Indian Journal of Agricultural Sciences **83** (10): 1095–8, October 2013/Short Communication

Enrichment of greengram (*Vigna radiata*) genotypes with iron through ferti-fortification

P DEBROY[1], R P NARWAL[2], R S MALIK[3] and NARENDER[4]

CCS Haryana Agricultural University, Hisar, Haryana 125 004

Received : 24 March 2013; Revised accepted: 14 June 2013

Key words: Foliar application, Genotypes, Greengram, Iron fortification

Pulses are the important source of protein for human nutrition. Greengram is one of the most widely cultivated pulse crops in the country and is grown on about 3.44 m ha with the annual production of 1.4 mt along with the productivity of 406.98 kg/ha (Anonymous 2010). Iron deficiency is a common micronutrient problem and occurs mostly in calcareous soil. On calcareous soil with high pH, Fe is converted to highly insoluble Fe oxides and hydroxides, making Fe limiting for plant growth (Lindsay and Schwab 1982). Besides, in aerobic condition solubility of iron compound become so low that plant suffers from iron deficiency. Greengram [*Vigna radiata* (L.) Wilczek] when grown under micronutrient deficient soils suffers from yield loss. These deficiencies are causing not only yield loss but also leading to lower content of mineral elements in different plant parts and ultimately leads to the micronutrient malnutrition problems. There is a clear evidence of a high prevalence of iron deficiency anaemia in population of developing countries and, to a lesser extent, in the more industrialized countries of the world. Most critically affected are infants, school-age children, and women of reproductive age (Hurrell 1997). In India, severe iron anemia was also reported in 34% adolescent girls of Bikaner, Rajasthan and Gujarat (Seshadri 1998).

There are several approaches which can be used to increase the concentration of Fe in foods, including food nutrient fortification, supplementation programmes, conventional breeding and genetic engineering, to diagnose and manage the problem of micronutrient malnutrition. Biotechnology and plant breeding currently show efficiency of nutrient enrichment of grains with Fe, but these approaches are long term processes, costly and may be affected from very low chemical solubility of Fe in soils due to high pH (Sarkar and Wyn Jones 1982) and low organic matter content. However, agriculture is the primary source of intervention for all nutrients required for crops and human health. Fertilization is the key factor of nutrient management in agronomic approaches (ferti-fortification) to enhance crop yield and quality, as fertilization is one of the sustainable and low cost strategies to improve Fe content in edible portions of food crops. Besides we can also go for searching responsive legume cultivar which can accumulate and utilize higher Fe in edible plant part. In Haryana, till date no work has been done on agronomic biofortification in pulse crops. Therefore, the present investigation was planned to study the influence of greengram genotypes for their tolerance to Fe stress conditions and agronomic biofortification.

The experiment was conducted in greenhouse from July to October 2010 at CCS HAU, Hisar. The soil used in the experiments was collected from village Balsamand, District Hisar, a Fe-deficient area. The soil was air dried, ground and passed through 2 mm sieve. The basal soil sample was again mixed and stored in cloth bags for further analysis of relevant physico-chemical characteristics of soil and initial Fe content as shown in Table 1.

Table 1 Initial physico-chemical characteristics of experimental soil

Characteristics	Value
Sand	95%
Silt	3%
Clay	2%
Textural class	Sand
pH (1:2)	8.72
EC (dS/m)	0.52
Organic carbon (%)	0.12
Calcium carbonate (%)	Nil
CEC [c mol(p$^+$)/kg]	3.70
DTPA-Fe (mg/kg)	2.88

[1]Assistant Professor cum Junior Scientist (e mail: partha.slg09 @gmail.com), [2]Professor (e mail: rpnarwal@gmail.com), [3] Senior Soil Scientist (e mail: ranbirsinghmalik@gmail.com), [4]Ph D Scholar (e mail: narenderhisar@gmail.com), Department of Soil Science

Four kg thoroughly mixed soil was filled in each plastic pot placed in completely randomized way and replicated thrice in the screen house. Iron levels, i.e. control (Fe_0), 10 kg Fe/ha, 0.1 % Fe foliar and 10 kg Fe/ha + 1% Fe foliar were applied in respective pots along with recommended doses of N, P and K. Ten seeds of each of the seven genotypes of greengram (Asha, Basanti, Satya. MH 421, MH 318, MH 565 and Muskan) were sown. Thinning was done after one week and four uniform plants per pot were allowed to grow up to maturity. At maturity, grain and straw yield was recorded and samples of straw and seed were ground in a stainless steel grinder and digested with di-acid mixture (HNO_3 and $HClO_4$, 4:1). The Fe concentration in plant digests was determined by atomic absorption spectrophotometry (Jackson 1967) method. Iron content in the initial soil sample was measured by DTPA (Lindsay and Norvell 1978) method. Three replications were maintained. Based on percent yield responses to applied Fe, the greengram genotypes were arranged in order of their efficiency to accumulate iron in grain.

$$\text{Iron response} = \frac{\text{Fe concentration in grain at optimum level of Fe}}{\text{Fe concentration in grain in control}}$$

Effect of Fe application on straw yield

Data in Table 2 revealed that straw yield of all greengram genotypes increased significantly over control with each level of iron application. Combined application of soil + foliar increased straw yield by 43.3% over control. On the contrary, only foliar application of iron increased straw yield to 27.1% over control, being lowest as compared to other treatment. Highest straw yield (63.3 g/pot) was recorded in Satya genotype and lowest in MH 318. The increase in straw yield (27.1%) over control was low in the treatments that received only foliar application. However, foliar application at late growth stage attributed to less vigorous vegetative growth as compared to basal application of recommended dose. Secondly, the amount of nutrient applied through foliar may not be sufficient to meet the requirement of plants.

Table 2 Effect of iron application on straw yield (g/pot) of greengram genotypes

Genotype	Fe level (Kg/ha)		Foliar (0.1% Fe)	10 kg Fe/ha + Foliar (0.1% Fe)	Mean
	Control	10			
Asha	52.5	61.8	58.8	62.0	58.8
Basanti	45.2	58.7	55.7	62.1	55.4
Satya	49.3	72.6	58.4	72.8	63.3
MH 421	31.9	45.2	42	47.6	41.7
MH 318	30.5	48.6	38.2	48.9	41.6
MH 565	31.1	46.9	49.3	47.2	43.6
Muskan	37.9	56.8	51.9	58.4	51.3
Mean	39.8	55.8	50.6	57.0	

CD (P=0.05) Treatment = 4.4, Genotype = 5.1
Treatment × Genotype = NS

Table 3 Effect of iron application on seed yield (g/pot) of greengram genotypes

Genotype	Fe level (Kg/ha)		Foliar (0.1% Fe)	10 kg Fe/ha + Foliar (0.1% Fe)	Mean
	Control	10			
Asha	7.47	8.91	8.26	11.65	9.1
Basanti	6.52	8.60	7.85	9.44	8.1
Satya	7.14	10.56	8.24	11.77	9.4
MH 421	4.47	6.35	5.73	7.39	6.0
MH 318	5.51	6.72	6.22	7.74	6.5
MH 565	4.76	6.73	7.48	7.75	6.7
Muskan	5.21	7.22	7.48	8.12	7.0
Mean	5.9	7.9	7.3	9.1	

CD (P = 0.05) Treatment = 0.58, Genotype = 0.69
Treatment × Genotype = NS

Effect of Fe application on seed yield

Seed yield of all the greengram genotypes also increased significantly over control at each levels of iron application. As reflected from the results in Table 3, soil + foliar application of iron gave the highest seed yield (9.1 g/pot) as compared to other treatments. The lowest seed yield, among iron fertilized pots, was found 7.3 g/pot, where only foliar application of iron was made. There was a significant difference among genotypes. Satya gave the highest seed yield of 9.4 g/pot, while lowest seed yield of 6.0 g/pot was recorded in MH 421 genotype. Increase of seed yield might be due to the fact that Fe application under their stress environment provided better conditions for the pod formation and subsequently resulted an increase in number of seeds per pod. Highest seed yield was obtained with soil + foliar application due to more availability of iron to plant during growth period.

Straw iron concentration as affected by Fe application

Application of iron increased Fe concentration in straw significantly over control. The result shown in Table 4 revealed that maximum iron concentration (370.3 mg/kg) in straw was achieved under soil + foliar application method. Iron concentration in straw was lowest (223.9 mg/kg), among all the iron fertilized pots, where iron was applied @ 10 kg Fe/ha. Among genotypes, highest concentration (301.5 mg/kg) of iron in straw was found in Basanti, while lowest (256.0 mg/kg) was obtained in MH 421. Moreover, a significant interaction between treatments and genotypes was also found.

Seed iron concentration as affected by Fe application

The data in Table 5 revealed that basal application of iron @ 10 kg/ha was not able to increase iron concentration in seed significantly over control. However, application of iron through foliar and soil + foliar application were able to

Table 4 Effect of iron application on iron concentration (mg/kg) in straw of greengram genotypes

Genotype	Fe level (Kg/ha) Control	Fe level (Kg/ha) 10	Foliar (0.1% Fe)	10 kg Fe/ha + Foliar (0.1% Fe)	Mean
Asha	192	228.2	339	363.1	280.6
Basanti	203.9	234	372	396.1	301.5
Satya	193.3	206.2	360.5	386.5	286.6
MH 421	167.2	196.1	324.3	336.4	256.0
MH 318	204	229.2	352.2	380.2	291.4
MH 565	192.4	244.5	311.3	337.1	271.3
Muskan	190.7	228.8	370.2	392.9	295.7
Mean	191.9	223.9	347.1	370.3	

CD (P=0.05) Treatment = 8.3, Genotype = 9.8
Treatment × Genotype = 21.9

increase seed iron concentration significantly over control. The highest seed iron concentration (50.9 mg/kg) was achieved under soil + foliar application method. Among genotypes, MH 421 was found to contained highest iron concentration of 55.1 mg/kg in seed and lowest Fe concentration in seed was obtained in Asha variety of greengram. However, soil application of iron was unable to fortify grain because of fast conversion of Fe^{2+} into Fe^{3+} in alkaline well aerated condition. Moreover, the oxidized iron (Fe^{3+}) has a very low solubility at basic pH, resulting in limited uptake by plant roots as it cannot absorb by root cells (Lucena *et al.* 2007). In case of foliar application, iron concentration increased 1.8 folds in straw and 1.1 folds in grain which may be due to poor translocation of applied iron within the plant (Chen and Barak 1982). According to Rengel *et al.* (1999) and Frossard *et al*. (2000) foliar application might be the only available fertilization practice that increases the iron concentration in grain slightly. However, in the present study best treatment for seed and straw Fe enrichment was of its soil + foliar application method that resulted in 1.9 fold increases in iron concentration in straw and 1.3 fold in seed. Besides in iron deficient calcareous soil plant suffers initially Fe deficiency that reduce the foliage yield as well as their capacity to absorb Fe when applied in later stage as foliar spray.

Table 5 Effect of iron application on iron concentration (mg/kg) in seed of greengram genotypes

Genotype	Fe level (Kg/ha) Control	Fe level (Kg/ha) 10	Foliar (0.1% Fe)	10 kg Fe/ha + Foliar (0.1% Fe)	Mean
Asha	31.1	34.3	35.7	42.5	35.9
Basanti	42.2	43.3	47.1	53.3	46.5
Satya	37.5	41.9	45.7	47.1	43.1
MH 421	48.2	50.4	57.5	64.1	55.1
MH 318	39.4	42.8	43.0	49.0	43.6
MH 565	37	38.2	42.6	51.0	42.2
Muskan	33.1	35	40.9	49.2	39.6
Mean	38.4	40.8	44.6	50.9	

CD (P=0.05) Treatment = 4.5 Genotype = 5.4
Treatment × Genotype = NS

Straw and seed Fe uptake

All the levels of Fe application increased its uptake in seed and straw of greengram genotypes significantly over control. The result shown in Fig 1 revealed that soil + foliar application of iron gave the highest Fe straw uptake of 23.6 mg/pot as compared to rest of the treatments. The lowest uptake of iron (12.5 mg/pot) in straw was found where Fe has been applied @ 10 kg/ha. Significant differences in uptake of zinc in straw among different genotypes were found. Maximum iron uptake in straw (18.9 mg/pot) was obtained in Satya and lowest (11.8 mg/pot) in MH 421 genotype.

Uptake of iron in seed of greengram genotypes also increased significantly over control. Soil + foliar applied iron gave the highest seed uptake of 0.46 mg/pot compared to other treatments. The lowest uptake of iron (0.32 mg/pot) in seed was found where iron was applied @10 kg/ha as basal and in the treatment where only foliar application of iron was given. A significant difference in uptake of seed iron among genotypes was found. However, no significant interaction among treatments and genotypes was obtained. Highest iron uptake in seed (0.44 mg/pot) was found in seeds of Satya and lowest (0.29 mg/pot) in seeds of Muskan.

Seed crude protein content

All the levels and methods of Fe application significantly increased crude protein content in seed over its control. Highest crude protein content in seed (26.5 %) was recorded in soil + foliar application. The possible explanation for increasing crude protein content is that iron is involved in protein metabolism through several enzyme systems (Singh and Badhoria 1984, Zhang *et al.* 2009).

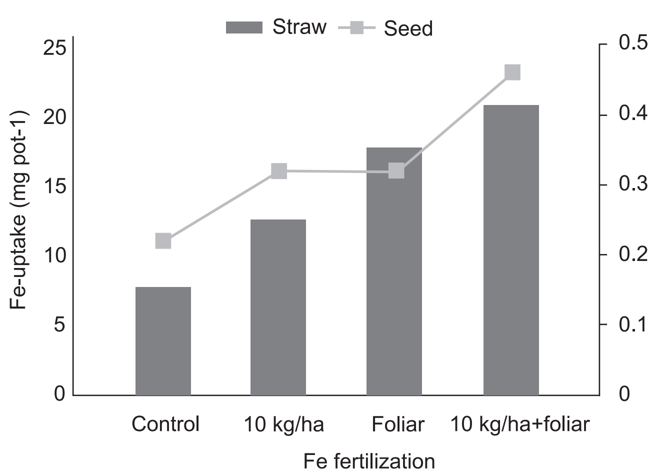

Fig.1 Effect of iron application methods on its uptake in straw and seed of greengram genotypes

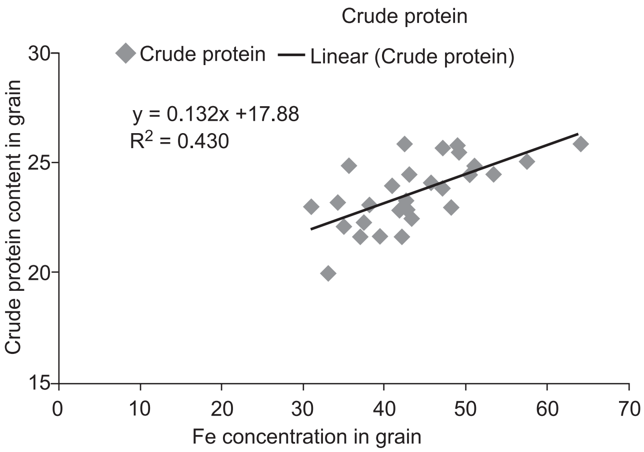

Fig. 2 Relationship between Fe concentration and crude protein (%) content of greengram seed

Fe response to greengram genotypes

Of the seven greengram cultivars tested, Fe response varied from 1.25 to 1.48 fold with a mean value of 1.33 for Fe application in soil plus foliar mode and from 1.09 to 1.21 fold with a mean value of 1.16 for foliar application of iron. Among different genotypes, Fe response was higher in Muskan followed by MH 565 and least response was obtained by MH 318 genotype with soil+foliar application of Fe. The higher Fe responsive cultivar for foliar application was also in Muskan followed by Satya and the least responsive was again MH 318. From this study it can be concluded that combined application of soil + foliar was more effective in increasing yield, grain Fe content and crude protein of all the greengram genotypes as compared to foliar application as the soil was iron deficient. If the experiment conducted in a iron sufficient soil condition may be foliar application alone give comparable result with soil+foliar application. To find the genotype that respond well for foliar and also for soil plus foliar application is prime target of the study. Among seven genotypes Muskan was the best performer genotype. So, proper fertilization along with responsive cultivar selection may not be sole but supplementary technique along with other methods of iron fortification.

SUMMARY

Iron enrichment in seven greengram [*Vigna radiata* (L) Wilczek] genotypes, viz Asha, Basanti, Satya, MH 421, MH 318, MH 565 and Muskan were studied in the screen house experiment. Iron through ferrous sulphate ($FeSO_4$) was applied @ 0, 10 kg Fe/ha, 0.1% Fe foliar application, 10 kg Fe/ha + 0.1 % Fe spray, respectively. Among all the treatments and methods of iron application, Fe enrichment of greengram was found maximum when applied through 10 kg Fe/ha + 0.1 % Fe foliar spray. Irrespective of greengram genotypes, the increase in the seed and straw Fe concentration was found to increase to the tune of 1.3 and 1.9 fold over control respectively. Of the seven greengram genotypes, Muskan gave best response to iron application when applied either through foliar or soil plus foliar.

REFERENCES

Anonymous. 2010. Area, production and productivity of moong bean in India. Directorate of Pulses development (DPD), Bhopal; Department of Agriculture and Co-operation; Ministry of Agriculture, Government of India, p 33.

Chen Y and Barak P. 1982. Iron nutrition of plants in calcareous soils. *Advance in Agronomy* **35**: 217–40.

Frossard E, Bucher M, Machler F, Mozafar A and Hurrell R. 2000. Potential for increasing the content and bioavailability of Fe, Zn and Ca in plants for human nutrition. *Journal of the Science of Food and Agriculture* **80**: 861–9.

Hurrell R F. 1997. Preventing iron deficiency through food fortification. *Nutrition Reviews* **55**: 210–22.

Jackson M L. 1967. *Soil Chemical Analysis*, p 498. Prentice Hall of India Pvt Ltd, New Delhi.

Lindsay W L and Norwell W A. 1978. Development of a DTPA soil test for zinc, iron, manganese and copper. *Soil Science Society of America Journal* **42**: 421–8.

Lindsay W L and Schwab A P. 1982. The chemistry of iron in soils and its availability in plants. *Journal of Plant Nutrition.* **5**: 821–40.

Lucena C, Romera F J, Rojas C L, Garcia M J, Alcantara E and Perez-Vicente R. 2007. Bicarbonate blocks the expression of several genes involved in the physiological responses to Fe deficiency of Strategy I plants. *Functional Plant Biology* **34**: 1002–9.

Rengel Z, batten G D and Crowley D E. 1999. Agronomic approaches for improving the micronutrient density in edible portions of field crops. *Field Crop Research.* **60**: 27–40.

Sarkar A N and Wyn Jones R G. 1982. Effect of rhizosphere pH on the availability and uptake of Fe, Mn and Zn. *Plant and Soil* **66**: 361–72.

Seshadri S. 1998. Oral iron supplementation to control iron anaemia in adolescent girls: *(In) Community trials of effectiveness of daily vs. weekly supplementation.* UNICEF Project of Department of Foods and Nutrition/WHO Collaborating Centre for Anaemia Control, Maharaja Sayajirao University, Baroda, Gujarat, p 26.

Singh B and Badhoria B S. 1984. Response of green gram to potassium and zinc application. *Journal of Agricultural Science* **102**: 253–5.

Zhang J, Wang M Y and Wu L H. 2009. Can foliar iron-containing solutions be a potential strategy to enrich iron concentration of rice grains (*Oryza sativa* L.). *Acta Agriculturae Scandinavica. Section B - Soil and Plant Science.* **59**: 389–94.