Annals of Plant and Soil Research 25(3): 381-388 (2023)
https://doi.org/10.47815/apsr.2023.10282

Iron biofortification for enhancing yield, nutrient uptake and iron nutrition in soybean (*Glycine max* L.)

**BADAPMAIN MAKDOH[1, 2], A. P SINGH*, L. T. LONGKUMER, T. GOHAIN, LANUNOLA TZUDIR,
D. NONGMAITHEM, REKHA YADAV AND L. TOUTHANG[2]**

Department of Agronomy, SASRD, Nagaland University, Medziphema - 797106, Nagaland

Received, May, 2023; Revised accepted, July, 2023

ABSTRACT

The study was undertaken in pots during *kharif* seasons of 2018 and 2019 at the Department of Agronomy, SAS, Nagaland University, Medziphema campus, Nagaland. The experiment was conducted to study the biofortification of iron in soybean with three soybean varieties *viz*., JS-335, JS-97-52 and local cultivar under six iron fertilization treatments laid out in Factorial CRD which was replicated thrice. Six iron treatments *viz*., Fe_0 as Control, Fe_1 (Foliar spray of 0.5% $FeSO_4.7H_2O$), Fe_2 (Foliar spray of 1.0% $FeSO_4.7H_2O$), Fe_3 (Foliar spray of 1.5% $FeSO_4.7H_2O$), Fe_4 (Foliar spray of 2.0 % $FeSO_4.7H_2O$), Fe_5 (Soil application 10 kg ha^{-1} $FeSO_4.7H_2O$). Two foliar applications of each concentration were applied at pre-flowering stage of the crop. Among varieties, JS 97-52 was superior in dry matter yield while in plant height local cultivar was superior. Many growth parameters were significantly enhanced with iron fertilization. Foliar spray application of 1.5% $FeSO_4.7H_2O$ was found most effective treatment in enhancing growth, yield and iron content of soybean. The seed yield and iron content in seed were enhanced by 27.6% and 18.5 % respectively over the control with foliar application of 1.5% $FeSO_4.7H_2O$. NPK uptake was found significantly higher in JS 97-52 among the varieties whereas among Fe fertilization, two foliar applications of 1.5% $FeSO_4.7H_2O$ significantly enhanced N and K uptake while P uptake was not affected significantly. Hence, it is concluded that foliar application of 1.5% iron sulphate can be recommended as an effective biofortification strategy for enhancing iron content of soybean seed and simultaneously improving its grain yield and quality as well.

Keywords: Biofortification, iron content, soybean yield, uptake

INTRODUCTION

Zinc and iron deficiency is the major concerns on public health and socio-economic issue particularly in developing countries (Welch and Graham, 2004). According to Stoltzfus and Dreyfuss (1998), iron deficiency is one of the most prevalent micronutrient deficiencies in the world, affecting an estimated two billion people. According to WHO (2002), iron deficiency is ranked 6th among the risk factors for death and disability in developing countries. Iron deficiency mainly results in anaemia, leading to functional impairments of the human body (Cappellini *et al.,* 2020). According to an estimate that of the world's total population, it was found that 60-70% has iron deficiency. The recommended daily allowance of iron is 13 mg per day for children, 17 mg per day for adult. Unfortunately, in India, due to lack of nutritional diversity deficiency in micronutrients, iron and zinc are prevalent (Anon., 2009). Apart from its importance on human health and nutrition, iron has many vital roles in plant systems. Iron is a structural component of porphyrin molecules, cytochromes, hematin, ferrichrome and leghaemoglobin involved in oxidation reduction reactions in respiration. Iron is an important constituent element for the nitrogenase enzyme which plays an important role in N-fixation through the N-fixing bacteria and also in the chloroplast for photosynthetic reduction processes.

Soybean is considered the world's most important seed legume crop and it shares 25% of the global edible oil and contributes almost two-thirds of protein concentrate required for livestock feed production globally. India is also the 4th largest producer of soybean in the world. Soybean is also considered to be an important crop in the North-Eastern states of India. This crop has always been an integral part in the

---

*Corresponding author- apsingh@nagalanduniversity.ac.in, [1] Ph.D. Scholar [2]ICAR for NEH Region, Umiam (Barapani)-793102, Meghalaya culture and farming systems of different ethnic groups and farming communities of North-Eastern India. Soybean has been traditionally known be an invaluable item of different cuisines and local delicacies of almost all communities of the region. It is consumed roasted, fermented, fresh pods as vegetable and local preparations by almost all the tribal communities of this region. In the state of Nagaland many tribes have been using soybean as an important ingredient for many local delicacies especially in fermented forms. Akhoni, a local fermented soybean of Sema tribe of Nagaland is a favoured preparations liked by many tribes of the Northeast. For alleviating the problem of iron deficiency in humans, strategies comprising of iron supplementation, fortification, dietary diversification/ modification and biofortification can be deployed. The approach of ‘biofortification’ of food crops gradually has gained global attention (Graham *et al.,* 2001). Biofortification is defined as ‘the process of increasing the bioavailable concentrations of essential elements in edible portions of crop plants through genetic selection or agronomic intervention’ (White and Broadley, 2005). Majority of soils in the NE region of India are in the sufficiency range with respect to available iron in the soil. Bandyopadhay *et al.* (2018) reported that out of total surveyed area in Nagaland, the DTPA-extractable Zn and Fe were mostly in sufficiency range of 85.60% and 99.6%, respectively and where only 14.40% and 0.40% was in the deficiency range. Similarly, as per the reports by Shukla *et al.* (2021) the soils of Nagaland were containing high amount of iron (68.7% of the samples).

In the recent past many agronomic biofortification programmes have been mainly emphasized on major cereals or staple food crops like wheat, maize and rice which indeed gave encouraging results for enhancing zinc and iron density in grain. Hence, the concept has since been gradually extended to different crops based on preferences or location specific. So, the ideas of executing the concept of iron biofortification on soybean being a commonly cultivated crop in the region become a good prospect for investigation. Hence, this study was conducted with the goal to evaluate the effect of iron on growth, yield and enhancing the bioavailability of iron in soybean seed.

MATERIALS AND METHODS

The pot experiment was conducted at Department of Agronomy, School of Agricultural Sciences (SAS), Nagaland University, Medziphema, Nagaland, situated at a latitude of $25^0 45^{'} 43^{''}$ North and longitude $95^0 53'04''$ East with altitude of 310 meters above mean sea level (MSL). The experimental site lies in a humid subtropical region with annual rainfall ranging from 2000-2500 mm. The experimental soil was sandy loam with pH 4.90 organic carbon 1.51%, available N 526.7 kg ha^{-1} available P_2O_5 32.8 kg ha^{-1} available K and DTPA extractable Fe was 264.82 kg ha^{-1} and 78.43 mg kg^{-1} of soil respectively. Cement pot 20 L capacity filled with 12 kg top soil (0-15 cm) was used for experiment. This experiment was conducted to study the biofortification of iron in soybean with three soybean varieties *viz*., JS-335 (V_1), JS-97-52 (V_2) and local cultivar (V_3) under six iron fertilization treatments laid out in Factorial completely randomized design with three replications. Six iron treatments *viz*., Fe_0 as Control, Fe_1 (Foliar spray of 0.5% $FeSO_4.7H_2O$), Fe_2 (Foliar spray of 1.0% $FeSO_4.7H_2O$), Fe_3 (Foliar spray of 1.5% $FeSO_4.7H_2O$), Fe_4 (Foliar spray of 2.0 % $FeSO_4.7H_2O$), Fe_5 (Soil application 10 kg ha^{-1} $FeSO_4.7H_2O$). Two foliar applications of each foliar treatment were applied at pre-flowering stage of the crop. The crop was sown on second week of July 2018 and 2019 by maintaining five plants each pot for initial crop establishment. Recommended dose of NPK @ 20:60:40 kg ha^{-1} was applied through urea, SSP and MOP along with 10 t ha^{-1} FYM as general dose to all the pots irrespective of treatments on soil weight basis. The seed was treated with rhizobium culture @ 20g kg^{-1} seed. For foliar application treatments, ferrous sulphate heptahydrate containing 32.8% iron was used and two foliar spays was executed during pre-flowering and flowering stage. Lime supernatant was used as spray solution to avoid any salt injury to the foliage. Data on plant height, number of branches $plant^{-1}$, number of pods $plant^{-1}$, pods length, number of seeds pod^{-1}, test weight and grain and stover yield were recorded. Oven-dried plant samples were grounded to a fine powder using a mechanical grinder. A representative grounded straw and grain sample of 0.5 g were digested using a di-acid mixture (HNO_3:$HClO_4$, 3:1) on an electric hot plate procedure as described by Prasad *et al.* (2006). For N content, seed samples were analysed by Kjeldahl method. Phosphorus in grain and stover samples were determined in diacid (HNO_3, $HClO_4$) extract by advocating standard procedure. Potassium content in the plant sample was determined by flame photometer. The Fe concentration was estimated from the digested plant extracts through ICP Spectrophotometer model: iCAP 7000 series in mg kg^{-1}. The Fe uptake in soybeans was calculated employing the following formula:

$$\text{Fe uptake in seed or stover } (\mu\text{g pot}^{-1}) = \frac{\text{Fe content } (\text{mg kg}^{-1}) \text{ X yield } (\text{g pot}^{-1})}{1000} \text{x}1000$$

$$\text{Macronutrient uptake } (\text{gpot}^{-1}) = \frac{\text{Nutrient content } (\%) \text{ X yield } (\text{g pot}^{-1})}{100}$$

All the replicated data obtained from the experiment were statistically analysed using the F test as per the procedure given by Gomez and Gomez (1984). Critical difference values at $P = 0.05$ were used to determine the significance of differences between treatment means.

RESULTS AND DISCUSSION

Growth parameters

Varieties differed significantly with plant height at all crop stages (Table 1). At harvest local cultivar (71.19 cm) registered the highest plant height while the lowest value observed in JS-335 (51.64 cm). Among the varieties, local cultivar found to be much superior in plant height being a traditionally taller cultivar when compared to short duration high yielding improved varieties. The main reason for this is due to varietal and genotypic differences which were shown in crop morphology. Iron fertilization failed to produce any significant difference on plant height of soybean at all crop stages. Although, there was no significant variations on imposition of Fe fertilization on plant height, however Fe_3 (Foliar spray application of $FeSO_4.7H_2O$@ 1.5% at pre-flowering stage) has numerical superiority (63.22 cm) over the rest. The reason for this could be due to increase in the availability of iron to plant which might have stimulated the metabolic and enzymatic activities thereby increasing the growth of the plant reported by (Trivedi *et al.,* 2011). It can also be explained by the fact that Fe treated plants have better uptake of plant nutrients due to which might enhance more photosynthesis in turn resulted to more leaves, leaf area and dry matter accumulation and ultimately on plant height. Balachandar *et al.* (2003), Kobraee *et al.* (2011), Dhaliwal *et al.* (2013) and Soni and Kushwaha (2020) also reported similar findings with respect to effect of Fe on plant height. There was significant variation in dry matter accumulation (DMA) among varieties at all crop stages. At harvest, the variety JS 97-52 (16.63 g plant^{-1}) was statistically at par with local cultivar (16.18 g plant^{-1}) and JS-335 (12.19 g plant^{-1}). Application of ferrous sulphate, Fe_3 resulted in maximum value (16.20 g plant^{-1}) followed by Fe_2 (15.79 g plant^{-1}) and the least was in control (13.91 g plant^{-1}). The positive effect of Fe nutrition in enhancing DMA might be enhanced growth and development of the crop. Iron tends to increase the synthesis of enzymes like IAA production and protein synthesis, which helps promote vegetative growth. The results of this investigation are in accordance with the findings of Kamble *et al.* (2021).

Yield attributes

Among varieties, JS 97-52 recorded the highest number of pods plant^{-1} (66.32) which was significantly higher than local cultivar (41.99) and JS 335 (35.89) (Table 1). Fe fertilization treatments did not result to any significant effect on the number of pods plant^{-1}. The number of seeds pod^{-1} was not affected significantly with verities and iron application. There was significant variation among the varieties on pod length of soybean where JS-335 recorded the highest value (3.79 cm) followed by local cultivar (3.39 cm). No significant result with iron application was found on this yield attribute although slightest higher value (3.56 cm) observed in Fe_3 (Foliar spray application of 1.5% $FeSO_4.7H_2O$ at pre-flowering stage). The maximum seed index was recorded in JS-335 (12.67 g) which was statistically superior over the other varieties. JS 97-52 recorded (11.27 g) with the least value observed in local cultivar (7.22 g). As revealed by the data there was no significant effect of Fe nutrition on yield attributes of soybean. The reason could be due to lesser impact from the applied Fe as the soil in the experimental site was sufficient in Fe range. However, when compared to the control plants the yield attributes slightly showed higher values which to some extent as $FeSO_4$ plays its role in the plant system. The slight improvement in the number of pods plant^{-1}, number of seeds pod^{-1}, 100-seed weight etc. may be attributed to the fact that favourable nutritional environment in rhizosphere and absorption of Fe by foliage led to enhanced photosynthesis and production of assimilates. This further led to more translocation of photosynthates in the reproductive structures and thus to the yield attributes. With foliar application of $FeSO_4$ entry and absorption of Fe in the plant system became easier which then might have enhanced availability of iron, increased the chlorophyll content and thus more to the accumulation of carbohydrates. This produces positive effect on flowering and pod development and ultimately on the yield attributes of soybean. Similar findings were also reported by Umamaheswari and Singh (2002) and Moosavi and Ronaghi (2011).

Table 1: Effect of varieties and iron application on growth yield attributes and yield of soybean (pooled)

Treatment	Plant height (cm)	Dry matter accumulation at harvest (g plant^{-1})	No. of pods plant^{-1}	No. of seeds pod^{-1}	Pod length (cm)	100-seed weight (g)	Seed yield (g pot^{-1})	Stover yield (g pot^{-1})
Varieties								
V_1	51.64	12.19	35.89	2.53	3.79	12.67	20.76	20.89
V_2	58.47	16.63	66.32	2.56	3.20	11.27	29.04	29.68
V_3	71.19	16.18	41.99	2.46	3.39	7.22	17.36	19.69
SEm±	1.10	0.32	1.14	0.03	0.04	0.19	*0.36*	*0.53*
CD (P=0.05)	3.10	0.89	3.22	NS	0.10	0.54	*1.00*	*1.49*
Fe fertilization								
Fe_0	55.96	13.91	44.11	2.43	3.39	9.65	19.50	20.51
Fe_1	59.60	15.08	46.83	2.49	3.43	10.34	22.66	22.94
Fe_2	62.65	15.79	47.64	2.56	3.49	10.48	21.87	23.91
Fe_3	63.22	16.20	52.19	2.54	3.56	10.74	24.89	26.28
Fe_4	59.57	14.30	47.25	2.50	3.46	10.37	22.44	23.31
Fe_5	61.58	14.72	50.36	2.57	3.43	10.73	22.96	23.55
SEm ±	1.55	0.45	1.61	0.04	0.05	0.27	*0.50*	*0.75*
CD (P=0.05)	NS	1.26	NS	NS	NS	NS	*1.42*	*2.11*

Yields

Seed yield varied significantly among the varieties (Table 1). JS-97-52 (29.04 g pot^{-1}) recorded highest seed yield which was followed by JS-335 (20.76 g pot^{-1}) and the lowest was recorded in local cultivar (17.36 g pot^{-1}). Improved variety like JS 97-52 markedly surpassed over the other two in yield potentials over the two years of experiment irrespective of the treatments imposed mainly is attributed to the effect of Genotype x Environment interaction. All the Fe treated pots were statistically at par with each other and significantly higher than the control. The per cent increase in seed yield with two foliar applications of 1.5% $FeSO_4.7H_2O$ was to the tune of 27.65 % over control followed by Fe_5 (Soil application of 10 kg ha^{-1} $FeSO_4.7H_2O$) (17.7 %). The increase in yield due to Fe application was attributed to better performance in the growth and yield parameters which could have influenced the physiological processes and photosynthates build up under adequate availability of major and micro nutrients in soil as explained by Tabassum *et al.* (2013). It can also be explained by the fact that Fe is part of ferrodoxin and cytochrome structures which are electron carrier and plays vital role in various metabolic processes *viz*., hormone production, nitrogen fixation, chlorophyll construction, photosynthesis, respiration, DNA synthesis (Vaghar *et al.,* 2020). Thus, significant increase in the biological yield of soybean might be due to the improved leaf and stem nutrition and intensification of photosynthesis due to foliar application of Fe (Rai *et al.,* 2021). Further, Fe application through different treatments might have increased number of enzymatic activities of

Fe-containing enzymes which cumulatively have positive effect on crop yield. The results of the present study were in concordance with the results obtained from Ghasemian *et al.* (2010) and Kobraee *et al.* (2011). JS 97-52 recorded the highest stover yield (29.68 g pot^{-1}) which was significantly higher than JS-335 (20.89 g pot^{-1}) and local cultivar (19.69 g pot^{-1}). The highest stover yield was observed in Fe_3 (26.28 g pot^{-1}). Application of 1.5% $FeSO_4.7H_2O$ foliar spray enhanced the stover yield by (28.12 %) over the control. Iron is known to improve photosynthesis and assimilates transportation to sinks which then finally increases stover yield of crop. Similar effect of foliar spray of iron was observed in cowpea in sandy loam soil of Kerala by Anitha *et al.* (2005) and Yadav *et al.* (2013) in mungbean.

NPK uptake

The N, P and K uptake by grain in JS 97-52 was (1.758, 0.108, 0.411 g pot^{-1}) respectively which was followed by JS-335 (1.262, 0.074, 0.286 g pot^{-1}) respectively (Table 2). Foliar spray application 1.5% of $FeSO_4.7H_2O$ at pre-flowering stage registered the highest N, P and K uptake by grain (1.553, 0.088, 0.357 g pot^{-1}) with the least value in control (1.118, 0.078, 0.272 g pot^{-1}). However, the P uptake by grain and stover was found non-significant. The N, P and K uptake by stover of soybean was also found significantly varied among the varieties where JS 97-52 registered the highest value (0.581, 0.088, 0.664 g pot^{-1}) followed by JS-335 (0.437, 0.059, 0.467 g pot^{-1}) respectively. The N and K uptake by stover as influenced by iron fertilization was found to be significantly higher in foliar application of 1.5% of $FeSO_4.7H_2O$ (0.564, 0.603 g pot^{-1}) while P uptake was non-significantly affected. The significantly higher value of grain and stover yield in certain varieties and iron fertilization have cumulatively added to overall significantly higher value in N and K uptake except for P due to antagonistic effect of P and Fe content (Mundra and Bhati, 1991). Iron nutrition in plants helps in number of enzymatic and physiological processes which are directly linked to photosynthetic activities and accumulation of photosynthates in grain, which is resulted in overall grain yield and thus to the nutrient uptake like nitrogen and other nutrients. The results are corroborated by the findings of Singh *et al.* (2004) and Abbas *et al.* (2012).

Table 2: Effect of varieties and iron application on N, P and K uptake in soybean (pooled)

Treatment	N uptake (g pot^{-1})		P uptake (g pot^{-1})		K uptake (g pot^{-1})	
	Grain	Stover	Grain	Stover	Grain	Stover
Varieties						
V_1	1.262	0.437	0.074	0.059	0.286	0.467
V_2	1.758	0.581	0.108	0.088	0.411	0.664
V_3	1.020	0.372	0.067	0.063	0.251	0.443
SEm±	0.023	0.013	0.002	0.002	0.006	0.016
CD (P=0.05)	0.065	0.038	0.006	0.006	0.017	0.046
Fe fertilization						
Fe_0	1.118	0.388	0.078	0.065	0.272	0.449
Fe_1	1.356	0.441	0.086	0.069	0.319	0.507
Fe_2	1.323	0.471	0.080	0.070	0.307	0.531
Fe_3	1.553	0.564	0.088	0.074	0.357	0.603
Fe_4	1.339	0.464	0.080	0.067	0.315	0.517
Fe_5	1.390	0.452	0.086	0.074	0.326	0.541
SEm ±	0.033	0.019	0.003	0.003	0.009	0.023
CD (P=0.05)	0.092	0.053	NS	NS	0.024	0.064

Iron content in grain and stover

Varieties significantly differed from each other in grain Fe concentration (Table 3). The highest grain Fe content (67.50 mg kg^{-1}) was found in the cultivar JS-335 (V_1) which was statistically at par with JS 97-52 (65.13 mg kg^{-1}) followed by local cultivar (56.78 mg kg^{-1}). The variation of grain Fe content among the varieties could be explained by the varietal difference in efficiency level with respect to Fe acquisition which was supported by the findings of Rengel and Graham (1996), Graham *et al.* (1997) and Dhaliwal *et al.* (2013). With application of

$FeSO_4.7H_2O$ there was positive effect on the Fe biofortification and enhancement in grain Fe concentration. Among the treatments, Fe_3 recorded the maximum grain Fe concentration (67.29 mg kg^{-1}) (Table 3). The per cent increase in grain Fe density upon Fe fertilization over the control was (18.50%). The possible reason for higher Fe content could be due to higher synthesis of photosynthetic product with specific absorption and transport sites which increases plant biomass. It is also elaborated that upon foliar spray of ferrous sulphate, Fe absorption in plant leaves is accompanied by its translocation in the plant system. Similar results have also been reported by Dhaliwal *et al.* (2010) and in the latest study reported by Dhaliwal *et al.* (2022) in soybean, it was indicated that maximum increase in Fe density can be achieved through either 2-3 foliar sprays of 0.5% $FeSO_4.7H_2O$. Their result substantiated the findings of our experiment, where spray concentrations of $FeSO_4.7H_2O$ at 1.5%, 1% and 0.5% yielded higher value of iron biofortification in soybean grain. As revealed by the data it was found that foliar application of iron sulphate was superior over soil application which further corroborated by the findings of Pal *et al.* (2019). Iron content in stover was found non-significantly upon varieties and Fe fertilization. However, higher value of Fe content in stover was observed in Fe application when compared to control.

Table 3: Effect of varieties and iron application on iron content and uptake in grain and stover (pooled)

Treatment	Fe content in seed (mg kg^{-1})	Fe content in stover (mg kg^{-1})	Fe uptake by grain (µg pot^{-1})	Fe uptake by stover (µg pot^{-1})
Varieties				
V_1	67.50	113.76	1404.54	2375.68
V_2	65.13	117.95	1902.28	3526.21
V_3	56.78	119.50	991.25	2361.03
SEm±	*0.94*	*2.07*	*35.69*	*79.73*
CD (P=0.05)	*2.64*	*NS*	*100.61*	*224.77*
Fe fertilization				
Fe_0	56.78	106.12	1118.93	2183.76
Fe_1	64.24	115.21	1466.67	2626.23
Fe_2	65.94	119.70	1464.40	2846.82
Fe_3	67.29	124.48	1695.93	3270.82
Fe_4	61.94	119.47	1398.79	2796.95
Fe_5	62.63	117.45	1451.42	2801.27
SEm ±	*1.32*	*2.93*	*50.47*	*112.75*
CD (P=0.05)	*3.73*	*8.25*	*142.29*	*317.87*

Iron uptake by grain and stover

Varieties of soybean were significantly differed in the grain Fe uptake. It was observed that variety JS 97-52 (1902.28 µg pot^{-1}) recorded significantly higher value of grain Fe uptake (Table 3). Foliar application of 1.5% of $FeSO_4.7H_2O$ at pre-flowering stage (Fe_3) resulted in significantly higher grain Fe uptake (1695.93 µg pot^{-1}) which was followed by Fe_5 and Fe_2 in the first year and the least Fe uptake recorded in control (1118.93 µg pot^{-1}). The percentage increased upon Fe fertilization treatments was found to be the highest in Fe_3 (51.56 %) over control. This result was in conformity to the findings reported by Dhaliwal *et al.* (2010) and Yadav *et al.* (2013). In stover, JS 97-52 (3526.21 µg pot^{-1}) recorded significantly higher value of Fe uptake. The treatment, Fe_3 recorded the highest value (3270.82 µg pot^{-1}) and was significantly higher than the rest of Fe treatments while they were statistically at par with each other. These finding was supported by the finding of Dhaliwal *et al.* (2022).

From the results of the present investigation, it may be concluded that among varieties, JS 97-52 was superior in growth, yield and iron uptake. However, iron nutrition with application of 1.5% of $FeSO_4.7H_2O$ was the most effective method in enhancing crop growth, seed yield with enrichment of Fe density in grain.

REFERENCES

Abbas, G., Hussain, F., Anwar, Z., Khattak, J.Z.K. and Ishaque, M. (2012) Effects of iron on the wheat crop (*Triticum aestivum* L.) by uptake of nitrogen, phosphorus and potassium. *Asian Journal of Agricultural Sciences* **4** (3): 229-235.

Anitha, S., Sreenivasan, E. and Purushothaman, S.M. (2005) Response of cowpea {Vigna unguiculata (L.) Walp.} to foliar nutrition of zinc and iron in the oxisols of Kerala. *Legume Research-An International Journal* **28** (4): 294-296.

Anon. (2009) Nutrient requirement and recommended dietary allowances for Indians. National Institute of Nutrition, ICMR, Hyderabad. 331-332.

Balachandar, D., Nagarajan, P. and Gunasekaran, S. (2003) Effect of organic amendments and micronutrients on nodulation and yield of blackgram in acid soil. *Legume Research-An International Journal* **26** (3): 192-195.

Bandyopadhyay, S., Ray, P., Padua, S., Ramachandran, S., Jena, R.K., Roy, P.D., Dutta, D.P., Singh, S.K. and Ray, S.K. (2018) Priority Zoning of Available Micronutrients in the Soils of Agro-ecological Sub-regions of North-East India Using Geo-spatial Techniques. *Agricultural research* **7**: 200-214.

Cappellini, M.D., Musallam, K.M. and Taher, A.T. (2020) Iron deficiency anaemia revisited. *Journal of internal medicine* **287** (2): 153-170.

Dhaliwal, S.S., Sadana, U.S., Khurana, M.P.S., Dhadli, H.S. and Manchanda, J.S. (2010) Enrichment of rice grains with zinc and iron through ferti-fortification. *Indian Journal of Fertilisers* **6** (7): 28-35.

Dhaliwal, S.S., Sadana, U.S., Manchanda, J.S., Khurana, M.P.S. and Shukla, A.K. (2013) Differential response of maize cultivars to iron applied through ferti-fortification. *Indian Journal of Fertilisers* **9** (8): 52-57.

Dhaliwal, S.S., Sharma, V., Shukla, A.K., Kaur, J., Verma, V., Kaur, M., Singh, P., Kaur, L., Verma, G., Singh, J. and Gaber, A. (2022) Biofortification of Soybean (*Glycine max* L.) through $FeSO_4.7H_2O$ to Enhance Yield, Iron Nutrition and Economic Outcomes in Sandy Loam Soils of India. *Agriculture* **12** (5): 586.

Ghasemian, V., Ghalavand, A., Zadeh, A.S. and Pirzad, A. (2010) The effect of iron, zinc and manganese on quality and quantity of soybean seed. *Journal Phytologist* **2** (11): 73-79.

Gomez, K.A. and Gomez, A.A. (1984) *Statistical procedures for agricultural research*. John Wiley & sons.

Graham, R.D., Senadhira, D. and Ortiz-Monasterio, I. 1997. A strategy for breeding staple-food crops with high micro-nutrient density. *Soil Science and Plant Nutrition* **43**: 1153-1157.

Graham, R.D., Welch, R.M., Bouis. H.E. (2001) Addressing micronutrient malnutrition through enhancing the nutritional quality of staple foods: Principles, perspectives and knowledge gaps. *Advances in Agronomy* **70**: 77-142.

Kamble, B.M., Meena, R. and Gajbhiye, P.N. (2021) Effect of Iron Nutrition on Growth, Quality and Yield of Soybean (*Glycine Max* L.) Grown on Problematic Inceptisol. *Journal of Experimental Agriculture International* **43** (12): 20-28.

Kobraee, S., Noor Mohamadi, G., HeidariSharifabad, H., DarvishKajori, F. and Delkhosh, B. (2011) Influence of micronutrient fertilizer on soybean nutrient composition. *Indian Journal of Science and Technology* **4** (7): 763-769.

Moosavi, A.A. and Ronaghi, A. (2011) Influence of foliar and soil applications of iron and manganese on soybean dry matter yield and iron-manganese relationship in a calcareous soil. *Australian Journal of Crop Science* **5**: 1550-1556.

Mundra, S.L. and Bhati, D.S. (1991) Effect of iron, manganese and Rhizobium inoculation on nutrient content and uptake by cowpea (*Vigna unguiculata*). *Indian Journal of Agronomy* **36**: 294-296.

Pal, V., Singh, G. and Dhaliwal, S.S. (2019) Agronomic biofortification of chickpea with zinc and iron through application of zinc and urea. *Communications in Soil*

Science and Plant Analysis **50** (15): 1864-1877.

Prasad, R. (2006) Zinc in soils and in plants, human & animal nutrition. Indian Journal of Fertilizers **2(**9): 103-119

Rai, S., Singh, P.K., Mankotia, S., Swain, J and Satbhai, S.B. (2021) Iron homeostasis in plants and its crosstalk with copper, zinc, and manganese. *Plant Stress* **1**:100008.

Rengel, Z. and Graham, R.D. (1996) Uptake of zinc from chelate-buffered nutrient solutions by wheat genotypes differing in zinc efficiency. *Journal of Experimental Botany* **47**: 217-219.

Shukla, A.K., Behera, S.K., Prakash, C., Tripathi, A., Patra, A.K., Dwivedi, B.S., Trivedi, V., Rao, C.S., Chaudhari, S.K., Das, S. and Singh, A.K. (2021) Deficiency of phyto-available sulphur, zinc, boron, iron, copper and manganese in soils of India. *Scientific reports* **11** (1):19760.

Singh, M., Chaudhary, S.R., Sharma, S.R. and Rathore, M.S. (2004) Effect of some micronutrients on content and uptake by chickpea (*Cicer arietinum*). *Agriculture Science Digest* **24** (4): 268-270.

Soni, J. and Kushwaha, H.S. (2020) Effect of foliar spray of zinc and iron on productivity of mungbean (*Vigna radiata* (L.) Wilczeck). *Journal of Pharmacognosy and Phytochemistry* **9** (1): 108-111.

Stoltzfus, R.J. and Dreyfuss, M.L. (1998) Guidelines for the use of iron supplements to prevent and treat iron deficiency Anemia. *International Nutritional Anemia Consultative Group* (INACG). ILSI Press. Washington DC, USA.

Tabassum, S., Khamparia, R.S. and Singh, S. (2013) Effect of zinc and organic manures on yield attributes and yield of rice. *Bioinfolet-A Quarterly Journal of Life Sciences*. **10** (3a): 879-881.

Trivedi, A.K., Hemantaranjan, A. and Pandey, S.K. (2011) Iron and sulphur application may improve growth and yield of soybean. *Indian Journal of plant physiology* **16** (3/4): 309-313.

Umamaheswari, P. and Singh, C.P. (2002) Influence of irrigation levels and micronutrients (Fe and Zn) on yield and yield attributes of Rajmash (*Phaseolus vulgaris L*.). *Legume Research* **25** (2): 121-123.

Vaghar, M.S., Sayfzadeh, S., Zakerin, H.R., Kobraee, S. and Valadabadi, S.A. (2020) Foliar application of iron, zinc, and manganese nano-chelates improves physiological indicators and soybean yield under water deficit stress. *Journal of Plant Nutrition* **43** (18): 2740-2756.

Welch, R. M. and Graham, R.D. (2004) Breeding for micronutrients in staple food crops from a human nutrition perspective. *Journal of Experimental Botany* **55**: 353-364.

White, P.J. and Broadley, M.R. (2005) Biofortifying crops with essential mineral elements. *Trends in Plant Science* **10**: 586-592.

WHO, (2002) The World Health Report 2002; Reducing Risks, Promoting Healthy Life. World Health Organization, Geneva, Switzerland. 1-168.

Yadav, G. S., Dinesh, K. and Subhash, B. (2013) Enhancing iron density and uptake in grain and straw of aerobic rice through mulching and rhizo-foliar fertilization of iron. *Global Journal of Agricultural Research and Reviews* **8** (44): 5447-5454.