did not significantly affect seed P content in the first years, however in the second year, seed P content was significantly influenced by Fe application (Fig. 6A). The Fe x P interaction significantly affected seed and straw P content, whereas all other interactions were non-significant. With higher Fe sprays levels resulted in a slight but significant reduction in seed P concentration. The highest seed P content was recorded in the control plots (0.30% in 2022 and 0.314 in 2023), while the lowest values were recorded at 0.6% Fe spray (0.28% in 2022 and 0.29% in 2023). In contrast, increasing P rates resulted in a consistent increase in seed P content with the highest values recorded at 90 kg P ha^{-1} (0.37% in 2022 and 0.39 in 2023), followed by 60 (0.34% in 2022 and 0.35% in 2023) and 30 kg P ha^{-1} (0.25% in 2022 and 0.27% in 2023), while lowest content was observed in the control treatments (0.20% in 2022 and 0.21% in 2023). Straw P

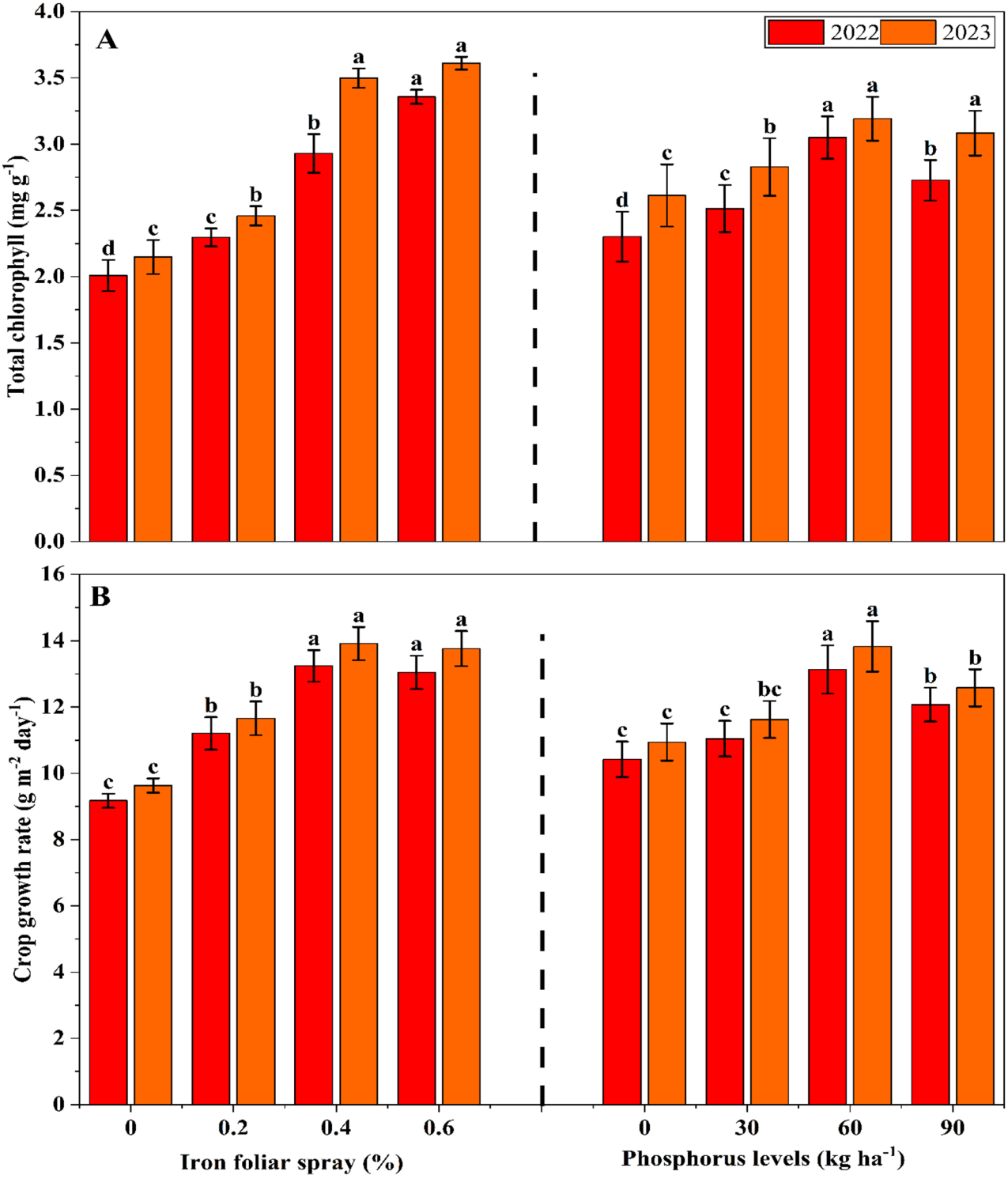


Fig. 3 Effects of iron foliar sprays and phosphorus levels on (**A**) total chlorophyll (mg g^{-1}) and (**B**) crop growth rate (g m^{-2} day^{-1}) of mung bean during 2022 and 2023 years. Vertical bars represent standard error ± mean. Means followed by different letters indicate differences among treatments at LSD ≤ 0.05 according to the LSD test

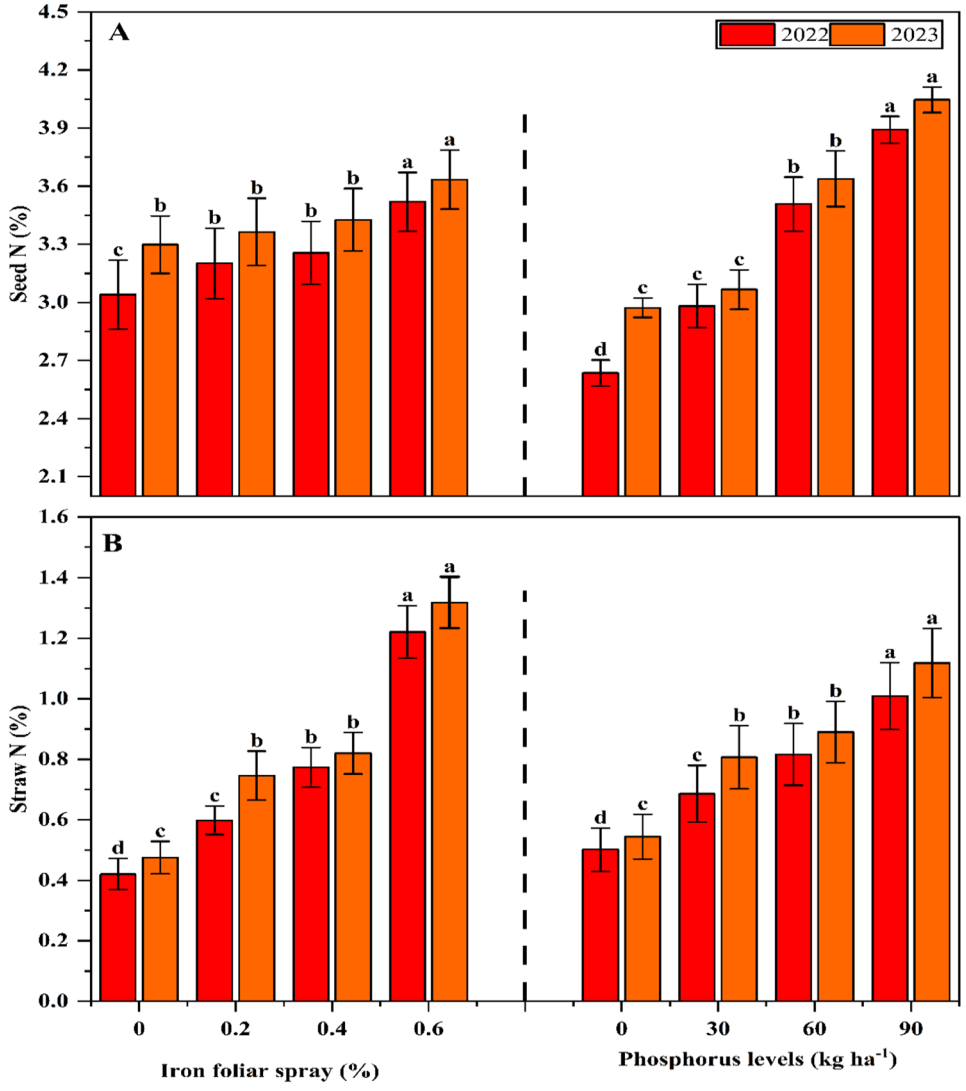


Fig. 4 Effects of iron foliar sprays and phosphorus levels on (**A**) seed N content (%) and (**B**) straw N content (%) of mung bean during 2022 and 2023 years. Vertical bars represent standard error ± mean. Means followed by different letters indicate differences among treatments at LSD ≤ 0.05 according to the LSD test

content was significantly affected by P rates and Fe foliar sprays during both experimental years (Fig. 6B). Straw P content increased markedly with increasing P rates, reaching maximum values at 90 kg P ha^{-1} (0.195% in 2022 and 0.205% in 2023), while the lowest values were recorded in unfertilized plots (0.100% in 2022 and 0.104% in 2023). Foliar application of Fe showed a decreasing trend in straw P content, with the highest P concentration observed in the control treatments (0.159% in 2022 0.166% in 2023), while the lowest values were recorded at 0.6% Fe spray (0.137% in 2022 and 0.146% in 2023).

Agronomic phosphorus efficiency, total N uptake, carbohydrates content and soil phosphorus content

Agronomic phosphorus efficiency (APE) was significantly impacted by both P rates and Fe foliar application in both years (Fig. 7A). The Fe x P and Year x Fe interactions were significant for APE, while all other interactions were non-significant. Among P treatments, APE increased markedly at 30 kg P ha^{-1} (5.45 kg kg^{-1} in 2022 and 3.07 kg kg^{-1} in 2023) followed by 60 kg P ha^{-1} (4.69 kg kg^{-1} in 2022 and 3.44 kg kg^{-1} in 2023), whereas lower APE was recorded at 90 kg P ha^{-1} (2.91 kg kg^{-1} in 2022 and 1.81 kg kg^{-1} in 2023). Regarding Fe application, APE was significantly enhanced with 0.4% Fe spray (5.75 kg kg^{-1} in 2022 and 2.83 kg kg^{-1} in 2023) followed by 0.6% (4.62 kg kg^{-1} in 2022 and 2.60 kg kg^{-1} in 2023) and 0.2% (3.98 kg kg^{-1} in 2022 and 2.74 kg kg^{-1} in 2023) compared with control (3.06 kg kg^{-1} in 2022 and 2.93 kg kg^{-1} in 2023). Year wise comparison showed that higher APE was noted during 2022 than 2023. Total N uptake showed significant respond to both P and Fe spray application in both

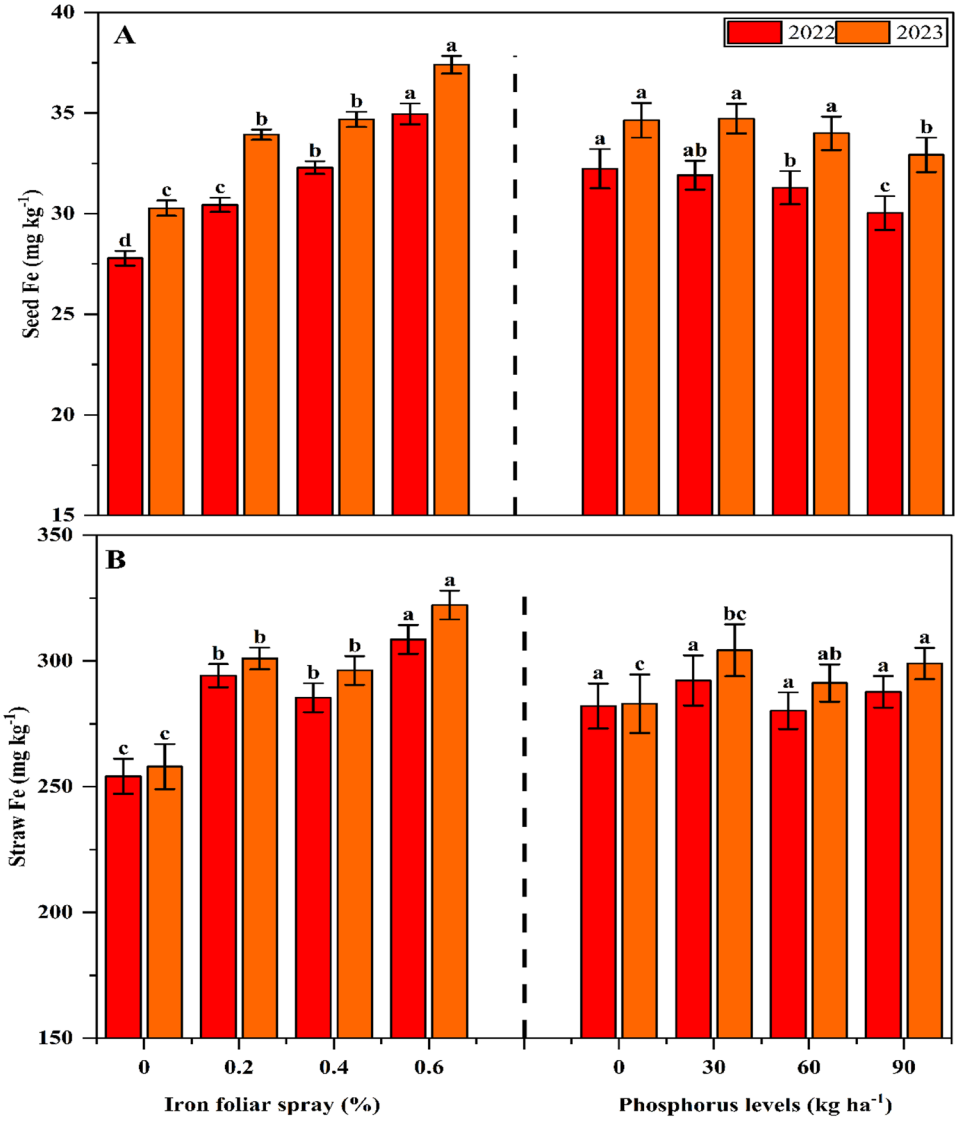


Fig. 5 Effects of iron foliar sprays and phosphorus levels on (**A**) seed Fe content (mg kg^{-1}) (**B**) straw Fe content (mg kg^{-1}) of mung bean during 2022 and 2023 years. Vertical bars represent standard error ± mean. Means followed by different letters indicate differences among treatments at LSD ≤ 0.05 according to the LSD test

years (Fig. 7B). The interactions between Fe x P, Year x Fe and Year x P were significant for total N uptake, except the Year x Fe x P interaction which was non-significant. Increasing P levels resulted in a progressive rise in N uptake, while maximum value was recorded at 90 kg P ha^{-1} (52 kg ha^{-1} in 2022 and 31 kg ha^{-1} in 2023) followed by 60 (47 kg ha^{-1} in 2022 and 29 kg ha^{-1} in 2023) and 30 kg P ha^{-1} (30 kg ha^{-1} in 2022 and 21 kg ha^{-1} in 2023), whereas the lowest values were recorded at control plots (27 kg ha^{-1} in 2022 and 17 kg ha^{-1} in 2023). Fe foliar spray also significantly increased N uptake with 0.6% (53 kg ha^{-1} in 2022 and 28 kg ha^{-1} in 2023) Fe producing the highest uptake followed by 0.4% (44 kg ha^{-1} in 2022 and 27 kg ha^{-1} in 2023) and 0.2% (36 kg ha^{-1} in 2022 and 23 kg ha^{-1} in 2023). The lowest values were recorded in control treatments (29 kg ha^{-1} in 2022 and 20 kg ha^{-1} in 2023).

Carbohydrates content of mung bean seeds was significantly affected by Fe foliar application and P fertilization in both years (Fig. 7C). The Fe x P interaction was significant for carbohydrates, whereas all other interactions were non-significant. Higher P levels increased carbohydrates content with higher values observed at 90 kg P ha^{-1} (62% in 2022 and 64% in 2023), whereas the lowest carbohydrates content was recorded at control plots (56% in 2022 and 57% in 2023). Among Fe treatments, 0.6% Fe spray (60% in 2022 and 63% in 2023) resulted in the highest carbohydrates content followed by 0.2% (58% in 2022 and 61% in 2023) and 0.4% (58% in 2022 and 60% in 2023). The lower carbohydrates were recorded in control

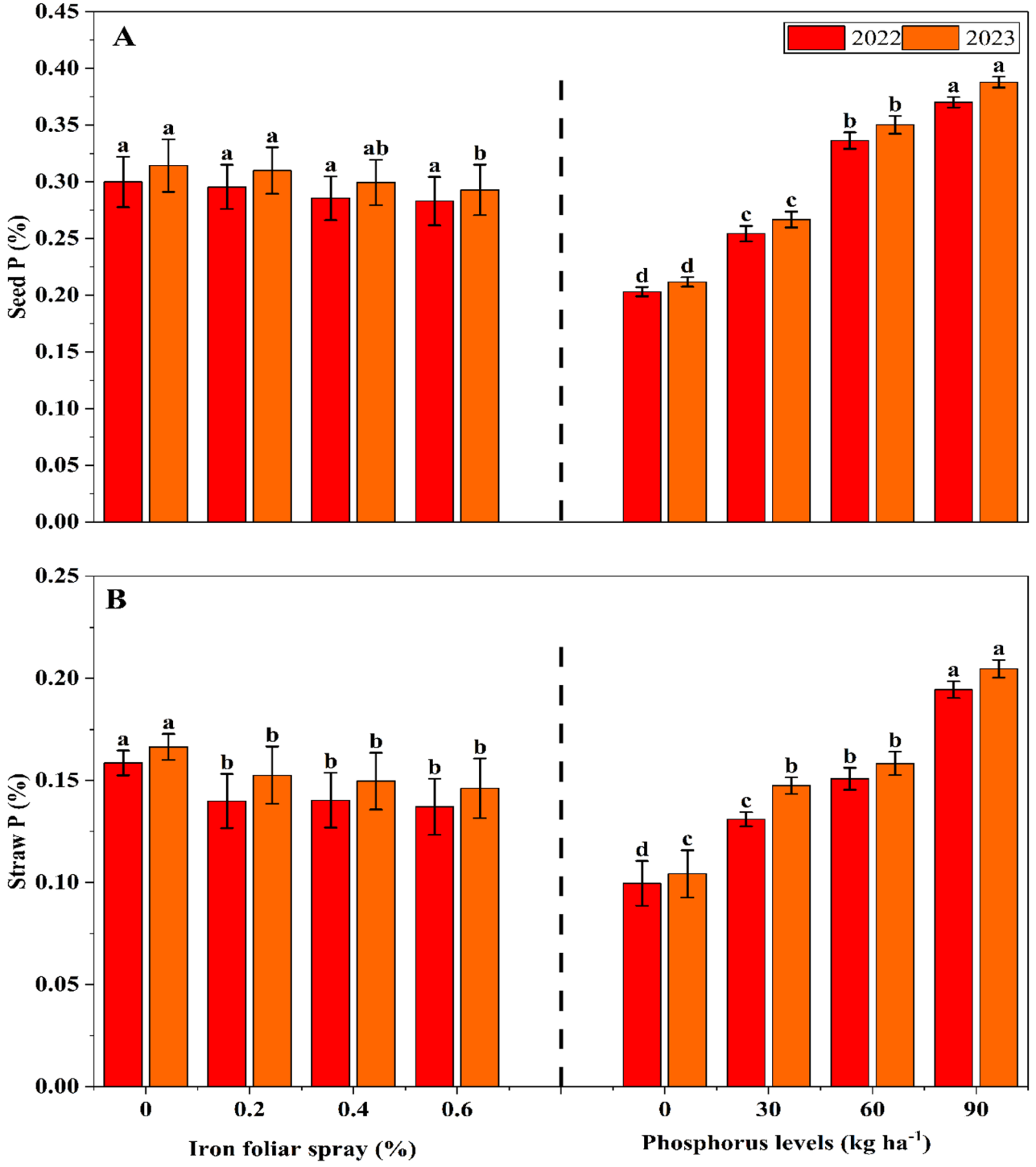


Fig. 6 Effects of iron foliar sprays and phosphorus levels on (**A**) seed P content (%) (**B**) straw P content (%) of mung bean during 2022 and 2023 years. Vertical bars represent standard error ± mean. Means followed by different letters indicate differences among treatments at LSD ≤ 0.05 according to the LSD test

treatments (57% in 2022 and 58% in 2023). Soil P content increased significantly with increasing P levels and Fe foliar spray (Fig. 7D). The Fe × P interaction was significant for soil P, while all other interactions were non-significant. Among P treatments, the highest soil P content was recorded at 90 kg P ha^{-1} (6.83 mg kg^{-1} in 2022 and 7.21 mg kg^{-1} in 2023) followed by 60 (5.84 mg kg^{-1} in 2022 and 6.73 mg kg^{-1} in 2023) and 30 kg P ha^{-1} (5.02 mg kg^{-1} in 2022 and 5.28 mg kg^{-1} in 2023), whereas the lowest soil P content was observed at control (4.27 mg kg^{-1}

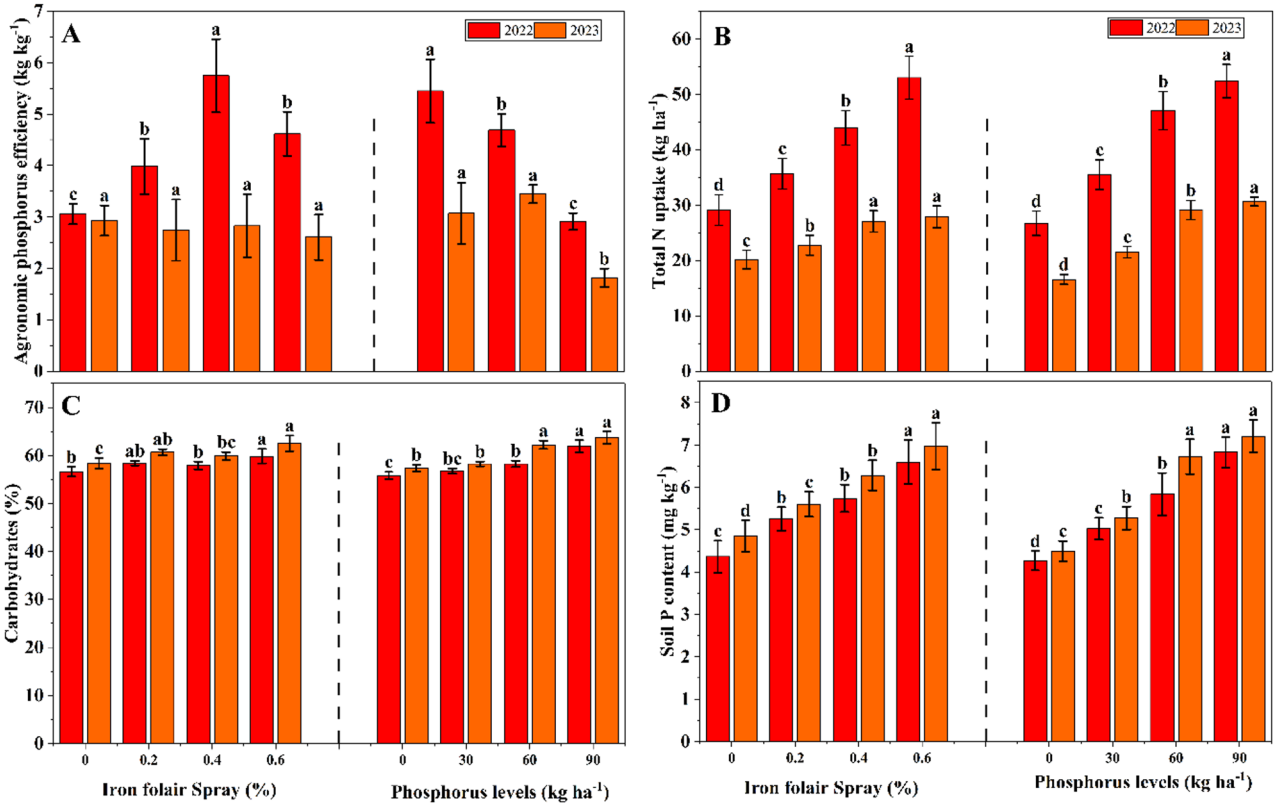


Fig. 7 Effects of iron foliar sprays and phosphorus levels on (**A**) agronomic phosphorus efficiency (kg kg^{-1}), (**B**) total N uptake (kg ha^{-1}), (**C**) carbohydrates (%) and (**D**) soil P content (mg g^{-1}) of mung bean during 2022 and 2023 years. Vertical bars represent standard error ± mean. Means followed by different letters indicate differences among treatments at LSD ≤ 0.05 according to the LSD test

in 2022 and 4.50 mg kg^{-1}). In Fe application, 0.6% Fe spray (6.60 mg kg^{-1} in 2022 and 6.97 mg kg^{-1} in 2023) resulted in the greatest increased in soil P content followed by 0.4% (5.74 mg kg^{-1} in 2022 and 6.28 mg kg^{-1} in 2023) and 0.2% Fe spray (5.26 mg kg^{-1} in 2022 and 5.60 mg kg^{-1} in 2023), whereas the lower soil P content was noted in control (4.37 mg kg^{-1} in 2022 and 4.85 mg kg^{-1} in 2023). Among the years, soil P content was higher in the second year compared with the first year.

Value-cost ratio analysis (VCR)

Economic analysis revealed that Fe foliar and P application influenced the value cost ratio of mung bean. Among the treatments, the combined application of 0.4% Fe with 60 kg P ha^{-1} produced the highest gross income (Rs. 369,653 ha^{-1}), net income (Rs. 311,908 ha^{-1}) and VCR (5.40) compared with all other treatments. The second highest economic return was recorded with 0% Fe combined with 60 kg P ha^{-1} which produced a VCR of 5.20, followed closely by 0.4% Fe combined with 40 kg P ha^{-1} with VCR of 5.11. These treatments displayed that moderate P application improved economic returns even at lower Fe levels. Across Fe treatments, foliar application of 0.4% Fe consistently enhanced profitability with control and higher Fe concentration (0.6%). In contrast, the control treatment (0% Fe and 0 kg P ha^{-1}) resulted in the lowest net income (179,425 ha^{-1}) and VCR (3.80) (Table 1). Overall, the integrated application of 0.4% Fe foliar spray with 60 kg P ha^{-1} proved to be the most economically efficient nutrient management strategy for mung bean cultivation in calcareous soils.

Regression analysis

Regression analysis of seed yield with yield related traits

Scatter plots were drawn to evaluate the relationship between seed yield and key growth, physiological and nutrient traits in mung bean (Fig. 8). Seed yield showed a strong positive association with biological yield ($R^2 = 0.646$) and thousand seed weight ($R^2 = 0.5492$), indicating that yield improvement was largely driven by biomass production and seed size. Similarly, total chlorophyll content exhibited a positive relationship with seed yield ($R^2 = 0.5241$), suggesting the role of photosynthetic efficiency in enhancing productivity. A positive association was also observed between seed yield and crop growth rate ($R^2 = 0.5478$), reflecting the importance of growth dynamics in yield formation. Nutrient- related traits showed comparatively weaker relationships, where seed phosphorus ($R^2 = 0.3523$) and total nitrogen uptake ($R^2 = 0.2231$) were positively but moderately associated

Table 1 Value cost-ratio (VCR) of mung bean as influenced by iron foliar and phosphorus application under calcareous soil conditions

Fe spray (%)	*P* rates (kg ha^{-1})	SY (kg ha^{-1})	STY (kg ha^{-1})	Seed value (Rs)	Straw value (Rs)	Gross income (Rs)	SSP cost (Rs)	Iron cost (Rs)	Urea cost (Rs)	Seed cost (Rs)	Cultivating charges (RS)	Labour charges (Rs)	Total expenditure (Rs)	Net income (Rs)	VCR
0	0	518	2429	207,196	19,429	226,625	0	0	10,200	10,000	15,000	12,000	47,200	179,425	3.80
0	30	604	2740	241,779	21,924	263,703	1800	0	10,200	10,000	15,000	12,000	49,000	214,703	4.38
0	60	727	3009	290,906	24,070	314,976	3600	0	10,200	10,000	15,000	12,000	50,800	264,176	5.20
0	90	709	2953	283,587	23,621	307,208	5400	0	10,200	10,000	15,000	12,000	52,600	254,608	4.84
0.2	0	575	2734	229,915	21,873	251,788	0	3473	10,200	10,000	15,000	12,000	50,673	201,115	3.97
0.2	30	674	2918	269,742	23,347	293,089	1800	3473	10,200	10,000	15,000	12,000	52,473	240,616	4.59
0.2	60	759	3188	303,756	25,501	329,257	3600	3473	10,200	10,000	15,000	12,000	54,273	274,985	5.07
0.2	90	711	3016	284,557	24,125	308,683	5400	3473	10,200	10,000	15,000	12,000	56,073	252,610	4.51
0.4	0	686	3074	274,207	24,596	298,802	0	6945	10,200	10,000	15,000	12,000	54,145	244,657	4.52
0.4	30	792	3089	316,925	24,714	341,639	1800	6945	10,200	10,000	15,000	12,000	55,945	285,694	5.11
0.4	60	859	3265	343,535	26,117	369,653	3600	6945	10,200	10,000	15,000	12,000	57,745	311,908	5.40
0.4	90	822	3102	328,671	24,812	353,484	5400	6945	10,200	10,000	15,000	12,000	59,545	293,939	4.94
0.6	0	619	2830	247,753	22,641	270,394	0	10,418	10,200	10,000	15,000	12,000	57,618	212,776	3.69
0.6	30	708	2918	283,154	23,342	306,496	1800	10,418	10,200	10,000	15,000	12,000	59,418	247,079	4.16
0.6	60	839	3164	335,777	25,315	361,092	3600	10,418	10,200	10,000	15,000	12,000	61,218	299,875	4.90
0.6	90	793	3127	317,161	25,012	342,173	5400	10,418	10,200	10,000	15,000	12,000	63,018	279,156	4.43

SY Seed yield, *STY* Straw yield, *RS* Pakistani rupees

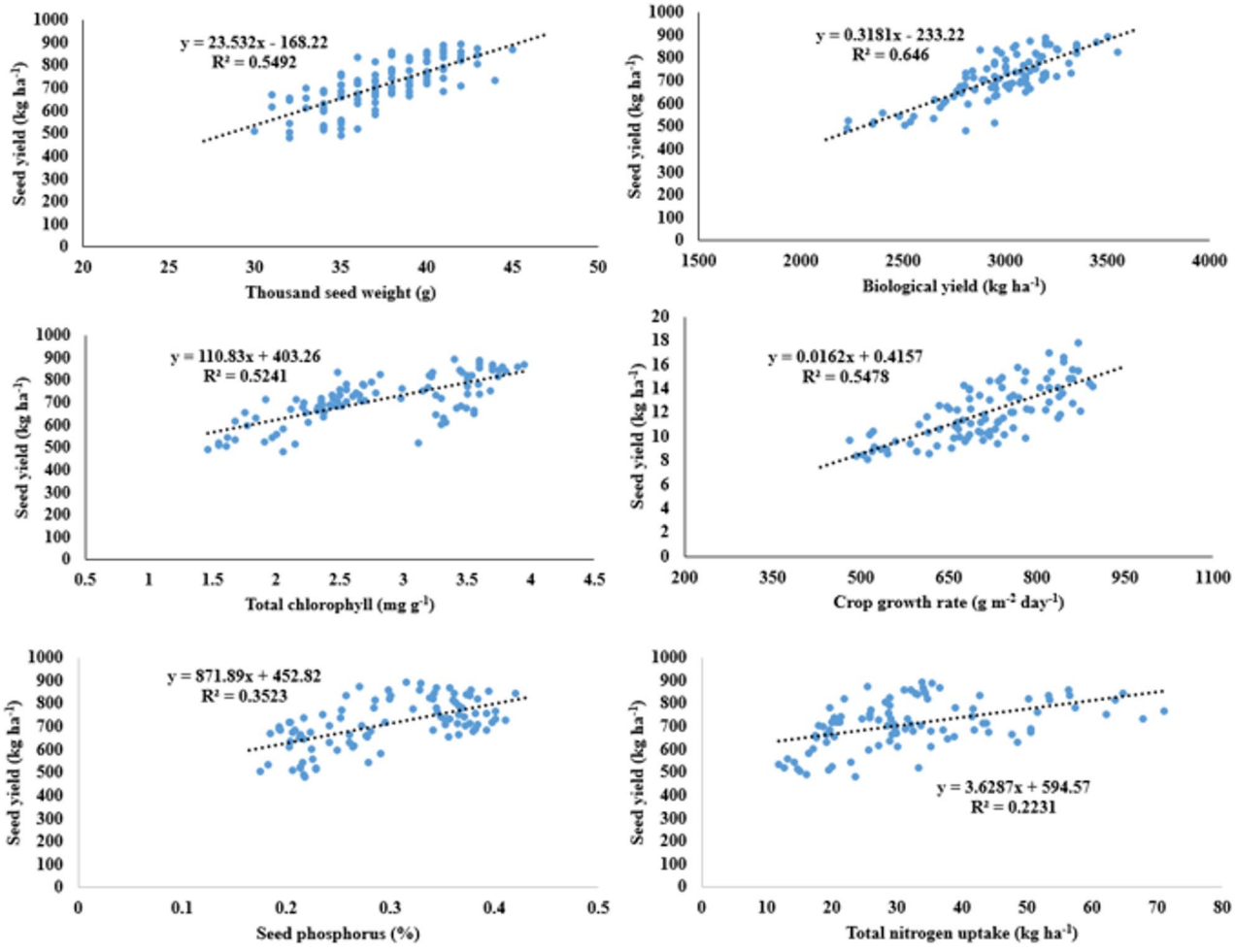


Fig. 8 Regression relationships between seed yield and yield related traits (thousand seed weight, biological yield, total chlorophyll, crop growth rate, seed phosphorus and total nitrogen uptake) of mung bean

with seed yield. Overall, the results indicate that growth and physiological traits contributed more strongly to seed yield variation than nutrient accumulation traits.

Correlation analysis

Correlation analysis revealed strong and positive associations among yield, nutrient uptake and soil fertility parameters in mung bean under different Fe foliar spray and P levels (Fig. 9). Grain yield traits showed strong positive association with growth parameters. Seed yield exhibited a strong positive correlation with BY ($r = 0.80$) and TSW ($r = 0.74$), indicating that higher biomass production and heavier seeds contributed significantly to increased yield. Similarly, CGR showed a strong positive correlation with total chlorophyll ($r = 0.80$) and SY ($r = 0.74$). Nutrient concentrations in plant tissues were also positively related to yield traits. Seed N had a moderate positive relation with SY ($r = 0.57$) and CGR ($r = 0.50$). Likewise, straw N was strongly correlated with CH ($r = 0.76$) and SY ($r = 0.62$), suggesting improved nutrient accumulation in plants under applied treatments. Micronutrient accumulation also showed important associations. Seed Fe had a positive correlation with CGR ($r = 0.67$) and straw Fe ($r = 0.58$), indicating coordinated nutrient uptake and translocation with the plant. However, seed Fe exhibited negative correlations with seed P (r = -0.29) and straw P (r = -0.35), suggesting a potential antagonistic relationship between Fe and P accumulation in plant tissues. Phosphorous content in plant tissues demonstrated strong positive relationships among themselves. Seed P was strongly correlated with straw P ($r = 0.76$), reflecting consistent P uptake and distribution in both grain and straw. In addition, seed P showed positive association with seed N ($r = 0.71$) and soil P ($r = 0.59$), indicating the beneficial role of P fertilization in enhancing nutrient accumulation. Soil fertility indicators also exhibited positive relationships with plant growth and yield traits. Soil P had positive correlations with SY ($r = 0.69$), CGR ($r = 0.64$) and straw N ($r = 0.73$). Similarly, carbohydrates showed positive correlations with BY

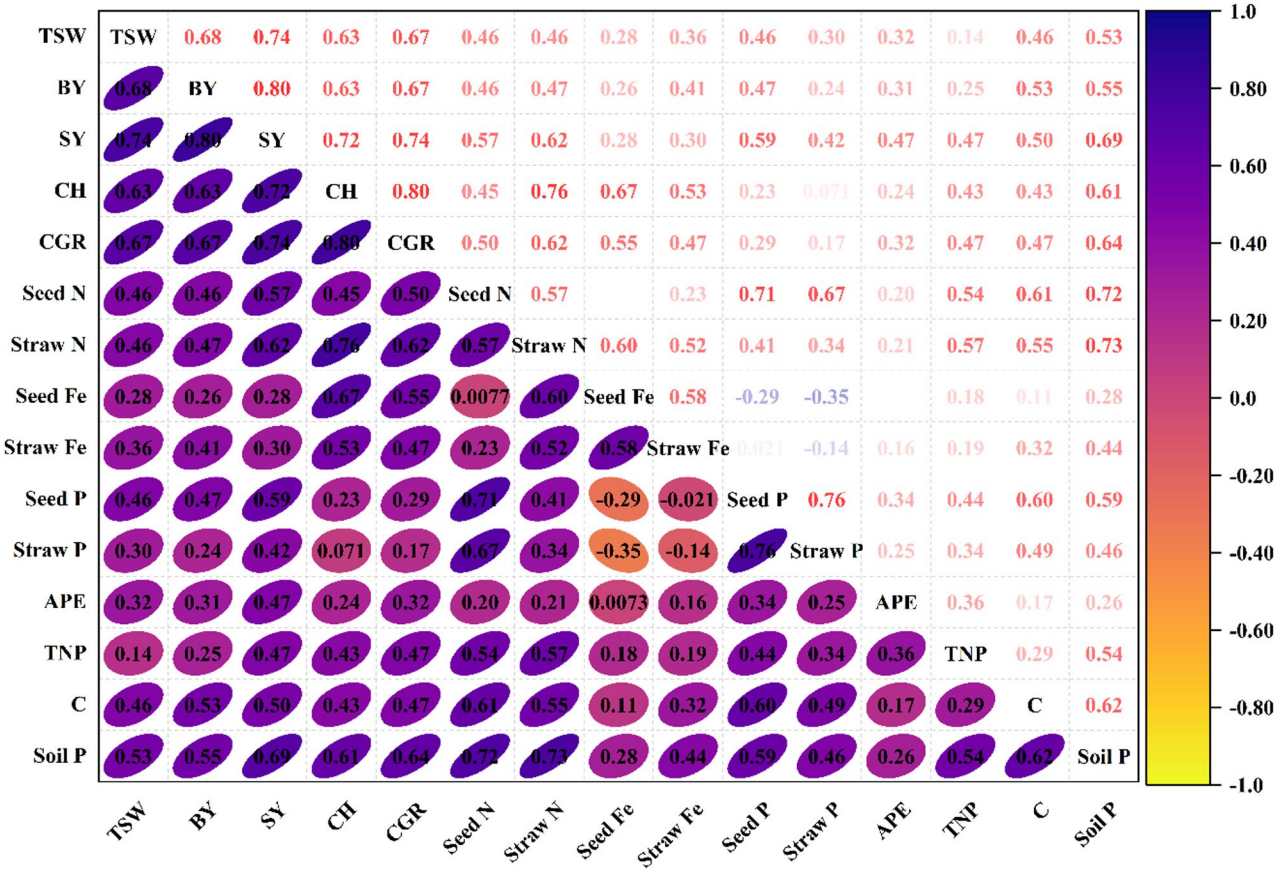


Fig. 9 Correlation analysis showing the relationships among the studied traits under iron foliar spray and phosphorus levels in mung bean. TSW = Thousand seed weight, BY = biological yield, SY = seed yield, CH = total chlorophyll, CGR = crop growth rate, Seed N = seed nitrogen content, Straw N = straw nitrogen content, Seed Fe = seed iron content, Straw Fe = straw iron content, Seed P = seed phosphorus content, Straw P = straw phosphorus content, APE = agronomic phosphorus efficiency, C = carbohydrates, soil P = soil phosphorus

($r = 0.53$) and SY ($r = 0.50$). The correlation analysis indicated that growth parameters, nutrient uptake and soil fertility traits were positively associated with mung bean yield, while some negative relationships were observed between Fe and P concentration in plant tissues.

PCA analysis

Principal component analysis (PCA) was performed to evaluate the relationships among growth, yield, nutrient accumulation and soil properties under different Fe spray and P levels during both years (Fig. 10). The first two principal components explained a substantial proportion of the total variation, where PC1 accounted for 55.02% and PC2 explained 18.72%, collectively explaining 72.84% of the total variability among the studied traits. Most of the growth and yield related traits, including seed yield, biological yield, thousand seed weight, crop growth rate and total chlorophyll content were strongly loaded on the positive side of PC1, indicating a strong positive association among these traits. These variables were closely related to treatments such as T31 (2023 + 0.6% Fe + 60 kg P ha^{-1}), T32 (2023 + 0.6% Fe + 90 kg P ha^{-1}), T27 (2023 + 0.4% Fe + 60 kg P ha^{-1}) and T15 (2022 + 0.6% Fe + 60 kg P ha^{-1}), suggesting that higher Fe concentrations combined with moderate to high P levels enhanced plant growth, chlorophyll formation and productivity. Nutrient related traits including seed N, seed P, straw P, total nitrogen uptake and soil P were also positioned along the positive quadrant of PC1, indicating a strong association between nutrient uptake and improved yield performance. Treatments such as T24 (2023 + 0.2% Fe + 90 kg P ha^{-1}), T28 (2023 + 0.4% Fe + 90 kg P ha^{-1}) and T30 (2023 + 0.6% Fe + 30 kg P ha^{-1}), representing higher P levels combined with Fe foliar spray were located close to these nutrient variables. Micronutrient traits, particularly seed Fe and straw Fe were strongly associated with PC2, indicating that Fe accumulation in plant tissues contributed independently to the overall variation. Treatments such T30 (2023 + 0.6% Fe + 30 kg P ha^{-1}) and T26 (2023 + 0.4% Fe + 30 kg P ha^{-1}) were positioned near these variables, suggesting enhanced Fe uptake under higher foliar Fe applications. Conversely, treatments such as T1 (2022 + 0% Fe + 0 kg P ha^{-1}), T2

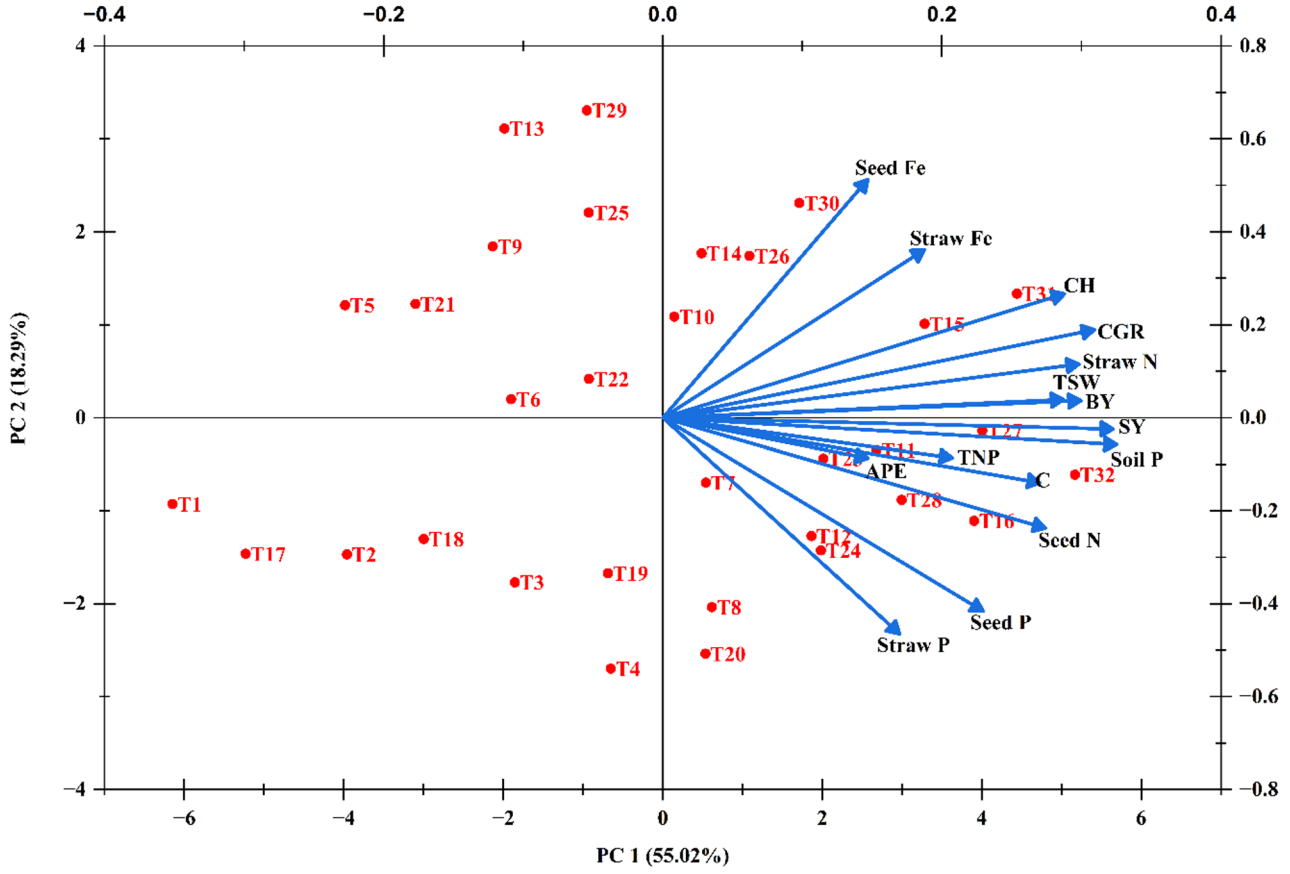


Fig. 10 Principal component analysis showing the effects of iron foliar spray nd phosphorus on mung bean different traits. Whereas, T1 = 2022 + 0% Fe + 0 kg P ha^{-1}, T2 = 2022 + 0% Fe + 30 kg P ha^{-1}, T3 = 2022 + 0% Fe + 60 kg P ha^{-1}, T4 = 2022 + 0% Fe + 90 kg P ha^{-1}, T5 = 2022 + 0.2% Fe + 0 kg P ha^{-1}, T6 = 2022 + 0.2% Fe + 30 kg P ha^{-1}, T7 = 2022 + 0.2% Fe + 60 kg P ha^{-1}, T8 = 2022 + 0.2% Fe + 90 kg P ha^{-1}, T9 = 2022 + 0.4% Fe + 0 kg P ha^{-1}, T10 = 2022 + 0.4% Fe + 30 kg P ha^{-1}, T11 = 2022 + 0.4% Fe + 60 kg P ha^{-1}, T12 = 2022 + 0.4% Fe + 90 kg P ha^{-1}, T13 = 2022 + 0.6% Fe + 0 kg P ha^{-1}, T14 = 2022 + 0.6% Fe + 30 kg P ha^{-1}, T15 = 2022 + 0.6% Fe + 60 kg P ha^{-1}, T16 = 2022 + 0.6% Fe + 90 kg P ha^{-1}, T17 = 2023 + 0% Fe + 0 kg P ha^{-1}, T18 = 2023 + 0% Fe + 30 kg P ha^{-1}, T19 = 2023 + 0% Fe + 60 kg P ha^{-1}, T20 = 2023 + 0% Fe + 90 kg P ha^{-1}, T21 = 2023 + 0.2% Fe + 0 kg P ha^{-1}, T22 = 2023 + 0.2% Fe + 30 kg P ha^{-1}, T23 = 2023 + 0.2% Fe + 60 kg P ha^{-1}, T24 = 2023 + 0.2% Fe + 90 kg P ha^{-1}, T25 = 2023 + 0.4% Fe + 0 kg P ha^{-1}, T26 = 2023 + 0.4% Fe + 30 kg P ha^{-1}, T27 = 2023 + 0.4% Fe + 60 kg P ha^{-1}, T28 = 2023 + 0.4% Fe + 90 kg P ha^{-1}, T29 = 2023 + 0.6% Fe + 0 kg P ha^{-1}, T30 = 2023 + 0.6% Fe + 30 kg P ha^{-1}, T31 = 2023 + 0.6% Fe + 60 kg P ha^{-1}, T32 = 2023 + 0.6% Fe + 90 kg P ha^{-1}

(2022 + 0% Fe + 30 kg P ha^{-1}), T3 (2022 + 0% Fe + 60 kg P ha^{-1}), T17 (2022 + 0.6% Fe + 90 kg P ha^{-1}) and T18 (2023 + 0% Fe + 0 kg P ha^{-1}) representing control or lower Fe and P levels were located on the negative side of PC1, indicating weaker associations with growth, yield, chlorophyll content and nutrient accumulation traits. Overall, the PCA results indicate that combined application of higher Fe spray (0.4–0.6%) and moderate to higher P levels (60–90 kg P ha^{-1}) positively influenced chlorophyll content, crop growth rate, nutrient uptake and ultimately mung bean productivity during both experimental years.

Discussion

The current investigation demonstrates that foliar application of Fe and phosphorus fertilization are important for improving the growth and productivity of mung bean. The application of micronutrients, including Fe, has been widely reported to enhance photosynthetic efficacy, chlorophyll production and enzymatic activity which facilitates better nutrient translocation and metabolic activity during the reproductive of legumes [44, 45]. Iron is particularly significant in nitrogen fixing crops such as mung bean as it plays a role in the synthesis and activity of nitrogenase enzymes which are essential for effective biological nitrogen fixation and overall plant vitality [46]. Iron plays a critical component of both nitrogenase enzyme complex and the oxygen- buffering heme protein leghemoglobin within root nodules. By supporting these functions, adequate iron enhances symbiotic nitrogen fixation, which promotes sustained chlorophyll synthesis, delays leaf senescence and increases the allocation of nitrogenous assimilates to developing seeds which are direct determinants of mung bean yield [25]. Phosphorus also plays a crucial role in legume growth by serving as a source of energy transfer (ATP), carbohydrate

metabolism and facilitating the partitioning of assimilates to developing seeds. Adequate phosphorus level promotes root development, nodule development and biomass accumulation, all of which are critical for vegetative and reproductive growth in mung bean [31, 47]. Phosphorus improves yield mainly by enhancing root development and nodulation efficiency in legumes. Stronger root systems improve nutrient uptake, while better nodulation increases biological nitrogen fixation, supporting reproductive growth and seed filling [48]. who reported that increased root nodulation and seed yield in mung bean with phosphorus up to 80 kg ha^{-1}, while [49] also found improved growth and yield components with phosphorus application. These support the present findings that phosphorus driven improvements in root and nodulation traits lead to higher yield. The complimentary relationship between Fe and P indicates that their combined application can synergistically enhance physiological and biochemical functions resulting in increased growth, improved assimilate allocation and higher yield.

The current results indicate that Fe and P fertilization are both vital in improving the physiological functioning of mung bean. The foliar application of micronutrients, especially Fe enhanced chlorophyll synthesis, photosynthetic efficiency and enzymes activities all of which contribute to improved energy capture and the translocation assimilation to actively growing tissues [13, 50]. The role of Fe in legumes is particularly crucial as it is involved in nitrogen fixation and supports the function of the nitrogenase and leghemoglobin, thereby promoting plant health and chlorophyll retention [51]. Phosphorus exerts a complementary effect by enabling energy production through ATP synthesis, supporting nitrogen fixation in root nodules and facilitating the development of roots and photosynthetic tissues. Adequate P supply enhances assimilate partitioning and biomass accumulation both of which are essential for sustaining crop growth and increasing crop growth rate and chlorophyll content [52]. The observed in increases in CGR can be attributed to increased photosynthetic activity and the efficient transportation of energy and metabolites to growing parts, demonstrating the combined influence of macro and micronutrient nutrition on plant growth. The synergistic effect of Fe and P shows the importance of balanced nutrient management for mung bean growth particularly in calcareous and alkaline soils where nutrient availability is often limited. Foliar application of Fe and P fertilization significantly influenced nitrogen accumulation in both mung bean seed and straw, underscoring the importance of balanced nutrient supply in legume nitrogen metabolism. An adequate supply of Fe promotes nodule development and nitrogenase activity, thereby enhancing the uptake of atmospheric nitrogen and its subsequent incorporation into plant tissues [53]. The higher nitrogen concentrations observed in seeds and vegetative parts under Fe application can be attributed to improved nodulation, enhanced enzymatic activity, and increased nitrogen assimilation rates in mung bean plants [54, 55]. Sufficient P supply stimulates root growth, enhances rhizobial activity in nodules, and improves nutrient uptake and translocation within the plant. Given that nitrogen fixation is an energy-intensive process requiring substantial ATP, an adequate phosphorus supply supports efficient nodule function and increases nitrogen concentrations in plant tissues. The beneficial effect of P on seed nitrogen content may also be associated with improved assimilate partitioning and enhanced transport of nitrogenous compounds to developing grains. The positive interaction between Fe and P is evident in the simultaneous increase in nitrogen uptake by both seed and straw following their combined application. Iron supports enzymatic reactions involved in nitrogen fixation, while P maintains the energy supply and root development necessary for efficient nutrient uptake and translocation. Previous research on mung bean has similarly shown that integrated micronutrient and phosphorus nutrition enhances nodulation, nitrogen fixation, and overall nitrogen content in plant tissues [56].

When applied foliar, Fe is directly absorbed and translocated within the plant, thereby increasing its concentration in developing seeds. The observed increase in seed Fe content following foliar treatment can be attributed to enhanced micronutrient uptake efficiency, greater translocation from source tissues to grains and heightened physiological activity during reproductive development. Similar studies on mung bean have reported that foliar Fe application significantly increases seed Fe concentration and improves grain nutrient composition by enhancing nutrient mobility and assimilation within plant tissues [57, 58]. This is further corroborated by [59] who demonstrated that various Fe application methods including foliar sprays, effectively enhanced Fe biofortification in mung bean and by [20], who showed that combined foliar application of Fe with boron and zinc markedly increased grain Fe levels and overall nutritional quality. Phosphorus nutrition can also influence Fe accumulation through nutrient interactions in the soil-plant system. Elevated P concentrations can promote the formation of insoluble Fe-phosphate complexes in the soil or within plant tissues, thereby limiting Fe uptake and its translocation to developing grains. Physiological and molecular evidence has demonstrated that P-Fe interactions are important in regulating nutrient homeostasis in plants, with high P supply potentially inhibiting Fe acquisition and reducing its accumulation in plant tissues [60, 61]. A recent genetic study in mung bean identified VrYSL3, a transporter gene that facilitates iron distribution within the plant under iron-limited conditions, often induced by high phosphate

availability in calcareous soils [62]. This finding provides molecular evidence that mung bean possesses a specific iron-handling mechanisms whose function or regulation may be directly comprised by elevated phosphorus, explaining the antagonistic effect of seed iron accumulation observed here.

Sufficient P supply enhances root growth, nutrient uptake efficiency, and the translocation of assimilates to developing seeds, ultimately leading to higher phosphorus concentrations in grain tissues. Previous studies on mung bean have similarly reported that increased phosphorus application significantly enhances grain P concentration by improving nutrient uptake and internal transport [63, 64, 65]. At moderate P levels, agronomic phosphorus efficiency tended to increase, likely because plants utilized moderately available phosphorus more productively, whereas higher application rates were less effective due to phosphorus fixation in calcareous soils. In alkaline soils, precipitation of applied P with calcium compounds is common, which reduces P availability for plant uptake and diminishes P-use efficiency [66, 67, 68]. Similarly, high pH and bicarbonate in these calcareous soils reduce iron solubility, while calcium fixes phosphorus as insoluble Ca-phosphates. Foliar Fe bypasses this soil-mediated immobilization by delivering iron directly to leaves and soil applied P supplies sufficient orthophosphate for root and nodule development despite partial fixation. The observed improvements can thus be attributed to these amendments overcoming the inherent nutrient constraints of calcareous soils [69].

A practical strategy for mung bean growers in calcareous soils is to combine soil applied phosphorous (60 kg ha^{-1}) with a 0.4% foliar iron spray at early vegetative stage. This approach is supported by [70, 71], who reported that P fertilization improved yield in P-deficient soils and a recent study confirming that foliar Fe is the most effective method for enhancing chlorophyll content and grain yield in mung bean [13]. In severely deficient field, increasing the rates to 90 kg P ha^{-1} and 0.6% Fe can further boost grain iron enrichment and overall productivity [16]. A more detailed consideration of the study limitations, including potential spatial variability in soil properties, environmental influences during the experimental period and the short-term nature of the field trial, would provide a more comprehensive and balanced interpretation of the findings while improving the understanding of their broader applicability under diverse agroecological conditions.

Conclusion

It can be concluded that foliar application of 0.4% Fe combined with 60 kg P ha^{-1} significantly enhances mung bean yield and profitability, while application of 0.6% Fe combined with 90 kg ha^{-1} P increased the nutrient accumulation. These findings suggest that integrated application of Fe foliar spray and phosphorus fertilization can be adopted as an effective nutrient management strategy to improve mung bean productivity and nutrient use efficiency in calcareous soils but also provides insights for developing sustainable biofortification strategies in other legume and field crops grown under micronutrient-deficient conditions and further studies evaluating nutrient bioavailability and anti-nutritional factors are needed for a complete assessment of biofortification efficacy. The use of 0.4% Fe combined with 60 kg P ha^{-1} may help farmers achieve higher yields under alkaline soil conditions where Fe and P availability are often limited. However, these findings should be further validated through multi-location field trials across soil types and agro-climatic conditions to ensure their broader applicability and stability under variable environmental conditions.

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Authors' contributions
M.K., M.A. and H.A: Writing – original draft, Data curation, Methodology, Formal Analysis. S.S.S., H.A.K., M.T.A., W.L., M.A.N., and M.A.: Writing – review & editing, Software, Methodology, Visualization. W.L.: Funding acquisition. All authors wrote and reviewed the paper.

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Data availability
All data generated or analysed during this study are included in this published article.

Declarations

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Competing interests
The authors declare no competing interests.

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