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Zinc and iron foliar feeding on different lathyrus varieties influencing the biofortification, bio-availability and productivity

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ABSTRACT

Globally two billion population is under micronutrient malnutrition due to poor quality food intake. Foliar fertilization of micronutrients is a deliberate attempt to increase its concentration in edible portion of crop and to enhance consumer diet with targeted elements. A two-year field experiment was designed to validate whether foliar application is effective for biofortification of iron (Fe) and Zinc (Zn) in lathyrus and its bioavailability as well. The experiment was carried out following split plot design taking three lathyrus varieties in main plot (V1: Nirmal, V2: Prateek, and V3: Ratan) and four foliar sprays at pre-flowering and pod development stages in sub plot (F1: Control, F2: 0.5% FeSO₄, F3: 0.5% ZnSO₄, and F4: 0.5% FeSO₄ + 0.5% ZnSO₄) with three replications. Zn (highest 3.73 ppm vs. 3.51 ppm in control), Fe (highest 6.68 ppm vs. 6.34 ppm in control) and protein concentration of lathyrus grain consistently increased with foliar fertilization of micronutrients either alone or in combination for all three varieties. F4 treated lathyrus had 21% reduction of phytic acid (PA) concentration, the main inhibitor of mineral absorption in human. With decreased PA concentration PA:Zn and PA:Fe molar ratio were reduced significantly. A maximum of 13% yield increase was also noticed with foliar fertilization as compare to control and the trend can be explained as F4 (13%) > F3 (12%) > F2 (10%).

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Introduction

Human require at least 22 mineral elements for their wellbeing (Welch and Graham 2004) and plant provides most of them through diet. Deficiency of these micronutrients causing 'micronutrient malnutrition' is a serious problem to human health throughout the world, primarily in resource-limited countries (Kennedy, Nantel, and Shetty 2003). Globally two billion population has been affected with dietary deficiency of iron (Fe), zinc (Zn), and other micronutrients (FAO 2015; WHO 2016). Out of 795 million malnourished population 98% belongs to developing countries (Riesgo et al. 2016). Most sustainable way to combat micronutrient deficiency is diversified diet, but a balance diet containing fruits, vegetables, animal products is out of reach to 78% of world's population who has an income of <1.25 USD person⁻¹ year⁻¹ (World Bank 2015). Biofortification is one of the effective strategies to produce nutrient dense plant edible parts by means of fertilization, breeding approaches, and microbial intervention (White and Broadley 2005). Biofortification through existing agronomic practices will be accommodative to resource poor people of developing countries. Soil

application of fertilizers undergo long-term complex process to reach the grain, viz. reaction with soil colloids, absorption by roots, xylem loading, transport to leaf, phloem loading, and subsequently to grain (Saha et al. 2017a). Availability of nutrients also depends on soil temperature, pH, humidity, and microbial population (Li et al. 2009). On the other hand, foliar fertilization can directly transport the mineral to targeted plant part (Niu et al. 2021).

Applied nutrient may enrich their concentration in grain but cannot ensure their bioavailability in cooked product. Like, in cereal grains, Zn and Fe concentration are higher in aleurone layer and embryo rather than endosperm (Brinch-Pedersen et al. 2007). But, as a matter of fact, people like to eat the starchy endosperm rather than aleurone layer and embryo, which compel cereals to go through a complex processing before it comes to plate (Raes et al. 2014). In contrary with that, pulses need not to go through extensive processing consequently lesser nutrient loss. Into the bargain, seed coat removal/dehulling of legumes is reported to improve the bioavailability of minerals, particularly Zn (Hemalatha, Platel, and Srinivasan 2007) and Fe (Ghavidel and Prakash 2007), ascribable to the reduction of anti-quality factors like phytate and polyphenols (Henry and Massey 2001). Phytate degradation not only takes place during food processing but also in gastrointestinal track, followed by increased availability of Zn and Fe (Hurrell et al. 1992; Sandberg 2002). In addition to that, Zn is said to be much bio-accessible from pulses then cereals as soluble amino acid complex from higher protein diet may potentially improve bio-accessibility of minerals (Hemalatha, Platel, and Srinivasan 2007). For Fe, plastidic ferritin is the main storage form in legumes, which is highly bioavailable (Lott, Goodchild, and Craig 1984; Davila-Hicks, Theil, and Lönnerdal 2004).

In the orbit of domesticated plants, cereals can be considered as the first choice in human history. However, pulse production ranked second after cereals worldwide (Boukid et al. 2019). India is the largest producer and consumer of pulse with a share of 26% global production, 27% world consumption, and 14% export (FAO (Food and Agriculture Organisation of the United Nation) 2020). Lathyrus is one of the underutilized pulses in India sharing 357 thousand hectare area and 287 thousand tonnes of production (India Agristat 2019). It is inherently capable of withstanding drought, waterlogging, salinity, temperature extremes, and various biotic stresses. Lathyrus is very rich source of vegetable protein (31%), carbohydrate (58%), fat (2%), and minerals especially calcium (90–110 mg), phosphorus (P; 317–500 mg), and Fe (Aletor, El-Moneim, and Goodchild 1994; Akalu, Johansson, and Nair 1998; Majumdar 2011). Besides, it is a very good source of the essential amino acids like arginine (7.8 g), lysine (7.4 g), isoleucine (6.7 g), leucine (6.6 g), and valine (4.7 g) per 100 g of protein (Parihar and Gupta 2016). Nutrient dense insurance crop like lathyrus can play an indispensable role in alleviating malnutrition.

Materials and methods

Location

The experiment was carried out during two successive *rabi* seasons (October to February) of 2016–2017 and 2017–2018 at the District Seed Farm (DSF), AB block, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal, India. The study site belongs to New Alluvial zone of West Bengal under sub-tropical humid climate with short and mild winter. The crop was grown exclusively under rainfed condition. The soil of the experimental field belonged to the textural class of sandy loam with pH 6.8–7.2. According to the criteria of critical soil test values laid by Muhr et al. (1965), the available nitrogen (N) content of the soil was low (163.6 and 168.3 kg/ha, respectively, during 2016–2017 and 2017–2018). While the available P (22.9 and 21.4 kg/ha, respectively, during 2016–2017 and 2017–2018) and potassium (137.3 and 141.3 kg/ha, respectively, during 2016–2017 and 2017–2018) content was medium in range. The organic carbon content of the soil was medium, i.e. 0.57% and 0.51% during 2016–2017 and 2017–2018, respectively. Zn and Fe content of initial soil sample was 3.1 and 0.23 ppm, respectively, during

2016–2017 and 3.4 and 0.26 ppm, respectively, during 2017–2018 (measured following DTPA-TEA extract method, Lindsay and Norvell 1978).

The experimental site falls under sub-tropical humid climate with short and mild winter. The day-to-day meteorological data during the period of experiment, collected from AICRP on Agrometeorology, Directorate of Research, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal. Weather condition during the period of experiment has been presented in monthly basis (Figure 1(a,b)).

Treatment and experimental details

The experiment was laid out in a split plot design taking three lathyrus varieties in main plot (V_1 : Nirmal, V_2 : Prateek, and V_3 : Ratan) and four foliar sprays at pre-flowering and pod development stages in sub plot (F_1 : No spray, F_2 : 0.5% FeSO_4 , F_3 : 0.5% ZnSO_4 , and F_4 : 0.5% FeSO_4 + 0.5% ZnSO_4) with three replications (Table 1). Lathyrus was grown as relay or paira crop with rice (IET 4786). Lathyrus seeds were broadcasted in standing rice field 10–15 d before harvesting of rice. Rice was harvested when the crops are fully matured (fourth week of October) with the help of sickle retaining a stubble height of 25 cm. Descriptions of three lathyrus varieties are presented in Table 2.

Crop husbandry

Land preparation was mainly done for the transplanting of rice seedlings as the lathyrus seeds were broadcasted in the standing rice as relay or paira or utera crop. The land was prepared with two deep plowing followed by leveling. Seed inoculation with *Rhizobium* culture (sp. *leguminosarum*) at the rate of 25 g kg^{-1} seed was done before Lathyrus sowing. Inoculated seeds were broadcasted on October 13 2016 and October 14 2017 with a seed rate of 60 kg ha^{-1} . The seeds were sown on accordance with the suitable moisture condition of the soil before the harvesting but after the flowering of rice. Recommended fertilizer dose of 20:40:20 kg N-P₂O₅-K₂O ha^{-1} was applied as basal using urea (46% N), single super phosphate (SSP, 16% P₂O₅), and muriate of potash (MOP, 60% K₂O), respectively, as sources. Full dose of N, phosphorous, and potassium was broadcasted at the time of lathyrus sowing. The crop was sprayed with 0.5% FeSO_4 , 7H₂O, and 0.5% ZnSO_4 , 7H₂O as per the treatments in two stages, i.e. Pre-flowering (November 23 2016 and November 25 2017) and Pod initiation (December 8 2016 and December 5 2017).

Data recording

The crop was harvested manually at physiological maturity, when the leaves become yellow and about 85–90% of green pods turned to golden straw color. The crop plants after harvesting from the ground level were allowed for sun drying for 6–7 d. After drying, the harvested produce of net plot size was tied separately into bundles, labeled and the bundle weight was recorded with the help of weighing balance.

The sun dried produce of the crops from each plot was threshed manually by beating the bundles with wooden sticks and seed yield were recorded.

The harvest index was determined from seed yield ha^{-1} and biological yield ha^{-1} at the time of harvest using formula given by Donald (1962).

$$\text{HI} = \frac{\text{Economic (seed) yield}}{\text{Biological yield}} \times 100$$

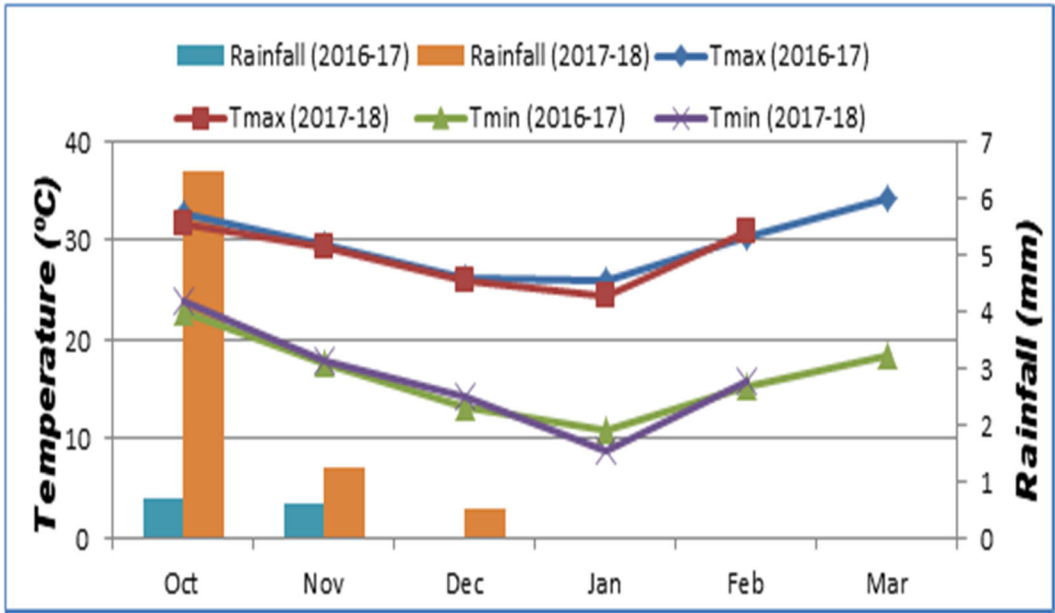


Figure 1a. Mean monthly maximum and minimum temperature and rainfall during the crop growth period of lathyrus in 2016–2017 and 2017–2018.

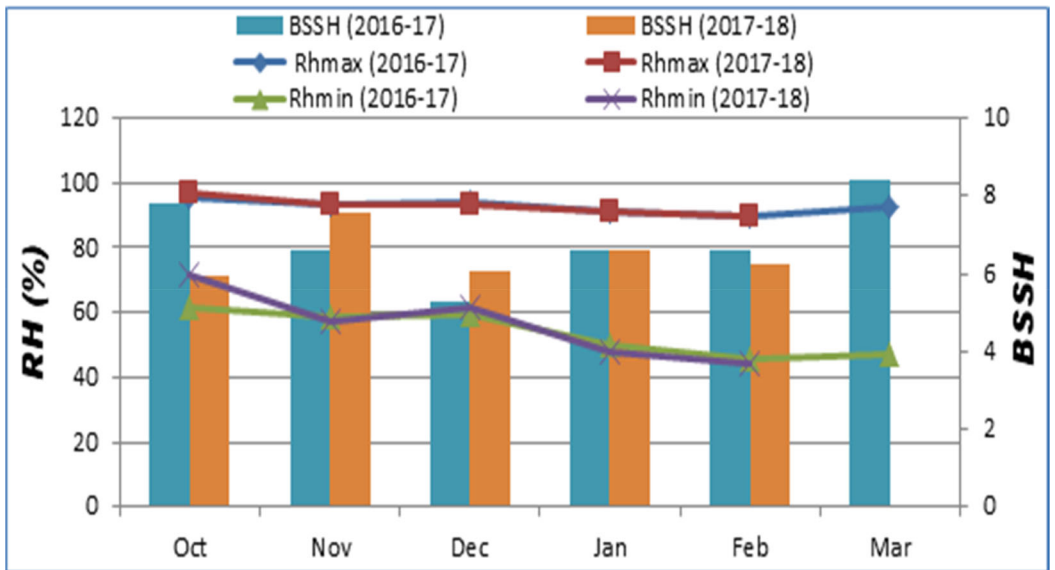


Figure 1b. Mean monthly maximum and minimum relative humidity and bright sunshine hour during the crop growth period of lathyrus in 2016–2017 and 2017–2018.

Plant analysis (qualitative parameters and nutrient content)

Sample preparation

The plant samples taken for the recording dry matter at harvest of lathyrus were utilized for the estimation of nutrient content (N, P, Fe, and Zn). After shed drying for few days, the plant samples were oven dried at 65°C for 8–10 h. Thereafter, the seeds were grinded separately to maximum fineness with the help of mechanical grinder.

Table 1. Treatment details of the experiment.

Treatment		Details
Main plot	Lathyrus variety	V1: Nirmal V2: Prateek V3: Ratan
Sub plot	Foliar spray at pre-flowering and pod development stage (RDF: 20:40:20 kg N:P ₂ O ₅ :K ₂ O ha ⁻¹)	F ₁ : RDF (control) F ₂ : RDF + 0.5% FeSO ₄ ·7H ₂ O at two stages (Pre-flowering and Pod initiation stages of lathyrus) F ₃ : RDF + 0.5% ZnSO ₄ ·7H ₂ O at two stages (Pre-flowering and Pod initiation stages of lathyrus) F ₄ : RDF + 0.5% FeSO ₄ ·7H ₂ O + 0.5% ZnSO ₄ ·7H ₂ O at two stages (Pre-flowering and Pod initiation stages of lathyrus)

Table 2. Varietal details.

Name	Developing center	Pedigree	Year of release	Average yield (q/ha)	Days to maturity	ODAP (%)	Others
Nirmal	PORS, West Bengal	Selection from local material	1981	16–20	120–130	0.2	–
Prateek	IGKV	Ratan × JRL2	2006	15–16	115–120	0.08	–
Ratan	IARI	Somaclone of P24	1997	15–16	115–120	0.05	Tolerant to moisture stress

Zinc and iron content

Fe and Zn in plant samples (seed and stover) were determined by using atomic absorption spectrophotometer (AAS). The plant samples prepared earlier were digested overnight with tri acid (HNO₃:HClO₄:H₂SO₄::10:4:1) method (Jackson 1967). After digestion, the samples were then heated on hot plate to evaporate the acid solution till the residue is colorless. The colorless digest was cooled, diluted with distilled water, and filtered with Whatman No. 1 filter paper. The final volume of the filtrate was made up to 50 mL and run through AAS to determine the micronutrient content.

Protein content

$$\text{Protein content (\%)} = \text{Nitrogen content (\%)} \text{ of the seed} \times 5.5^*$$

*Nitrogen to protein conversion factor for grain legumes (Mosse 1990; FAO (Food and Agriculture Organisation) 1982; Ezeagu et al. 2002).

Total N in plant samples were determined by the Micro-Kjeldahl method as per procedure suggested by (AOAC 1970).

Phytic acid content

According to Cosgrove (1980), Phytate (inositol hexaphosphate) is the major form of storage P and contributes about 60–80% P in cereals, legumes and oilseed (Skoglund, Carlsson, and Sandberg 2009).

The phytic acid (PA) content of a sample was calculated as follows:

$$\text{Phytic acid} \left(\frac{\text{g}}{100 \text{ g}} \right) = \frac{\text{phosphorus} \left(\frac{\text{g}}{100 \text{ g}} \right)}{0.282^*}$$

*Based on the fact that PA comprises 28.2% P (McKie and McCleary 2016).

Table 3. Effect of variety and foliar spray of zinc and iron on zinc, iron, protein, and phytic acid content of lathyrus grain.

Treatment	Fe in grain (ppm)		Zn in grain (ppm)		Protein in grain (%)		Phytic acid in grain (%)	
	2016–2017	2017–2018	2016–2017	2017–2018	2016–2017	2017–2018	2016–2017	2017–2018
Variety								
V1	6.27 ^b	6.41 ^b	3.55 ^b	3.66 ^b	25.33 ^b	24.69 ^b	0.65 ^a	0.63 ^a
V2	6.50 ^a	6.68 ^a	3.63 ^a	3.72 ^a	27.92 ^a	28.31 ^a	0.37 ^c	0.52 ^b
V3	6.59 ^a	6.70 ^a	3.57 ^b	3.68 ^{ab}	28.24 ^a	28.66 ^a	0.57 ^b	0.51 ^b
Foliar spray								
F1	6.26 ^c	6.43 ^b	3.43 ^d	3.60 ^c	25.43 ^c	25.77 ^b	0.63 ^a	0.61 ^a
F2	6.54 ^{ab}	6.74 ^a	3.55 ^c	3.65 ^b	28.05 ^a	28.11 ^a	0.54 ^b	0.58 ^{ab}
F3	6.37 ^{bc}	6.49 ^b	3.66 ^b	3.74 ^a	26.74 ^b	26.48 ^b	0.47 ^c	0.54 ^b
F4	6.63 ^a	6.73 ^a	3.71 ^a	3.76 ^a	28.42 ^a	28.50 ^a	0.48 ^c	0.50 ^c
Least significant differences of means (5% level)								
V	0.21	0.22	0.03	0.06	1.61	1.37	0.037	0.044
F	0.20	0.22	0.05	0.04	0.78	0.95	0.067	0.036
V × F	NS	NS	0.07	0.07	NS	NS	NS	0.063

Note: V1-Nirmal.; V2-Prateek; V3-Ratan; F1- No Spray; F2-FeSO₄. 7H₂O Spray (twice); F3- ZnSO₄ 7H₂O spray(twice); F4- FeSO₄. 7H₂O + ZnSO₄ 7H₂O spray (twice). Different lowercase letters (a-d) within the columns are significantly different at p < 0.05 according to Duncan test.

Total P content in plant sample was determined spectrophotometrically by Vanado-molybdate phosphoric acid yellow color method (Piper 1966).

Estimation of zinc and iron bioavailability

Bioavailability of Zn and Fe in human nutrition was estimated by molar ratios of PA:Zn and PA:Fe (Tran et al. 2021; Morris and Ellis 1989).

$$\text{PA : Zn molar ratio} = \frac{(\text{Phytic acid content in mg kg}^{-1})/660}{(\text{Zn content in mg kg}^{-1})/65}$$

$$\text{PA : Fe molar ratio} = \frac{(\text{Phytic acid content in mg kg}^{-1})/660}{(\text{Fe content in mg kg}^{-1})/56}$$

where 660, 65 and 56 are molecular weight of PA, Zn, and Fe, respectively.

Statistical analysis

The results obtained were analyzed using GenStat software following split plot design. Mean effect of three replications was estimated through Duncan test ($p < 0.05$) and significance of treatments was accessed through two-way ANOVA (Fisher's analysis of variance) (Gomez and Gomez 1984). The PCA biplot and the chart correlation were visualized by using the R programme, Version 3.6.1 (Vienna, Austria).

Result

Zinc, iron, and protein content in grain

Different foliar fertilization treatments significantly altered grain Zn, Fe and protein content of three lathyrus varieties (Table 3). Maximum Zn content was noticed in Prateek when FeSO₄ and ZnSO₄ were combinedly applied @ 0.5% at pre flowering and pod development stages during both the years. The same foliar fertilization treatment caused maximum increase in protein and Fe content of lathyrus grain followed by sole application of FeSO₄ @ 0.5% in variety Ratan with 1–5% magnitude of superiority over Prateek and Nirmal. Zn and Fe loading in lathyrus grain did not reflect any antagonism effect rather they possess a positive linear correlation (Figure 2).

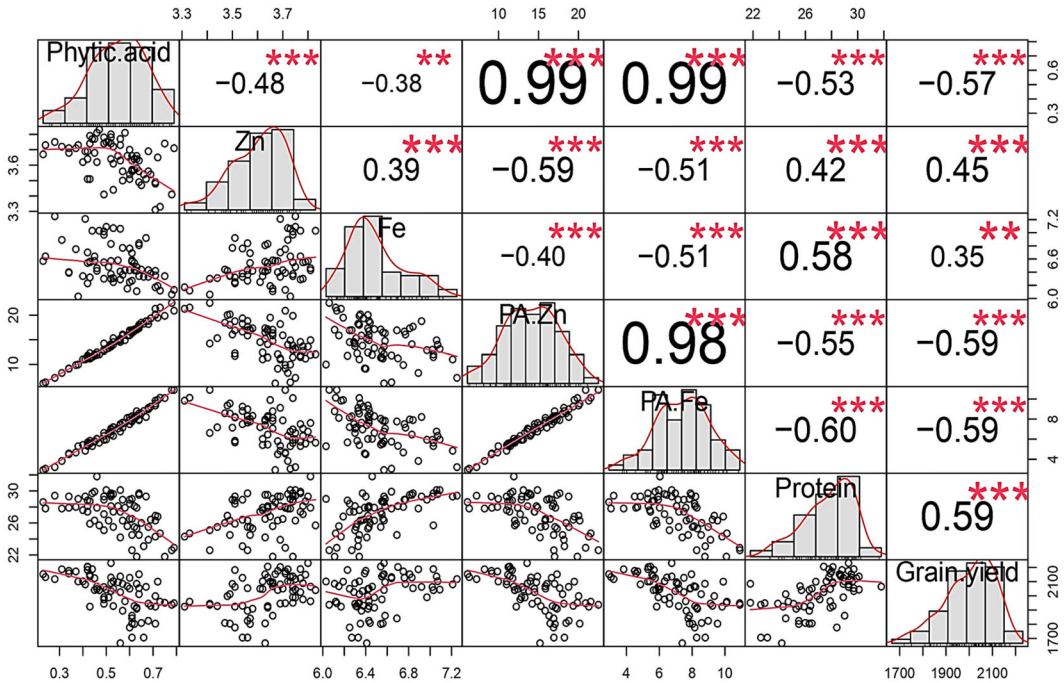


Figure 2. Correlation among different components (grain yield, Zn, Fe, Protein, Phytic acid, PA:Zn, PA:Fe).

Phytic acid content of grain

PA content was significantly influenced by both lathyrus varieties and micronutrient foliar application (Table 3). Zn and Fe foliar fertilization were found to reduce PA concentration in both the experimental years. The estimated decrease of PA was upto 21%, when grain raised with F4 (0.49%) and F3 (0.51%). F2 also reported a considerable decrease in PA content to the tune of 14% and 5% during 1st and 2nd years, respectively. Maximum PA content was estimated with variety of Nirmal and plants which were not supplemented with micronutrients. PA concentration had a significant negative correlation with grain yield and nutrient content, i.e. Zn, Fe, and protein (Figure 2).

PA: Zn and PA: Fe molar ratio in grain

Significant difference in terms of PA:Zn and PA:Fe molar ratio was noticed among the varieties and also among foliar micronutrient applications (Table 4). Lowest PA:Zn and PA:Fe molar ratio was observed with F3 and F4 followed by F2. In comparison with control, micronutrient application reduces PA:Zn and PA:Fe by 16–29% and 17–28% during 1st year, whereas, in second year it ranges between 7–26% and 13–26%, respectively. Among the varieties PA:Zn and PA:Fe molar ratio were lowest in Prateek.

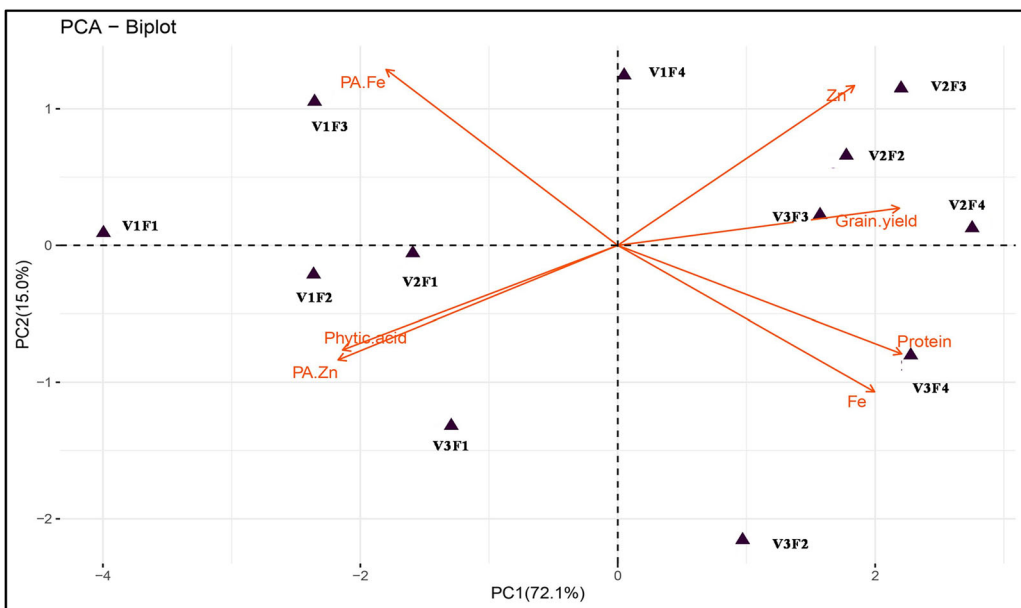
Grain yield

Different lathyrus varieties, micronutrient foliar applications and their interaction significantly affected the grain yield. During first year, highest yield was registered by Prateek (2094 kg ha⁻¹) followed by Nirmal (2011 kg ha⁻¹), and Ratan (2010 kg ha⁻¹). Whereas during second year, Ratan (2089 kg ha⁻¹) recorded maximum seed yield followed by Prateek (2029 kg ha⁻¹). Zn and Fe foliar

Table 4. Effect of variety and foliar spray of Zinc and Iron on seed yield of lathyrus.

Treatment	Grain yield (kg/ha)		PA:Zn		PA:Fe	
	2016–2017	2017–2018	2016–2017	2017–2018	2016–2017	2017–2018
Variety						
V1	2011 ^b	1860 ^c	18.1 ^a	16.9 ^a	8.8 ^a	8.3 ^a
V2	2094 ^a	2029 ^b	10.1 ^c	13.9 ^b	4.9 ^c	6.6 ^b
V3	2010 ^b	2089 ^a	15.7 ^b	13.8 ^b	7.3 ^b	6.5 ^b
Foliar spray						
F1	1890 ^c	1821 ^c	18.1 ^a	16.8 ^a	8.5 ^a	8.1 ^a
F2	2073 ^b	1997 ^b	15.2 ^b	15.6 ^b	7.1 ^b	7.3 ^b
F3	2103 ^a	2063 ^a	12.7 ^c	14.3 ^c	6.3 ^b	7.2 ^b
F4	2088 ^{ab}	2090 ^a	12.8 ^c	12.5 ^d	6.1 ^b	6.0 ^c
Least significant differences of means (5% level)						
V	20.4	34.31	1.01	1.24	0.60	0.75
F	23.56	39.61	1.83	0.98	0.96	0.59
V × F	38.31	64.06	2.92	1.07	NS	1.04

Note: V1-Nirmal; V2-Prateek; V3-Ratan; F1- No Spray; F2-FeSO₄ · 7H₂O Spray (twice); F3- ZnSO₄ · 7H₂O spray(twice); F4- FeSO₄ · 7H₂O + ZnSO₄ · 7H₂O spray (twice). Different lowercase letters (a-d) within the columns are significantly different at p < 0.05 according to Duncan test.

**Figure 3.** Principal component analysis (PCA) showing the position of different variables in the orthogonal space.

application in lathyrus at pre flowering and pod development stages, increased grain yield upto 13% as compare to control and the trend can be explained as F4 (13%) > F3 (12%) > F2 (10%).

Principal component analysis

The principal component analysis (PCA biplot) showed the position of different variables in the orthogonal space and reduced the twelve treatment combinations of lathyrus variety and micro-nutrient foliar nutrition into two orthogonal components (PC1 and PC2) (Figure 3). PC1 accounted for 72% of the total variance which was positively driven by nutrient concentrations (Zn, Fe, and protein content) and grain yield. Among all the variables represented in this biplot, only three (PA:Zn, PA:Fe, and PA) were negatively loaded in PC1. Significantly highest positive loading was observed for Protein content (0.406) followed by yield (0.403) (Table 5a). PC2

Table 5a. Loadings (Eigen vectors) of the correlation matrix of the significant principal components (PCs).

	PC1	PC2
Zn	0.338	-0.472
PA:Zn	-0.4	0.337
Yield	0.403	-0.11
Phytic acid	-0.393	0.308
Fe	0.367	0.431
Protein	0.406	0.319
PA:Fe	-0.331	-0.519
Eigenvalues	5.044	1.051
Explained variance (%)	72.1	15

Table 5b. Principal component scores from correlation matrix under different treatments.

	PC1	PC2
V1F1	-1.779	-0.089
V1F2	-1.052	0.208
V1F3	-1.049	-1.026
V1F4	0.022	-1.215
V2F1	-0.709	0.056
V2F2	0.791	-0.642
V2F3	0.98	-1.121
V2F4	1.226	-0.123
V3F1	-0.576	1.286
V3F2	0.432	2.102
V3F3	0.7	-0.218
V3F4	1.014	0.783

explained only 15% variability with highest loading of Fe (0.431). V2F4 treatment combination can be considered as best followed by V3F4 as V2F4 recorded a significantly highest principal component score in PC1 (Table 5b).

Discussion

Introduction of pulse in cereal-based diet can adjunct sufficient protein but not micronutrient. Dual biofortification of Zn and Fe in lathyrus through foliar fiddling was initiated with the objective of increasing micronutrient concentration in edible part and its bioavailability to human without sacrificing the yield.

The positive effect of Zn application on grain yield may be due to improved plant stand, improved photosynthetic efficiency, photo-assimilate transport, and seed setting (Ullah et al. 2020a, 2020b). Similarly, increase in leaf area, enzyme activation and chlorophyll synthesis, as a consequence of Fe application helps plant to harvest more sunlight and transfer the assimilates from source to sink, which results improved grain yield (Majeed et al. 2020). Moreover, Fe, Zn, Cu, and Mn application has been reported to increase yield contributing characters like, 1000 grain weight, number of grain and ultimately grain yield (Majeed et al. 2020).

Zn and Fe concentration of lathyrus grain consistently increased with foliar fertilization of micronutrients either alone or in combination for all three varieties of lathyrus. These results were also affirmed by Ghasemi et al. (2013) and Li-na et al. (2022). Olsen and Palmgren (2014) stated that if Zn is applied late, when the flowers come, it can easily reach the sink through phloem. In the matter of phloem mobility, Zn and Fe are known to be intermediate or conditionally mobile and mobility depends on several factors like plant species, variety, plant anatomy, plant phenology, and environment (Fernández and Brown 2013). Accordingly, in this study also Zn and Fe concentration of grain showed a significant variation in three lathyrus varieties

(Nirmal, Prateek, and Ratan), due to foliar fertilization. A significant positive correlation was noticed between Zn and Fe concentration of grain (Figure 1), though, an antagonism is well established between Zn and Fe which describes that Fe deficiency results higher Zn accumulation and higher Zn causes Fe deficiency (Briat et al. 2015; Shanmugam, Tsednee, and Yeh 2012; Xie et al. 2019). Remobilization of Zn and Fe from source to sink experience less antagonism or mostly unaffected (Saha et al. 2017a) rather than in case of soil-to-plant transport (Saha et al. 2015). Synthesis of limited transporter (substrate-specific transporter) required for xylem transport of Zn and Fe at vegetative stage leads to hazardous competition, reciprocally, toward maturity the competition reduces due to abundance of transporter (Saha et al. 2017b). Hess and Brown (2009) did not find any adverse effect of Fe absorption with the addition of Zn in food grain rather Podder, Glahn, and Vandenberg (2021) noticed better Fe absorption with Zn-fortification.

Maximum seed protein content was observed in F4 (combined application of ZnSO_4 and FeSO_4 @ 0.5% at pre flowering and pod development stages) and lowest was observed with F1 (No spray). Fe fertilization has been reported to enhance both protein (Pingoliya et al. 2015; Nadi, Aynehband, and Mojaddam 2013) and Fe (Borowski and Michalek 2011) concentration in pulse grain which was in agreement with the present investigation. Nasar and Shah (2017) also had similar observation who reported improvement in total N uptake, and protein % of lentil with foliar application of 0.5% FeSO_4 . Fe is associated mostly with heme-protein and certain non-hem proteins (Hochmuth 2011). Ferritin, a protein, store Fe in nontoxic form and mobilize it in required sites, is present in both plants and human (Seckback 1982; Briat et al. 2010). As a structural component of protein, Zn fertilization also increased crude protein content of lathyrus seed (Broadley et al. 2007). More protein content with Zn application is due to synergistic effect of Zn on N uptake, crucial role in protein metabolism, its involvement in leg-hemoglobin biosynthesis (Rehman et al. 2018a,b; Kryvoruchko 2017). This finding is supported in literature (Krishna 1995). Zn is well known to take part in almost 300 enzyme activation; some of these are involved in protein synthesis (Bao et al. 2003).

Molar ratios of PA:Zn and PA:Fe are considered as the indicators of bioavailability of Zn and Fe (Morris and Ellis 1989; Hussain et al. 2012). In intestine, PA form certain chelates with Fe and Zn which are insoluble and unavailable for absorption (Wise 1983; Sandstrom 1989). A PA:Zn molar ratio of 12 can be considered critical (Hemalatha, Platel, and Srinivasan 2007) and above that (15 or 20) human body experiences critical Zn deficiency (Sandberg et al. 1987; Ferguson et al. 1989). Whereas Bel-Serrat et al. (2014) stated that PA:Zn molar ratio of >15% has an inhibitory effect on functional Zn absorption. Higher concentration of Zn in grain is equivalent to higher percentage to bioavailable Zn because it reduces anti-nutritional factors like PA in grain (Rehman et al. 2018b; Ullah, Farooq, and Hussain 2019). During both the experimental years Zn and Fe foliar application had a significant effect in reduction of PA concentration. Zn is antagonistically related to P as Zn deficiency increase P accumulation at toxic level and vice-versa (Dwivedi, Randhawa, and Bansal 1975; Zhao, Shen, and McGrath 1998). On the other hand, PA represents 50–80% P of plant (Reddy, Sathe, and Salunkhe 1982). Existence of antagonism between Fe and P is also there in literature (Zheng et al. 2009). In present experiment lowest PA:Zn was noticed in F4, i.e. 12.6. F3 also recorded PA:Zn molar ratio lower than 15. According to Chan et al. (2007) and Mitchikpe et al. (2008), phytate:Fe molar ratio greater than 1 indicates poor Fe bioavailability. Ideally phytate:Fe molar ratio should be <0.4 for adequate Fe bioavailability (Hurrell 2004). Though phytate:Fe molar ratio is not the major inhibitory factor in Fe bioavailability (El et al. 2010). Molar ratio of phytate:Fe of lathyrus calculated here is lesser than $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$ fortified lentil (Podder et al. 2021).

Conclusion

Zn and Fe can be considered as the most abundant micronutrient in human, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ foliar fertilization is a potential vehicle to elevate Zn and Fe deficiency. Overall,

result of this study revealed that Zn, Fe, and protein content were increased significantly with foliar fertilization without sacrificing the production as well. PA concentration has decreased up to 21%. Moreover, micronutrient supplemented lathyrus had lower PA:Zn and PA:Fe molar ratio as compare to control.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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