BURKHOLDERIA PHYTOFRIMANS PSJN ASSISTED-IRON BIOFORTIFICATION OF RICE IN BIOCHAR AMENDED PH MANIPULATED CALCAREOUS SOIL

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ABSTRACT: One of the major problems associated with high pH calcareous soils is micronutrient unavailability. Paddy grown in such soils is usually low in total bio-available microelements in general, and, Iron in particular. In present study bio-available contents of iron i.e. ferritin, were enhanced by exploiting the positive interaction of plants and microbial biomass in the presence of biochar in elemental sulfur treated calcareous soil. First, pH of soil was manipulated to 6.5 units by carrying out an incubation study. Four rates i.e. 0, 1.5, 2, 2.5, 3 g kg^{-1} soil of elemental sulfur were used. To maximize translocation and uptake of DTPA extractable Fe i.e. 15 $kgha^{-1}$iron fertilizer was applied in combination with biochar, 1% (w/w) and *Burkholderiaphytofirmans* PsJN inoculation. Results showed that combined application of Fe, *Burkholderiaphytofermans* and biochar enhanced physiology, crop stand and over all rice crop turn out in pH manipulated soil relative to control. Bio-available Fe i.e. ferritin was increased by 2.54 folds while phytate contents were reduced by 35% compared to control. Quality of grain was assessed by quantifying protein, fat, ash and crude fiber that were improved by 36%, 37%, 69% and 48% respectively in pH modified soil over control. It may be concluded that integrated use of *endophytic* PsJN and carbon-rich biochar in pH modified soil can be a good option for iron biofortification in rice.

Keywords: Biochar: *Burkholderia phytofirmans*strain PsJN: Calcareous soil: Ferritin: Iron biofortification.

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INTRODUCTION

Climate change and bourgeoning population have become two most daunting challenges of the age. It is alarming to note that by 2050 population of the world is going to increase by 9.8 billion, 34% what it is today (UN, 2017). Food security has become a major concern that demands increase production per unit area focusing its nutrient availability. Most cereals staple food i.e. wheat, maize, rice are naturally poor in micronutrients like Fe, Zn and vitamins (Kumar *et al*. 2019, Wakeel *et al*. 2018). Particularly rice grains roughly contain Fe in the range between 1 - 11 mg/kg bioavailable iron (Kenzhebayeva *et al*. 2020; Maganti *et al.* 2019) which is in not enough to assure the everyday intake of iron.

Despite being micronutrient iron controls and maintains variety of biochemical and physiological functions in living systems (Adnan *et al.* 2020). It is iron that involves in regulating mitochondrial functionalities in plants and also responsible for growth retardation if not available in adequate quaintly (Greenfield 2020). It also supplements chlorophyll synthesis and nitrogen assimilation (Sangeetha *et al.* 2021); while insufficient availability of iron results in yellowing of leaves i.e. interveinal chlorosis and decline in yield While in humans it plays the key role in making of blood carrying protein i.e. hemoglobin, helps in binding of oxygen to Red Blood Cells, aids in brain development and regulates muscles activities (Cortese-Krott *et al*. 2020; Bianco *et al*. 2018). Contrarily, anemia, impaired nervous system, loss of immunity against disease and poor workability of children are some of the issue related to inadequate supply of iron to human body (Price 2018; Liberal *et al.* 2020).

Calcareous soils are not deficient in iron; rather its inaccessibility for plant absorption in these soils is major issue associated with these soils (Ramzani *et al*. 2017). A number of soil factors are involved in determining solubility, mobility and availability of iron in calcareous soils (Moradi and Karimi 2021). Nonetheless, solubility and translocation of iron from soil to plants controls by single dominating factors that is soil pH (Antoniadis *et al*. 2021). In high pH calcareous soils adsorption of iron on the soil particulate matter results in its deficiency in soil solution. (Dhaliwal *et al*. 2021). Besides, dominating species of iron in soil solution is highly soluble ferrous ions (Fe^{2+}), and which with increase in pH transforms into less or sparingly soluble ferric ions (Fe^{3+}) reported by White (2018). So soil pH coupled with redox reaction in calcareous soils promotes non-availability of iron for plant absorption (Ramzan*i et al.* 2017)

Besides soil factors some anti-nutritional factors i.e. phytate and phenolic compounds which affects the bioavailability of Fe (Magalhaes *et al*. 2017; Mann and Truswell 2017). Of the key concerns, grain phytate, stocked phosphorous which deposits when seed develops, obstruct Fe absorption in intestinal tract (Pavord *et al*. 2019). During digestion, it gets bind to Fe and forms insoluble compounds thus decrease the absorption of Fe along with different essential micronutrients in humans (Nissar *et al.* 2017; Corte-Rea and Bohn 2018; Milman 2020). Contrary to ferritin- a stable iron storage protein keeps the bioavailability of iron constant in grains (Boonyaves *et al*. 2017, liberal *et al*. 2020) as well during digestion as it does not undergo denaturation (Yin 2021).

Soil pH manipulation using some acidifying agent i.e. elemental sulfur speeds up solubility and availability of micro-nutrients (Fe) for plants (Osman 2018; Hartz 2020). Two protons (H^+) are ought to be obtained from mineralization of elemental sulphur, consequently, increasing the acidity and mobilizing micro elements adsorbed on particulates into micro environment (Zaccheo *et al*. 2021; Pot *et al*. 2021). To tackle with the micronutrient deficiency one may either go through clinical medication i.e. supplementation, food fortification i.e. enrichment of food with some mineral or vitamins (iodized salt and Vitamin D- enrich milk) or by making some modification in cooking and processing (Man *et al.* 2021). However, it needs several precautionary measures, smooth and unbroken public and political interests backed by heavy financial supports that may last long (Fiedler *et al*. 2020). Contrary to these conventional strategies, biofortification is a profitable, sustainable and targeted strategy that provides sufficient quantity of micronutrients in balance during the whole life span (Bouis 2019; Rehman *et al.* 2019)

It is important and bear reporting that since last 20 years, scientists have explored the biochar science to maximize nutrient cycling as well as crop output (Rawat *et al*. 2019). As the matter of the fact, biochar itself keeps reserves of nutrients (Kopecký *et al*. 2020) releases slowly but steadily, making the nutrients availability possible in the most nutrient limiting condition as well (Bolan *et al.* 2021). Large surface area, towering cation exchange capacity and porous nature of biochar significantly contribute to make it an effective and sustainable amendment especially for biofortification program (Das and Ghosh 2020; Gaffar *et al*. 2021)

To chart a new course, researchers are struggling hard to exploit as much as possible eco-friendly and sustainable approaches. Microbial inoculation proves a reasonable solution in this regard. Endophytes inhabit the plant internal organs like root and shoots (Krishnamoorth *et al.* 2021; Bomfim *et al.* 2020). They not only improving plant growth, but also induce immunity against biotic and abiotic stresses (Harman and Uphoff 2019; Iasur Kruh *et al.* 2020). These tiny creatures are capable to regulate important biochemical reaction i.e. nitrogen fixation and auxin production in plants (Ullah *et al*. 2019; Rana *et al*. 2020). One of the most significant feature of the said microbe is the production Fe-siderophores, that accelerates iron in soil system (Walitang *et al*. 2017; García-Latorre *et al.* 2021) making it a suitable candidate for Fe-biofortification studies. (Kaur *et al*. 2020). Plethora of works on biofortification of rice, maize and wheat is on record using either biotechnology or improving its breeding techniques (Dapkekar *et al*. 2020; Rhowell *et al.* 2021). Current study was designed to maximize solubility and translocation of soil and seed bounded iron by exploring the potential of endophytes in biochar enriched sulfur treated soil.

MATERIAL AND METHODS

Experimental soil: Soil used in lab and pot study was collected from Soil and Environmental Science research area; University of Agriculture Faisalabad. Well before use for incubation and pot trail various soil was shade dried, thoroughly mixed and passed through a 2-mm sieve and removing pieces of stones. After homogenization, composite soil was tested different physical and chemical properties. Daily temperature values kept nearly 28-30ºC during day time, night temperature 20-23ºC. Level of relative humidity was maintained between 44.9 to 75% soil-air water percentages at given temperature.

pH manipulation of soil: To manipulate the pH of soil crude sulphur was employed. Four rates of S^o i.e. 0, 1.5, 2, 2.5, 3 g kg^{-1} soil were chosen in order to select the rate of sulfur to create acidity in soil to 6.5 units. Elemental sulfur was first ground to a powder with the help wooden spatula, sieved to remove all the impurities well before use in incubation study. Small plastic trays were filled with 500 g soil and were incubated at room temperature and 64% WHC for 105 days. Crude sulfur treated soil was manually mixed twice a day thereby to ensure aerobic conditions for sulfur oxidizing microbial population. On the other hand, to maintain moisture level in experimental soil deionized water was applied. Soil pH was measured once a week using distilled water in soil:water ratio of 1:5. Rate of sulphur to lower down the soil pH to 6.5 units was as under (Table 1).

Inoculum preparation: For inoculums preparation, a loop of said microbe i.e. *B. phytofirmans*strain PsJN was provided by Microbiology and Biochemistry Lab. Institute of soil and Environmental Science; University of Agriculture Faisalabad. For multiplication of the microbe, 250 mL LB broth was prepared and autoclaved in a 500-mL Erlenmeyer flask and incubated at 28°C and 150 rpm for 48 hours. Spectrophotometer (SPECTRONIC GENSIS 5 MULTON ROY)) was used to adjust optical density of broth 0.5 and was recorded at K 535 nm. All this was done to get a homogeneous bacterial biomass i.e. 10^8–10^9 colony forming units (CFU) mL^{-1}in the LB liquid media. After transplantation, 2 week aged healthy seedlings were inoculated with 50 mL of *B. phytofirmans* PsJN::gusA10 containing about 10^9 cells ml^{-1} per pot.

Soil characteristics	
Soil pH (soil saturated paste) (HI 2211-pH/ORP meter, HANNA Instrument).	8
Soil textural class (Gee and Bauder, 1986; Moodie *et al*. 1959).	Loam
Soil organic matter (Walkley-Black method, Nelson and Sommers, 1982)	0.54%
Soil EC	1.88dSm^{-1}
Calcium carbonate ($CaCO_3$) (Allison and Moodie 1965).	4.8%
Plant available (DTPA-extractable) Fe and Zn (Lindsay and Norvell 1978).	3.7 and 0.49 mg kg^{-1}
Phosphorous (Watanabe and Olsen 1965)	7.9mg kg^{-1}
Nitrogen (Bremner and Mulvaney 1982)	0.42%
Extractable K (Flame Photometer at 767-nm wavelength).	107 mg kg^{-1}

Pot experiment: For pot experiment, plastic pots of capacity10 kg, with 9 kg of sulfur amended soil, collected lab study were filled. For biochar preparation eucalyptus as a feed stock was used while pyrolysis was done at 400^0C. Chemical and biochemical attributes of biochar were measured as pH 6.66, organic carbon 52.8 %, Electrical Conductivity 1.90dS m^{-1}, Iron 298.4 mg kg^{-1}, Manganes 131.8mg kg^{-1}, Cupper 155.6 mg kg^{-1}, Zinc 111.7 mg kg^{-1} BC and Carbon to Nitrogen ratio 53. By following treatment plan, experimental soil was mixed with eucalyptus biochar @1% w/w (Table 1). Hydrated ferrous sulphate ($FeSO_4.H_2O$) as iron fertilizer, @7.5 mg kg^{-1} soil was also applied. Healthy rice seedlings of variety Basmati 515 aged 28 days were shifted to amended soil. Recommended dose of N:P:K 2:1:1.5 were applied using urea, K_2SO_4 and SSP. Other half of N fertilizer i.e. 0.03 g N kg^{-1} soil was added after 3 weeks of seedlings transplantation. Submerged condition for uniform growth of rice seedlings were maintained using distilled water. Physiological parameters like photosynthesis (A), stomatal conductance (g_s), transpiration rate (E), vapor pressure deficit (VPD) and sub-stomatal conductance (C_i) using CIRAS-3 (PP System Amesbury, MN, USA) of rice plants were recorded when reached aged 2 months. When rice karnals were turned golden yellow crop was harvested and threshed manually and grains were separated. These were then washed thoroughly using deionised water. After shade drying of root and shoot samples these were oven dried at 70°C till the constant weight (Tokyo Rikakikai, Eyela WFO-600 ND, Tokyo, Japan). Root and shoot dried weights were recorded.

Chemical and biochemical analysis: Oven dried plant root and shoot samples were ground using mill (IKA Werke, MF 10 Basic, Staufen, Germany) and digested in nitric acid and percholoric acid mixture in ratio 2:1 (Jones and Case 1990) for their chemical and biochemical measurements. DTPA- extractable Fe was measured from the digested samples of root, shoot and grain were run on atomic absorption spectrophotometer (PerkinElmer, AAnalyst 100 and Waltham USA). While saturation percentage was calculated by following methods prescribed in AOAC (2003). Protein contents were determined by following Bradford (1976) colorimetric method. For ash contents sample was digested in muffle furnace at 550ºC till the gray white ash collection (AOAC, 2003). Fat determination was carried out using Soxhlet apparatus (AOAC 2003). Ether extraction followed by digestion in H_2SO_4 and KOH solution of rice grain was done. After digestion, left over residues were again hated in the furnace, loss in weigh named as crude fiber. While Nitrogen free extract was calculated using difference method as,

NFE = (100-% moisture + % crude fat + % crude protein + % ash + % crude fiber).

For the quantification of Phytate in grain sample Haug and Lantzsch (1983) were followed. Briefly, 55.9 g of grain sample was prepared by grinding the whole grain and was extracted for 120 minutes at 25 degree centigrade with 10 mL of 0.2 N HCl. Using spectrophotometer (Shimadzu, UV-1201, Kyoto, Japan) phytic acid contents were calculated from extracted samples. For ferritin content measurement, grain sample was prepared as method described by Lukac et al. (2009). However, bioavailable Fe i.e. ferritin was determined by Rebecca et al. (2009) with some modifications and final readings were (absorbance) taken at 450 nm by developing direct ELISA.

Statistical analysis: A completely randomized design, each treatment in triplets was arranged for pot study. One way ANOVA was used to determine treatment effect on plant growth. Treatment mean comparison was carried out by using Duncan's multiple range test (DMRT) ($p < 0.05$) at SPSS statistic software version 22 (SPSS, Inc., Somers, NY, USA).

RESULTS

Photosynthetic measurements in rice leaves: In rice leaves, photosynthetic activity *(A*) as significantly decreased in calcareous soil (Table 2). When compared with control, either sole or in combination with biochar and microbial inoculation, Fe fertilizer significantly increased the photosynthetic activity; nevertheless, results were different in both acidified and normal soil. Photosynthesis was increased up to1.24 folds when pH manipulated soil was amended with Iron, biochar and PsJN as a single treatment over its respective control.

Likewise, all organic and mineral treatments positively influenced transpiration rate (*E*) by the first order and second order interaction compared with control (Table 2). It was observed that upon integrate use of biochar, endophyte and iron fertilizer transpiration activity increased upto 98% in sulfur treated soil over control. Similarly, 58% to 68% improvement in stomatal gas exchange (g_s) was observed in S amended soil where soil was treated with biochar, PsJN and iron, compared with 50% increase in normal soil with same treatments (Table 2). However, sub-stomatal conductance (C_i) was reduced with Fe fertilization, biochar and microbial inoculation in both normal and pH manipulated soils (Table 2). Highest reduction (18%) in sub-stomatal CO_2 conductance was recorded where organic (biohar and PsJN) and inorganic 9Fe and S) amendments were used over its respective control.

Plant dry matter and grain yield: Application of biochar and microbial inoculation increased he effectiveness of Fe fertilizer that ultimately influenced the root and shoot dry weight ($p <0.05$) (Table 3). A significant increase in dry matter yield was observed in sulfur amended soil relative to control. In root dry weight, 28% increase while 63% increase in short dry weight was recorded,where biochar was applied in combination with PsJN and Fe fertilizer in acidified soil relative to control. Besides increasing total number of tiller plant^{-1}(40%) integrated use of biochar, microbial inoculation and Fe fertilizer increased the rice grain yield (16%) in S treated calcareous soil relative to control (Table 3).

Table 1 Effect of sulfur applied with Fe, BC and PsJN on soil pH. Sulfur applied with treatments in each pot having 8 kg soil and its effect on soil pH after sulfur oxidation as observed in each pot before sowing crop.

Treatments	Abbreviation	Initial soil pH	Iron solubilizing agent (Elemental Sulfur)		Soil pH before crop sowing
			Sulfur (g kg^{-1} soil)	Corresponding H^+ ions (mmol kg^{-1} soil)	
Control	C	8±0.01	-	-	8±0.01
Biochar	BC	8±0.01	-	-	7.9±0.03
PsJN	PsJN	8±0.01	-	-	8±0.02
Biochar + PsJN	BC + PsJN	8±0.01	-	-	7.9±0.02
Iron	Fe	8±0.01	-	-	7.9±0.02
PsJN + Iron	PsJN + Fe	8±0.01	-	-	7.9±0.02
Biochar + Iron	BC + Fe	8±0.01	-	-	7.9±0.01
Biochar + PsJN + Iron	BC + PsJN + Fe	8±0.01	-	-	7.9±0.02
Sulfur	S	8±0.01	2.5	156.2	6.5±0.03
Sulfur + Biochar	S + BC	8±0.01	2.5	156.2	6.5±0.03
Sulfur + PsJN	S + PsJN	8±0.01	2.5	156.2	6.5±0.03
Sulfur + Biochar + PsJN	S + BC + PsJN	8±0.01	2.5	156.2	6.5±0.03
Sulfur + Iron	S + Fe	8±0.01	2.5	156.2	6.5±0.03
Sulfur + PsJN + Iron	S + PsJN + Fe	8±0.01	2.5	156.2	6.5±0.03
Sulfur + Biochar + Iron	S + BC + Fe	8±0.01	2.5	156.2	6.5±0.03
Sulfur + Biochar + PsJN + Iron	S + BC + PsJN + Fe	8±0.01	2.5	156.2	6.5±0.02

Fe in soil and different organs of rice crop: Application of Fe fertilizer in combination with microbial inoculation and biochar improved iron contents in root, shoot and grain by first order and second order interactions (Table 4). Increasing soil acidity using elemental sulfur, increased DTPA-Fe in plant dry matter (root, shoot, grain). The effectiveness of biochar, PsJN and Fe fertilizer significantly increased in sulfur treated soil which was evident from 1.19 foldsincrease in DTPA extractable Fe relative to control. An increase of 1.17 fold was recorded for shoot Fe concentration in pH manipulated soil compared with 69% increase in un-amended control. However microbial inoculation, biochar and iron fertilizer improved the grain by1.22 foldsin sulfur treated soil relative to control.

Grain proximate composition: Rice plants grown in both of the soil i.e. normal and manipulated pH showed varied degree of significant ($p < 0.05$) increase in grain proximate composition when treated with sulfur and amended with biochat, Fe and PsJN (Table 5). It was observed that under soil acidification, biochar, microbial inoculation and Fe fertilizer 68% increase in ash contents was recorded relative to 64% in un-amended control. Likewise, 36% increase in crude fat and 38% increase in protein contents were recorded when iron was applied with PsJN and C-rich biochar compared to control (Table 5). Similarly, crude fiber contents were significantly ($p < 0.05$) improved from 32 to 48% by the application of PsJN, organic amendment and mineral fertilizer in sulfur affected soil.A significant increase i.e. 30% in moisture contents were also observed in acidified soil, when Fe fertilizer was tested against biochar and PsJN relative to control

Grain phytate and ferritin concentration: A significant ($p<0.05$) reduction in phytic acid concentration was recorded in treatment iron, biochar and bacterial inoculation with and without sulphur application (Figure 2). Phytic acid contents were decreased up to 32% with integrated use of biochar and microbial inoculation in pH manipulated soil relative to control. Nevertheless, highest reduction i.e. 35% in phytate concentration was recorded when Fe fertilizer was mixed with biochar and microbial inoculation with respect to control in Sulphur treated soil. On the other hand, plants provided with iron fertilizer significantly improved bioavailable Fe i.e. ferritin contents in both original andacidified soil (Figure 1-A, B). Highest increase in grain ferritin consents i.e. 2.54 folds were recorded when PsJN, iron and biochar were applied as single treatment in sulfur treated soil with respect to control.

Table 2 Effect of Biochar, PsJN and Fe on physiological parameters of ricecrop. Means bearing same letter(s) are statistically non-significant according to Duncan's multiple range test (*p < 0.05*).

Treatments	Control	Bc	PsJN	Bc+PsJN	Fe	PsJN+Fe	Bc+Fe	Bc+PsJN+Fe
A								
pH 8	9.00k ±0.08	11.13i ±0.01	11.47hi ±0.01	12.01h ±0.07	14.93e ±0.01	15.65e ±0.03	16.73d ±0.03	18.99c ±0.06
pH 6.5	10.06j ±0.04	13.18fg ±0.00	12.92g ±0.02	13.92f ±0.01	18.94c ±0.03	21.58b ±0.01	21.17b ±0.11	22.56a ±0.04
E								
pH 8	3.75k ±0.02	3.88jk ±0.15	3.88jk ±0.05	4.02j ±0.05	4.91g ±0.01	5.41e ±0.01	5.47e ±0.02	6.15c ±0.02
pH 6.5	3.45l ±0.09	4.37i ±0.02	4.58h ±0.03	5.16f ±0.01	5.90d ±0.05	6.74ab ±0.01	6.76ab ±0.01	6.89a ±0.02
Ci								
pH 8	277.66a ±0.33	266.04b ±0.61	262.22b ±1.06	259.01b ±0.66	212.07de ±0.58	213.67de ±0.45	204.23def ±0.88	202.66ef ±0.88
pH 6.5	238.55c ±0.66	227.99c ±0.65	231.58c ±0.76	215.05d ±0.56	202.23ef ±0.28	197.56f ±0.27	195.52f ±0.28	194.66f ±0.33
G_s								
pH 8	317.33k ±2.33	335.9jk ±2.40	367.00i ±3.05	416.40fg ±2.64	437.00ef ±1.15	459.70cde ±3.52	474.90c ±1.85	477.70c ±1.45
pH 6.5	357.00ij ±1.73	396.30gh ±2.33	378.30hi ±1.85	444.30de ±2.40	465.70cd ±2.60	586.30a ±3.05	555.70b ±2.08	595.30a ±2.90
VPDd								
pH 8	3.93a ±0.01	3.85b ±0.01	3.81b ±0.00	2.93d ±0.01	2.42f ±0.01	2.36g ±0.01	2.33g ±0.01	2.14h ±0.01
pH 6.5	3.15c ±0.02	2.70e ±0.02	2.93d ±0.03	2.44f ±0.01	2.32g ±0.01	2.07i ±0.01	2.15h ±0.03	2.02i ±0.02

Table 3 Effect of Biochar, PsJN and Fe on growth of ricecrop. Means bearing same letter(s) are statistically non-significant according to Duncan's multiple range test (*p < 0.05*).

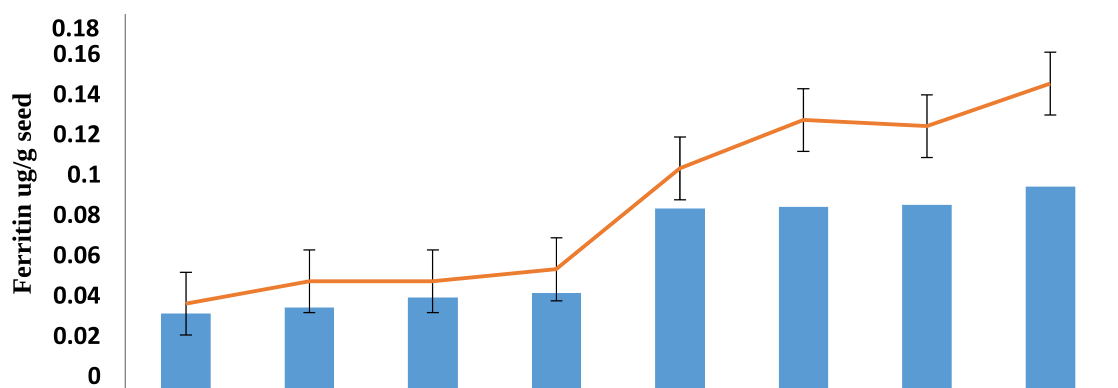
Treatments	Control	Bc	PsJN	Bc + PsJN	Fe	PsJN + Fe	Bc+ 15 Fe	Bc + PsJN + Fe
Plant height								
pH 8	71.67i ±0.33	75.47gh ±0.98	74.33h ±0.88	76.87fg ±0.46	76.50fg ±0.33	80.20e ±1.34	80.40e ±0.55	86.10c ±0.14
pH 6.5	74.10h ±0.92	76.73fg ±0.63	75.33gh ±1.20	77.73f ±0.96	83.93d ±1.57	98.53a ±0.33	95.33b ±0.56	100.33a ±0.88
Root dry weight								
pH 8	1.86j ±0.10	2.28f ±0.14	2.21g ±0.05	2.42d ± 0.01	2.12h ±0.04	2.47c ±0.18	2.41d ±0.02	2.54b ±0.10
pH 6.5	2.06i ±0.02	2.36e ±0.15	2.26f ±0.19	2.42d ±0.08	2.47c ±0.07	2.62a ±0.03	2.52b ±0.01	2.64a ±0.02
Shoot dry weight								
pH 8	11.50i ±0.22	12.68gh ±0.16	12.53h ±0.13	15.09d ±0.27	12.85gh ±0.14	12.51h ±0.18	14.14ef ±0.37	18.00c ±0.40
pH 6.5	12.59h ±0.12	14.31e ±0.37	13.96ef ±0.51	18.12c ±0.31	13.44fg ±0.53	19.73b ±0.24	19.23b ±0.14	20.58a ±0.31
Total no. of tiller								
pH 8	6.00e ±0.66	7.33cd ±0.33	6.67cde ±0.33	7.67bc ±0.33	6.33de ±0.33	7.00cde ±0.00	7.00cde ±0.66	7.67bc ±0.33
pH 6.5	6.67cde ±0.67	7.33cd ±0.33	7.00cde ±0.00	8.67ab ±0.33	7.33cd ±0.67	9.00a ±0.66	9.00a ±0.33	9.33a ±0.33
1000 grain weight								
pH 8	11.53f ±0.02	12.26de ±0.02	11.95ef ±0.01	11.79ef ±0.16	12.04ef ±0.16	12.18de ±0.12	12.68cd ±0.11	13.06bc ±0.03
pH 6.5	11.83ef ±0.14	12.36de ±0.17	12.31de ±0.28	12.71cd ±0.04	13.04bc ±0.02	13.61ab ±0.01	13.57ab ±0.00	13.79a ±0.01

Table 4 Plant available Fe in soil after crop harvest and iron concentration (mg kg^{-1}) in root, shoot and grain of rice. Means bearing same letter(s) are statistically non-significant according to Duncan's multiple range test (*p < 0.05*).

Treatments	Control	Bc	PsJN	Bc + PsJN	Fe	PsJN + Fe	Bc + Fe	Bc + PsJN +Fe
DTPA extractable Fe								
pH 8	4.07fg ±0.08	3.92g ±0.05	4.96e ±0.02	4.93e ±0.01	7.07cd ±0.04	6.57d ±0.16	8.36b ±0.25	8.42a ±0.06
pH 6.5	4.61efg ±0.05	4.81ef ±0.11	4.77ef ±0.02	7.18cd ±0.48	7.61c ±0.09	9.95a ±0.09	9.73a ±0.01	10.14a ±0.02
Grain Fe								
pH 8	42.98j ±0.64	36.60k ±0.63	52.28h ±0.52	50.55h ±0.64	70.81e ±1.06	65.91f ±0.68	82.55d ±0.16	88.75c ±0.75
pH 6.5	46.53i ±1.15	50.11h ±0.40	58.14g ±0.85	65.80f ±0.55	80.15d ±0.77	97.41b ±0.37	88.50c ±1.41	103.63a ±0.93
Root Fe								
pH 8	145.00ij ±2.30	139.67j ±2.27	152.00hi ±1.15	168.67fg ±0.67	179.67de ±0.33	177.67def ±0.67	191.33bc ±0.88	198.33b ±0.33
pH 6.5	155.00h ±2.08	166.33hi ±1.15	158.67hi ±0.33	172.33efg ± 1.20	185.33cd ±2.18	215.00a ±1.00	210.00ja ±1.45	229.33a ±0.67
Shoot Fe								
pH 8	73.67j ±1.45	67.33k ±1.00	80.00hi ±0.88	83.50gh ±0.33	97.30e ±0.88	88.67f ±1.52	109.00d ±0.33	124.67c ±2.18
pH 6.5	76.67ij ±1.20	76.00j ±1.15	74.67j ±0.33	84.00g ±2.64	125.67c ±1.76	151.33a ±0.57	138.00b ±1.45	166.67a ±2.33

Table: 5 Grain biochemical attributes as affected by different organic and inorganic amendments. Means bearing same letter(s) are statistically non-significant according to Duncan's multiple range test (*p < 0.05*).

Treatments	Control	Bc	PsJN	Bc + PsJN	Fe	PsJN + Fe	Bc+ Fe	Bc + PsJN + Fe
Ash%								
pH 8	0.31i ±0.00	0.34h ± 0.00	0.34h ±0.00	0.38g ± 0.01	0.44ef ± 0.00	0.46de ±0.00	0.43f ±0.01	0.51c ±0.01
pH 6.5	0.35h ±0.00	0.45def ± 0.01	0.40g ± 0.01	0.47d ± 0.01	0.50c ±0.00	0.55b± 0.00	0.50c ± 0.01	0.59a ±0.01
C. Fat%								
pH 8	0.91h ±0.00	0.96fg ± 0.01	0.94gh ±0.01	1.13c ± 0.03	0.99ef ± 0.02	1.10d ±0.03	1.02e ±0.01	1.15c ±0.01
pH 6.5	0.95fg ±0.01	1.07d ±0.01	0.96fg ± 0.01	1.17c ± 0.01	1.07d ±0.05	1.25b ±0.02	1.02e ±0.01	1.30a ±0.01
C. Protein%								
pH 8	5.31k ±0.01	5.62h ±0.00	5.54hi ±0.01	5.79g ±0.01	6.31e ± 0.00	6.83d ±0.01	6.37e ±0.03	7.19b ±0.01
pH 6.5	5.44ij ±0.00	5.96g ±0.01	5.87g ±0.02	6.14f ±0.01	6.84d ±0.03	7.29ab ± 0.04	7.01c ± 0.01	7.52a ±0.14
C. Fiber								
pH 8	0.33g ±0.00	0.35g ±0.00	0.35g ± 0.00	0.40de ±0.01	0.38f ±0.00	0.37f ±0.00	0.39ef ±0.00	0.44b ±0.00
pH 6.5	0.35g ±0.00	0.39ef ± 0.00	0.38f ±0.00	0.43c ±0.00	0.42cd ±0.00	0.49b ±0.00	0.43c ±0.00	0.52a ± 0.00
NFE								
pH 8	83.00a ±4.40	82.28ab ±3.06	81.83abc ±3.32	80.90bcd ±4.10	80.66be ±3.17	79.19ef ±3.23	78.68fg ±3.50	77.33ghi ±3.95
pH 6.5	81.75abc ±3.45	79.85def ±3.87	80.33cde ±4.37	79.12ef ±4.48	78.37fgh ±3.67	76.33i ±1.64	76.83hi ±3.48	75.90i ±3.11
Moisture								
pH 8	10.17h ±0.03	10.45g ± 0.07	11.01f ±0.08	11.22ef ±0.02	11.29e ±0.07	12.20d ±0.06	12.91c ±0.02	13.29b ±0.12
pH 6.5	11.19ef ±0.03	12.31d ±0.04	12.16d ± 0.02	12.77c ±0.05	12.88c ±0.23	14.19a ± 0.02	14.13a ±0.05	14.60a ±0.25



	Control	Biochar	PsJN	Biochar+ PsJN	Fe	PsJN + Fe	Biochar+Fe	Biochar + PsJN + Fe
Soil pH 8	0.038	0.041	0.046	0.048	0.09	0.091	0.092	0.101
Soil pH 6.5	0.043	0.054	0.054	0.06	0.11	0.134	0.131	0.152

Fig. 1(A) Grain ferritin concentration µg/ seed under Fe, Biochar and *Burkholderiaphytofrmans*PsJN in pH modified soil

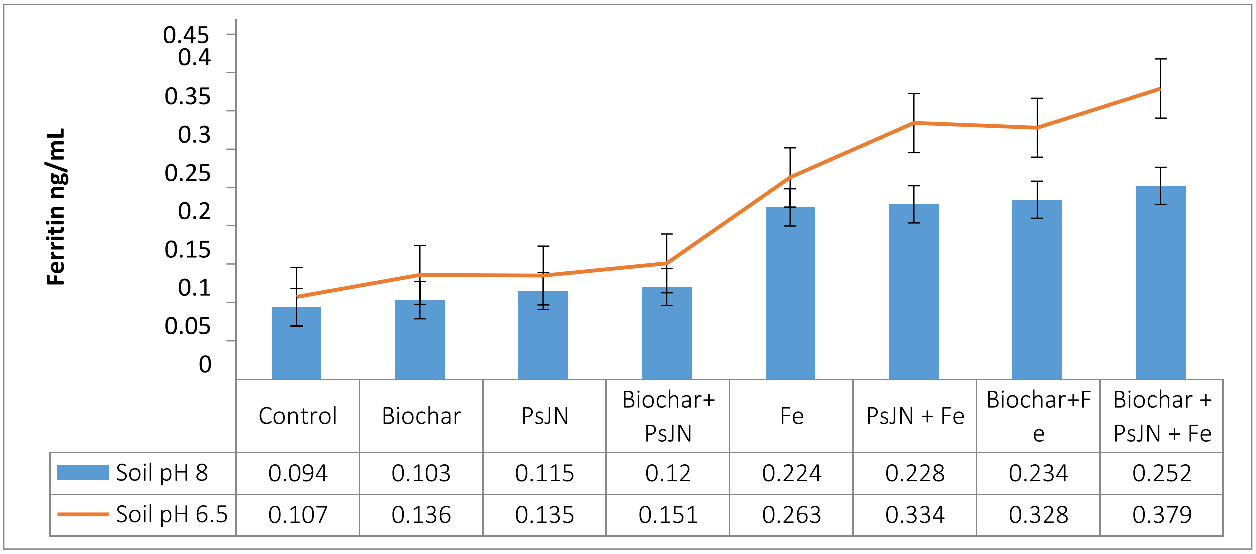


Fig. 1(B) Grain ferritin concentration ng/ ml under Fe, Biochar and *Burkholderiaphytofrmans*PsJN in pH modified soil

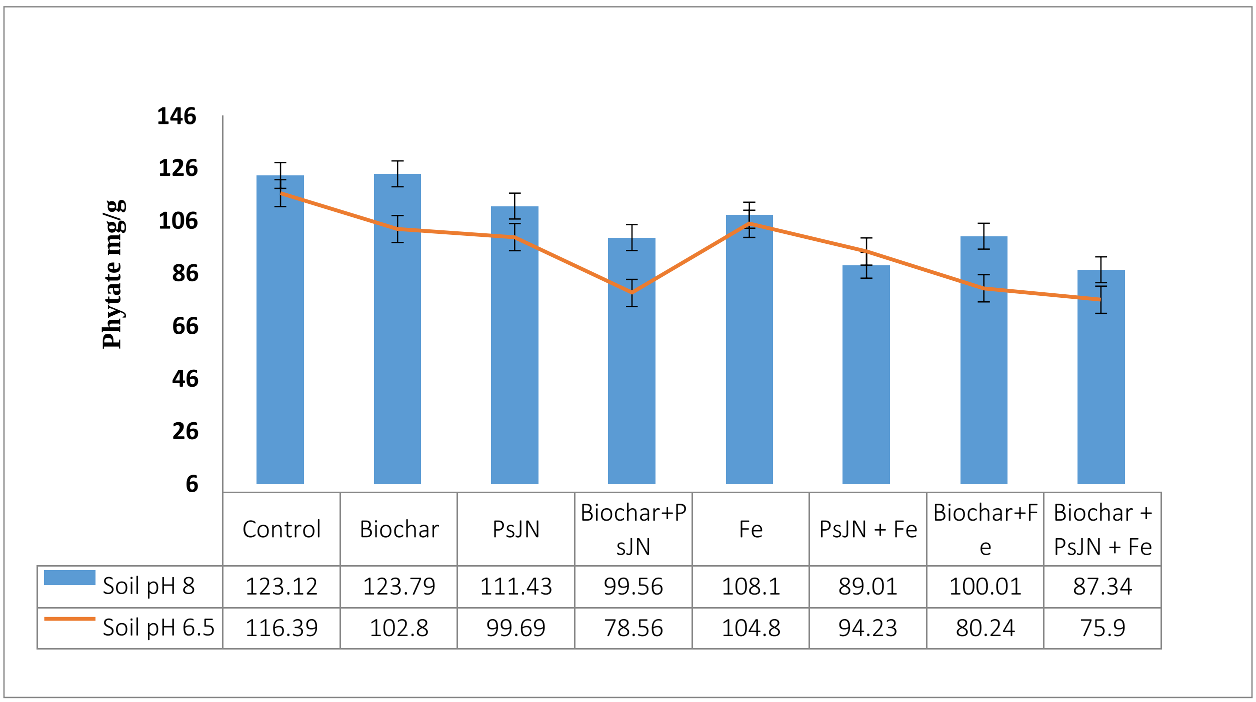


Fig. 2 Phytate concertation mg/ g under Fe, Biochar and *Burkholderiaphytofrmans*PsJN in pH modified soil

DISCUSSION

Soil acidification and nutrient solubility: In soil system, availability of iron is regulated by soil pH. Most of the available iron i.e. ferrous (Fe^{+2}) in alkaline soil rapidly gets converted into insoluble or sparingly soluble Fe^{+3} oxides and hydroxides and thereby apparently become unavailable to plants (Rajniak *et al.* 2018; Xing *et al.* 2020). Researcher have reported the potential of sulfur as acidifying agent which creates acidity in micro environment i.e. rhizosphere as it promote protonation through nutrient oxidation thus increase mineralization and consequently bioavailability of important micro-elements i.e. Fe and zinc (Mahala *et al.* 2020; Ali *et al.* 2020). In current study chemical analysis of soil, root, shoot and grain both showed a significant increase in Fe concentration (Table 4) when soil was treated with sulfur to lower down the pH from 8 to 6.5 (Table 1). It is soil pH and calcareous nature of soil that are responsible for such a low bioavailability of Fe in soil by reducing its mineralization and mobilization (Turan *et al*. 2018; Das *et al*. 2020).

Effect of B.phytofirmansPsJN inoculation on plant growth and plant physiology: It is worth mentioning that paddy physiological, growth and yield parameters were significantly increased in current study when soil was treated biochar, microbial inoculation i.e. PsJN and Fe fertilizer in S amended soil (Table 2, 3). Microbial mediated sulfur oxidation and resultantly release of H^+ in the rhizosphere (Mahala *et al*. 2020) might be the driving force behind this improvement. Besides, PsJN is known to produce Fe chelating compounds i.e. siderophores that might have aided in Fe uptake by plants (Aziz *et al*. 2020; Naveed *et al*. 2020). The mode of action of siderophores released by *B.phytofirmans* strain PsJN is very similar to mugeic acid (Alvarado *et al*. 2019) that might have increased the solubility and the mobility of micronutrients i.e. Iron, Zn (Rosales-Segovia *et al*. 2021). Thanks to the physiology plant and microbes- they release sideophores, that in turn help the plant to sustain in Iron limiting conditions. Sidephores are one of the key factors in iron mineralization and uptake by plants (Verma *et al*. 2021; Nuzzo *et al*. 2018). PsJN inoculation only influences plant physiology but also plant heath as it releases plant growth hormones (Naveed *et al*. 2020).The activity and effectiveness of the said microbes is unquestionably remained unchanged in stress conditions as described by Aziz *et al*. (2020). PsJN worked irrespective of the stress situation and improved the photosynthesis and transpiration of maize; similar results were recorded in current study (Table 2). These are microbes that may induce modifications in plant morphology thus regulates gaseous exchange and control photosynthetic rate (Martínez-Arias, *et al.* 2021; Liu and Wei 2021) these studies support the current observations (Table 2). Furthermore, it was recorded that, sulfur treated treatments were bearing tillers which were only healthy but also more in number compared with other treatments. It was because upon acidification Fe and other important micronutrients released in soil solution which were otherwise bond to soil particulates (Falcone *et al.* 2021) Further, remaining portion of nutrients supplemented by carbon rich biochar (Gong *et al.* 2020; Dhir 2021). Clearly (Table 3) optimum plant nutrition and endophytic inoculation improved total number of tillers, plant growth and yield (Maqsood et al., 2021).

Mechanisms of Biochar for improving Fe bioavilability: Biochar- a carbon rich product provides variety of services in micro and macro environment thus attracting plant growth promoting micro-flora (Bolo *et al*. 2019; Shind *et al.* 2019) High surface area, sorption capacity and porous nature of biochar aids Fe and other essential micronutrients to adsorb on its surface, moreover, high cations exchange capacity and microbial aided protonation enables slow but steady release of Fe thereby keeps nutrient supply constant (Table 3). In submerged conditions, thio-oxidansuses ferric Fe^{3+} as alternative electron acceptor because respiratory activity increases right after either partial utilization or complete absence of O_2 (Fan 2020; Oladosu *et al.* 2020). Translocation of Fe into plants increases that cause overall improvement in plant growth and development (Al-Amri *et al.* 2020). Application of bichar influences moisture contents in soil as well as in plants (Kanwal *et al.* 2018; Panahi *et al.* 2020) which ultimately improves yield and quality of crop (Saha *et al.* 2019) as discussed in current study (Table 2).

Influence of Fe nutrition in seed biochemistry: In grains most of the Fe is stored and available as ferritin and does not undergo any transfusion digestion in gastrointestinal tract. However, important staple cereals i.e. maize, wheat are naturally low in bioavailable Fe i.e. ferritin contents. Results suggested that by manipulating the pH of soil application of PsJN and biohar significantly ($P<0.05$) increased ferritin contents in grains with its respective control (Figure 1-A, B). Studies revealed that it is accelerated activity of enzyme i.e. aconitase which is responsible for ferritin accumulation in rice grain when growth media was enriched with Fe fertilization (Mendoza-Cózatl *et al*. 2019; Pappas *et al*. 2018).

Contrarily to this, a significant ($P < 0.05$) reduction in phytic acid contents in rice grains were recorded when soil was treated with sulfur and biochar, PsJN and iron fertilizer was applied as single treatment relative to its control (Table 4; Figure 2). Scientists have suggested that mature embryo and aleurone layer of rice act as sink for phytate (Silva *et al.* 2021; Che *et al*. 2021). Phytate- an anti-nutrient, inhabit the absorption of essential micro-elements i.e. Fe, Zn etc. by forming complexes in intestinal tract during digestion (Umar *et al.* 2019; Dapkekar *et al*. 2020) Availability of minerals like Zn and Fe in root zone influences the entry of phosphorus through root interception and storage in grains (Bindraban *et al*. 2020; Haider *et al*. 2020). Furthermore, rice genome has been explored and low phytic acids mutants have been discovered in order to find the best possibilities of lowering phyate concentration in rice (Ashokkumar *et al*. 2020). Successful attempts have been made to identify quantitative trait loci (QTL) for grain phytatecontents rice (Ali *et al*. 2020) hereby increasing bioavailability of micro-elements.

Farm modernization has changed the concepts of profit based farming rather quality produce has replaced it over the time. A good quality grain is characterized by proximate composition i.e. Ash, fiber, protein, fat and moisture contents. In current study we also took this parameter into consideration. Results showed all quality parameter were positively influenced when soil was treated with sulfur and amended with biochar and PsJN along with Fe fertilizer (Table 5); our results were in coherence with Yadav *et al*. (2013) and Ramzani *et al*. (2016b)

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REFERENCES

Zaccheo P, Crippa ., Giuffrida F (2021) Understanding and optimising the chemical properties of growing media for soilless cultivation. In *Advances in horticultural soilless culture* (pp. 139-170). Burleigh Dodds Science Publishing.

Pot S, De Tender, C, Ommeslag S, Delcour I, Ceusters J, Gorrens E, ... Vancampenhout K. (2021). Understanding the shift in the microbiome of composts that are optimized for a better fit-for-purpose in growing media. *Frontiers in microbiology*, *12*, 757.

Al-Amri N, Tombuloglu H, Slimani Y, Akhtar S, Barghouthi M, Almessiere M, ... Ozcelik S (2020) Size effect of iron (III) oxide nanomaterials on the growth, and their uptake and translocation in common wheat (*Triticum aestivum L.). Ecotoxicology and environmental safety 194*, 110377.

Ali Q, Afzal I, Ayyub M, Rehman A, Zahir Z A (2020) Genetic Engineering to Enhance Rice Survival in Nutrient-Deficient Soil. In *Rice Research for Quality Improvement: Genomics and Genetic Engineering* 407-436. Springer, Singapore.

Ali W, Mao K, Zhang H, Junaid M, Xu N, Rasool A, ... Yang Z (2020) Comprehensive review of the basic chemical behaviours, sources, processes, and endpoints of trace element contamination in paddy soil-rice systems in rice-growing countries. *Journal of hazardous materials*, 122720

Alvarado AM, Aguirre-Becerra H, Vázquez-Hernández MC, Magaña-Lopez E, Parola-Contreras I, Caicedo-Lopez LH, ... Feregrino-Perez AA (2019) Influence of elicitors and eustressors on the production of plant secondary metabolites. In *Natural Bio-active Compounds* 333-388. Springer, Singapore

Antoniadis V, Levizou E, Shaheen, SM, Ok YS., Sebastian A, Baum C, ... Rinklebe J (2017) Trace elements in the soil-plant interface: Phytoavailability, translocation, and phytoremediation–A review. *Earth-Science Reviews 171*, 621-645

Ashokkumar K, Govindaraj M, Adhimoolam Karthikeyan VG, Warkentin,T D (2020) Genomics-integrated breeding for carotenoids and folates in staple cereal grains to reduce malnutrition. *Frontiers in genetics*, *11*.

Aziz MZ, Yaseen M, Naveed, M, Wang Xi, Fatima K, Saeed Q, Mustafa A (2020) Polymer-*Paraburkholderia phytofirmans* PsJN Coated Diammonium Phosphate Enhanced Microbial Survival, Phosphorous Use Efficiency, and Production of Wheat *Agronomy* 10, 1344.

Bianco CL, Savitsky A, Feelisch M, Cortese-Krott M M (2018). Investigations on the role of hemoglobin in sulfide metabolism by intact human red blood cells. *Biochemical pharmacology*, *149*, 163-173.

Bindraban PS, Dimkpa CO, Pandey R (2020) Exploring phosphorus fertilizers and fertilization strategies for improved human and environmental health. *Biology and Fertility of Soils*, *56*, 299-317.

Bolan N, Hoang SA, Beiyuan J, Gupta S, Hou D, Karakoti A ...Van Zwieten L (2021) Multifunctional applications of biochar beyond carbon storage. *International Materials Reviews* 1-51.

Bolo PO (2019) Effects of longterm application of organic residues and inorganic fertilizers on soil microbial biomass development, diversity and activities in Nyabeda Siaya Count, Keyna, (Doctoral dissertation, School of Environmental Studies Kenyatta University)

Bomfim CSG, da Silva VB, Cursino LHS, da Silva Mattos W, Santos JCS, de Souza LSB, ... Fernandes-Júnior PI (2020) Endophytic bacteria naturally inhabiting commercial maize seeds occupy different niches and are efficient plant growth-promoting agents. *Symbiosis 81*, 255-269.

Boonyaves K, Wu TY, Gruissem W. Bhullar NK (2017) Enhanced grain iron levels in iron-regulated metal transporter, nicotianamine synthase, and ferritin gene cassette. Frontiers in Plant Science 8, 130

Bouis H E, Saltzman A, Birol E (2019) Improving nutrition through biofortification. *Agriculture for improved nutrition: Seizing the momentum 47*.

Che J, Yamaji N, Ma JF (2021) Role of a vacuolar iron transporter OsVIT2 in the distribution of iron to rice grains. *New Phytologist 230*, 1049-1062.

Corte-Real J, Bohn T(2018) Interaction of divalent minerals with liposoluble nutrients and phytochemicals during digestion and influences on their bioavailability–a review. *Food chemistry 252*, 285-293.

Cortese-Krott MM (2020) Red Blood Cells as a ―Central Hub‖ for Sulfide Bioactivity: Scavenging, Metabolism, Transport, and Cross-Talk with Nitric Oxide. *Antioxidants & Redox Signaling* 33, 1332-1349.

Dapkekar A, Deshpande P, Oak MD, Paknikar KM, Rajwade JM (2020). Getting more micronutrients from wheat and barley through agronomic biofortification. In *Wheat and Barley Grain Biofortification* 53-99 (Woodhead Publishing)

Das SK, Ghosh GK (2020) Soil health management through low cost biochar technology. In *Biochar applications in agriculture and environment management* 193-206 Springer Cham.

Dhaliwal SS, Naresh R K, Mandal A, Singh R, Dhaliwal M K (2019) Dynamics and transformations of micronutrients in agricultural soils as influenced by organic matter build-up: A review. *Environmental and Sustainability Indicators 1*, 100007

Dhir B. (2021) Biochar Amendment Improves Crop Production in Problematic Soils. *Handbook of Assisted and Amendment: Enhanced Sustainable Remediation Technology*, 189-204.

Falcone EE, Federico C, Bellomo S, Brusca L, D'Alessandro W, Longo M, Calabrese S (2021) Impact of acidic volcanic emissions on ash leaching and on the bioavailability and mobility of trace metals in soils of Mt. Etna. *Italian Journal of Geosciences*, *140*, 57-78.

Fan L (2020) Anaerobic Oxidation of Methane in Paddy Soil (Doctoral dissertation, Georg-August-Universität Göttingen).

Fiedler, K. (2020). On the task, the measures and the mood in research on affect and social cognition. In *Emotion and social judgments* 83-104) Garland Science.

Gaffar S, Dattamudi S, Baboukani AR, Chanda S, Novak JM, Watts DW, ... Jayachandran K (2021) Physiochemical Characterization of Biochars from Six Feedstocks and Their Effects on the Sorption of Atrazine in an Organic Soil. *Agronomy 11*, 716.

García-Latorre C, Rodrigo S, Santamaría O (2021) Endophytes as Plant Nutrient Uptake-Promoter in Plants. *Endophytes: Mineral Nutrient Management 3*, 247-265.

Gong H, Li Y, Li S (2020) Effects of interaction between biochar and nutrients on soil organic carbon sequestration in soda saline-alkalized grassland: A review *Global Ecology and Conservation*, 01449.

Greenfield B (2020) *Boundless* (Victory Belt Publishing)

Haider MZ, Saeed F, Ali A, Ali Q, Habib N, Javed MT, ... Afzaal M (2020) Involvement of Microbes in Different Abiotic Stress Environments of Cropping Lands. In *Sustainable Agriculture in the Era of Climate Change* (441-479) Springer, Cham.

Hartz TK (2020) *Efficient Nutrient Management in California Vegetable Production* 3555 (UCANR Publications)

Iasur Kruh L, Bari VK, Abu-Nassar J, Lidor O, Aly R (2020) Characterization of an endophytic bacterium (Pseudomonas aeruginosa), originating from tomato (*Solanum lycopersicum* L.) and its ability to inhabit the parasitic weed *Phelipanche aegyptiaca*. *Plant Signaling and Behavior 15*, 1766292

Kanwal S, Batool A, Ghufran MA, Khalid A (2018) Effect of dairy manure derived biochar on microbial biomass carbon, soil carbon and *Vitis vinifera* under water stress conditions. *Pakistan. Journal of Botany 50*, 1713-1718.

Kaur T, Rana KL, Kour D, Sheikh I, Yadav N, Yadav AN, ... Saxena AK (2020) Microbe-mediated biofortification for micronutrients: present status and future challenges. *Trends of microbial biotechnology for sustainable agriculture and biomedicine systems: perspectives for human health. Elsevier, Amsterdam* 1-17.

Kenzhebayeva S, Abekova A, Atabayeva S, Yernazarova G, Omirbekova N, Zhang G, ... Wang Y (2019) Mutant lines of spring wheat with increased iron, zinc, and micronutrients in grains and enhanced bioavailability for human health. *BioMed research international*, *2019*.

Kopecký M Kolář L, Konvalina P, Strunecký O, Teodorescu F, Mráz, P, ... Bucur D. (2020). Modified Biochar—A Tool for Wastewater Treatment. *Energies 13*, 5270

Krishnamoorthy A, Gupta A, Sar P, Maiti MK (2021) Metagenomics of two gnotobiotically grown aromatic rice cultivars reveals genotype-dependent and tissue-specific colonization of endophytic bacterial communities attributing multiple plant growth promoting traits. *World Journal of Microbiology and Biotechnology 37*, 1-16.

Kumar S, Palve A, Joshi C Srivastava RK (2019) Crop biofortification for iron (Fe), zinc (Zn) and vitamin A with transgenic approaches. *Heliyon 5*, 01914.

Libera AAl, José P , Ana M VQ, Isabel CFR. Ferreira, Lillian B (2020) Fighting Iron-Deficiency Anemia: Innovations in Food Fortificants and Biofortification Strategies. Foods 9, 1871.

Liu Y, Wei X (2021) Dark septate endophyte improves the drought-stress resistance of Ormosia hosiei seedlings by altering leaf morphology and photosynthetic characteristics *Plant Ecolog,* 1-11.

Magalhaes S, Taveira M, Cabrita A, Fonseca A, Valentão P, Andrade P (2017) European marketable grain legume seeds: Further insight into phenolic compounds profiles. *Food Chemistry* 215, 177–184.

Maganti S, Swaminathan R, Parida A (2019) Variation in iron and zinc content in traditional rice genotypes. *Agricultural research*, 1-13.

Mahala DM, Maheshwari HS, Yadav RK, Prabina BJ, Bharti A, Reddy KK. ...Ramesh A. (2020) Microbial Transformation of Nutrients in Soil: An Overview. *Rhizosphere Microbes* 175-211.

Man Y, Xu T, Adhikari B, Zhou C, Wang Y, Wang B (2021) Iron supplementation and iron-fortified foods: a review. *Critical Reviews in Food Science and Nutrition*, 1-22.

Mann J, Truswell A (2017) Essentials in Human Nutrition (Mann J, Truswell A, Eds.Oxford University Press: Oxford, UK, 2017)

Maqsood A, Shahid M, Hussain S, Mahmood F, Azeem F, Tahir M, ... Basit F (2021) Root colonizing *Burkholderia sp. AQ12* enhanced rice growth and up-regulated tillering-responsive genes in rice. *Applied Soil Ecology*, *157*, 103769.

Martínez-Arias C, Sobrino-Plata J, Medel D, Gil L, Martín JA, Rodríguez-Calcerrada J (2021) Stem endophytes increase root development, photosynthesis, and survival of elm plantlets (Ulmus minor Mill.) *Journal of Plant Physiology 261*, 153420.

Mendoza-Cózatl DG, Gokul A, Carelse MF, Jobe,TO, Long TA, Keyster M (2019) Keep talking: crosstalk between iron and sulfur networks fine-tunes growth and development to promote survival under iron limitation. *Journal of experimental botany*, *70*, 4197-4210.

Milman, NT (2020). A review of nutrients and compounds, which promote or inhibit intestinal iron absorption: making a platform for dietary measures that can reduce iron uptake in patients with genetic haemochromatosis. *Journal of Nutrition and Metabolism 2020*.

Moradi N, Karimi A (2021) Fe-Modified common reed biochar reduced cadmium (Cd) mobility and enhanced microbial activity in a contaminated calcareous soil. *Journal of Soil Science and Plant Nutrition 21*, 329-340.

Naveed M, Ramzan N, Mustafa A, Samad A, Niamat B, Yaseen M, Ahmad Z, Hasanuzzaman M, Sun N, Shi Weiqi, Xu Minggang (2020) Alleviation of Salinity Induced Oxidative Stress in *Chenopodium quinoa* by Fe Biofortification and Biochar—Endophyte Interaction *Agronomy* 10, 168.

Nissar J, Ahad T, Naik, HR, Hussain, SZ (2017). A review phytic acid: As antinutrient or nutraceutical. *Journal of Pharmacognosy and Phytochemistry 6*, 1554-1560.

Nuzzo A, De Martino A, Di Meo V, Piccolo A (2018) Potential alteration of iron–humate complexes by plant root exudates and microbial siderophores. *Chemical and Biological Technologies in Agriculture 5,* 1-7.

Oladosu Y, Rafii MY, Arolu F, Chukwu SC, Muhammad, I, Kareem I. ... Arolu IW (2020) Submergence tolerance in rice: Review of mechanism, breeding and, future prospects. *Sustainability 12*, 1632.

Osman KT (2018) *Management of soil problems*. Springer.

Panahi HKS, Dehhaghi M, Ok YS, Nizami AS, Khoshnevisan B, Mussatto SI. Lam SS (2020) A comprehensive review of engineered biochar: Production, characteristics, and environmental applications. *Journal of Cleaner Production* 122462.

Pappas AC, Godlewska K, Surai PF (2018) Dietary Food and Feed Supplements with Trace Elements. *Recent Advances in Trace Elements*, *421*.

Pavord S, Daru J, Prasannan N, Robinson S, Stanworth S, Girling J (2019) UK guidelines on the management of iron deficiency in pregnancy. (Br J Haematol 2019, 188, 819–830)

Price K (2018) Iron Deficiency, Depression, and Other Affective Disorders in Female State Fair Attendees.

Rajnia, J, Giehl RF, Chang E, Murgia I, von Wirén N, Sattely ES (2018) Biosynthesis of redox-active metabolites in response to iron deficiency in plants. *Nature chemical biology 14*, 442-450.

Ramzani PMA, Khalid M, Anjum S, Khan WUD, Iqbal M, Kausar S (2017) Improving iron bioavailability and nutritional value of maize *(Zea mays L.*) in sulfur-treated calcareous soil. *Archives of Agronomy and Soil Science* 63, 1255-1266

Rana KL, Kour D, Kaur T, Devi R, Yadav N, Subrahmanyam G,…Yadav AN (2020) Biotechnological applications of seed microbiomes for sustainable agriculture and environments. *Trends of microbial biotechnology for sustainable agriculture and biomedicine systems: diversity and functional perspectives. Elsevier, Amsterdam* 127-143.

Rawat N, Neelam K, Tiwari VK, Dhaliwal HS (2013): Biofortification of cereals to overcome hidden hunger. *Plant Breed* 132, 437-445.

Rehman HM, Cooper J W, Lam HM, Yang SH (2019) Legume biofortification is an underexploited strategy for combatting hidden hunger. *Plant cell and environment 42*, 52-70.

Rhowell Jr NT, Fernie AR, Sreenivasulu N (2021) Meeting human dietary vitamin requirements in the staple rice via strategies of biofortification and post-harvest fortification. *Trends in Food Science and Technology*.

Rosales-Segovia K, Bai Y, Wang C, Huang L, Cheng W, Yamashita Y, ... Bounab N (2021) May. P1: Building an effective concentration signature for Zn availability from 4 techniques to a stream. In *Program and Abstract* 21.

Saha A, Basak BB, Gajbhiye NA, Kalariya K. Manivel P (2019) Sustainable fertilization through co-application of biochar and chemical fertilizers improves yield, quality of *Andrographis paniculata* and soil health. *Industrial Crops and Products 140*, 111607.

Shinde R, Sarkar PK, Thombare N (2019) Soil conditioners. *Agriculture and Food:Newsletter, 1* 1-5.

Silva VM, Putti FF, White PJ, Dos Reis AR (2021) Phytic acid accumulation in plants: Biosynthesis pathway regulation and role in human diet. *Plant physiology and biochemistry 164*, 132-146.

Turan V, Ramzani PMA, Ali Q, Abbas F, Iqbal M, Irum A, Khan WUD (2018) Alleviation of nickel toxicity and an improvement in zinc bioavailability in sunflower seed with chitosan and biochar application in pH adjusted nickel contaminated soil. *Archives of Agronomy and Soil Science 64,* 1053-1067

Ullah A, Nisar M, Ali H, Hazrat A, Hayat K, Keerio AA, ... Yang X (2019) Drought tolerance improvement in plants: an endophytic bacterial approach. *Applied microbiology and biotechnology 103*, 7385-7397.

Umar M, Nawaz R, Sher A, Ali A, Hussain R, Khalid MW (2019) Current Status and Future Perspectives of Biofortification in Wheat. *Asian Journal of Research in Crop Science*, 1-14.

United Nations (2017) The *World Population Prospects: The 2017 Revision*.

Verma S, Chakdar H, Kumar M, Varma A, Saxena AK (2021) Microorganisms as a Sustainable Alternative to Traditional Biofortification of Iron and Zinc: Status and Prospect to Combat Hidden Hunger. *Journal of Soil Science and Plant Nutrition*, 1-18.

Wakeel A, Farooq M, Bashir K, Ozturk, L (2018) Micronutrient malnutrition and biofortification: Recent advances and future perspectives. *Plant micronutrient use efficiency* 225-243.

Walitang DI, Kim K, Madhaiyan M, Kim YK, Kang Y, Sa T (2017) Characterizing endophytic competence and plant growth promotion of bacterial endophytes inhabiting the seed endosphere of Rice. *BMC microbiology 17*, 1-13.

Xing Y. (2020) Iron isotope fractionation in arable soil and graminaceous crops.

Yin S, Davey K, Dai S, Liu Y, Bi J (2021) A critical review of ferritin as a drug nanocarrier: Structure, properties, comparative advantages and challenges. *Particuology*.

Adnan M, Abbas B, Asif M, Hayyat MS, Raza A, Khan, BA, ... Khalid, M. (2020) Role of micro nutrients bio-fortification in agriculture: A review. *International Journal of Environmental Sciences & Natural Resources 24*, 209-213.

Sangeetha SS, Jawahar D, Chitdeshwari T, Babu C, Lakshmanan L (2021) The Influence of Iron Chelates on Chlorophyll Content and Yield of Bajra Napier. *Current Journal of Applied Science and Technology* 1-6.

Allison LE Moodie CD (1965) Carbonate. In: Black CA (ed.) Methods soil Analysis Part 2: Chemical and Microbiological Properties. *American Society of Agronomy* (pp.1379–1396) Madison, USA.

AOAC. 2003. Official methods of analysis of the association of official’s analytical chemists, 17th edn. Association of official analytical chemists, Arlington, Virginia.

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72, 248-254.

Bremner JM, Mulvaney CS (1982). Nitrogen total. In: Page AL (ed.) Methods of soil analysis. Agron. No. 9, Part 2: Chemical and microbiological properties, 2nd ed. *American Society* of Agronomy (pp. 595–624) Madison, WI, USA.

Gee GW, Bauder. JW (1986) Particle-size analysis. In: Klute A (ed.) Methods soil anal. Part 1: Physical and mineralogical methods. Agronomy Monogr 9: *Soil Science Society of America* (pp. 383–409).Madison, USA.

Haug W, Lantzsch H (1983). Sensitive method for the rapid determination of phytate in cereals and cereal products *Journal* of the *Science* of *Food* and *Agriculture* 34, 1423-1424.

Jones, JRJ, Case VW (1990) Sampling, handling, and analyzing plant tissue samples. *In:*Westerman RL (ed.) Soil testing and plant analysis. *Soil Science Society of America* (pp. 389-428) Madison, USA.

Lindsay WL, Norvell WA (1978) Development of a DTPA soil test for zinc, iron, manganese and copper. *Soil Science Society of . American Journal* 42, 421-428.

Lukac RJ, Aluru MA, Reddy MB (2009) Quantification of ferritin from staple food crops. *Journal of Agriculture and Food Chemistry* 57, 2155-2161.

Nelson DW, Sommers LE (1982) Total carbon, organic carbon and organic matter. In: Klute A (ed.) Methods of soil analysis, Part 2: chemical and microbiological properties. *Soil Science Society of America (*pp 570-571) Madison, USA.

Ramzani, PMA, Khan WUD, Iqbal M, Kausar S, Ali S, Rizwan M, Virk ZA (2016b) Effect of different amendments on rice (*Oryza sativa* L.) growth, yield, nutrient uptake and grain quality in Ni-contaminated soil. *Environmental Science and Pollution Research* 23, 01-11.

Rebecca JL, Aluru RM, Reddy BM (2009). Quantification of Ferritin from Staple Food Crops. J*ournal of Agricultural Food Chemistry* 57, 2155–2161.

Watanabe FS, Olsen S.R (1965) Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts from soil. *Soil Science Society of American Proceedings* 29: 677-678.

Yadav GS, Shivay YS, Kumar, Babu S (2013) Enhancing iron density and uptake in grain and straw of aerobic rice through mulching and rhizo-foliar fertilization of iron. *African Journal of Agricultural Research* 8, 5447-5454.