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Enrichment of Iron and Zinc Content in Pigeonpea Genotypes through Agronomic Biofortification to Mitigate Malnutrition

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A B S T R A C T

Iron (Fe) and Zinc (Zn) deficiencies are well-documented public health issue and an important soil constraint to crop production. Fe and Zn biofortification, which aims to enhance Fe and Zn concentration as well as bioavailability of pigeonpea grain, is considered as the more sustainable and economical solution to address human micronutrient deficiency. Generally, there is a close geographical overlap between soil deficiency and human deficiency of Zn and Fe, indicating a high requirement for increasing concentrations of micronutrients in food crops. Fertilizer strategy (agronomic biofortification) is one of the most cost-effective strategies to address the problem. Little research has been completed on the role of micronutrient fertilizers in increasing the micronutrient density of grain, suggesting that where fertilizers are available, making full use of fertilizers can provide an immediate and effective option to increase grain micronutrient concentration and productivity in particular. Field study was conducted to evaluate the effect of micronutrient fertilization on increasing iron and zinc content of pigeonpea genotypes. A total of 64 pigeonpea genotypes were sowed Lattice Design with three replications. Each genotype was sown in 6 rows of 3 meter length with a spacing of 90 cm and 30 cm between the rows and plants respectively. Results revealed that, in control plot the iron and zinc content was ranged from 23.67 to 79.48 ppm and 6.14 to 38.07 ppm, respectively. Foliar application of iron @ 0.5 % and zinc @ 0.5 % effectively improved the iron and zinc content in pigeonpea genotypes. However, among the genotypes, ICPL 96061 in both the years for iron and GRG-160 Zn showed good response to foliar application. Foliar application of iron and zinc offer a practical and useful option to improve Fe and Zn content in pigeonpea genotypes.

Keywords

Iron, micronutrient, pigeonpea, zinc, seeds

Introduction

Pigeonpea [*Cajanus cajan* (L.) Millsp] is an important grain legume that has primarily originated in the Indian sub-continent. Pigeonpea a major source of protein for several resource poor rural and urban families of Asia, Africa. Being a pulse crop, pigeonpea enriches soil through symbiotic nitrogen fixation, releases soil-bound phosphorous, recycles the soil nutrients, adds organic matter and other nutrients that make pigeonpea an ideal crop for sustainable agriculture (Saxena, 2008). Besides, it is mainly used as dry dehulled splits, its tender green seeds and pods are used as vegetable. Its dry crushed seeds have high protein content (20-25%) which act as major source of protein for vegetarian people, it is also used in preparation of sweet dishes, baby foods and its leaves are used as fodder to animals while, the dry stems make quality fuel wood.

More than 85 per cent of the world's pigeonpea is produced and consumed in India. It is commonly known as redgram or arhar or tur or thogari. The world acreage of pigeonpea is 4.86 m ha with an annual production of 4.1 mt and productivity of India is the largest producer and consumer of pigeonpea with an annual production of 3.29 mt in an area of 3.88 m ha with a productivity of 849 kg per ha.

Green revolution has helped in increasing the food production, thereby greatly reduced starvation, calories and protein malnutrition. However, this caused greater depletion of micronutrient reserve in soil and thereby accentuated wide spread deficiencies of micronutrients. Micronutrient deficiency is the fifth major global challenge to human health. The iron and zinc deficiency leads to crop yield losses and human health problem. Iron and zinc deficiency the most common and widespread, afflicting more than half of the human population (WHO, 2002; White and Broadley, 2009). More than 2 billion people suffer from iron deficiency alone, and the estimates of zinc deficiency are also close (Thacher *et al.*, 2006). Deficiency of iron and zinc, also known as 'Hidden hunger', results in poor growth and compromised psychomotor development of children, reduced immunity, fatigue, irritability, weakness, hair loss, wasting of muscles, sterility, morbidity and even death in acute cases (Stein, 2010).

The recommended daily allowance (RDA) of 13 mg per day (children), 17 mg per day (adult) for iron and 7 mg per day (children), 12 mg per day (adult) for zinc is necessary. but in India mostly people have monotonous diets which are deficient in micronutrients, iron and zinc (Anon., 2009). In India, consumption of pigeonpea as food provides daily per capita iron content (14.93 mg) which is less than RDA.

Currently, there is growing concern to address micronutrient malnutrition through different interventions. Typically, these interventions are categorized into 4 major groups: pharmaceutical supplementation, industrial fortification, dietary diversification, and biofortification (Meenaski *et al.*, 2007). Recently new approach called biofortification has been developed for alleviating malnutrition problem. Biofortification strategy involves developing crop varieties with superior nutrient qualities and it includes both increasing nutrient levels in the edible parts of fruit crops as well as their bioavailability.

Biofortification can be achieved by two approaches; conventional breeding and Agronomic biofortication. In conventional breeding method, locally adopted high yielding varieties are cross breed with the variety with naturally rich in nutrients to produce high yielding and nutritious plants. Agronomic biofortication involves application of micronutrients, iron and zinc to the soil or through foliar feeding.

Application of iron and zinc fertilizers to soil and/or foliar seems to be a practical approach for improving grain Fe and Zn concentration (*e.g.* agronomic biofortification). The micronutrient fertilizer strategy represents an important complementary approach to on-going breeding programmes for developing new genotypes with high iron and zinc density in seeds. As described by Cakmak (2008), biofortification of cereal grains through use of iron and zinc fertilizers is required for i)

Keeping sufficient amount of available Fe and Zn in soil solution, ii) Maintaining adequate Zn transport to the seeds during reproductive growth stage and iii) Optimizing the success of biofortification of staple food crops with Fe and Zn through use of breeding tools. In the past, numerous studies have been published on the role of soil and foliar-applied Fe and Zn fertilizers in order to correct Fe and Zn deficiency and increase yield. However, there are only few studies that investigated the effects of Fe and Zn fertilizers on grain Fe and Zn concentrations (or in other edible parts). Hence, the present study was planned and executed to enrich the pigeonpea seeds with iron and zinc through foliar feeding of iron and zinc.

Materials and Methods

Field location

A field experiment was conducted during *kharif*, 2014 and 2015 at Main Agricultural Research Station, Raichur. Raichur is situated on 16°12' North latitude, 77°20' East longitude and at an elevation of 389 meters above mean sea level and is located in North Eastern Dry Zone of Karnataka and laboratory studies were carried out in the Department of Seed Science and Technology, College of Agriculture, UAS, Raichur.

The experiment was laid out on deep block soil with optimum pH (7.27) and electrical conductivity was normal (0.42 dsm^{-1}). The nitrogen content in the soil was low (137.98 kg ha^{-1}), whereas the phosphorous was high (17.06 kg ha^{-1}) and the potash was medium (60.34 kg ha^{-1}). The organic carbon content was high (16.3 %) besides, zinc (0.39 ppm) content found to be slightly below the normal and iron (4.14 ppm) content was sufficient.

Experimental design and treatment

The experiment was laid out in two consecutive years and total of 64 pigeonpea genotypes were sowed Lattice Design with three replications. Each genotype was sown in 6 rows of 3 meter length with a spacing of 90 cm and 30 cm between the rows and plants respectively.

The foliar spray of 0.5 per cent FeSO4 and 0.5 per cent ZnSO4 were applied at pod setting stage to assess the influence of micronutrient spray on iron and zinc content in pigeonpea genotypes.

Estimation of Iron and Zinc content (ppm)

Processing of seeds

Before analyzing the pigeonpea seeds samples for iron and zinc estimation, the seeds of all the 64 genotypes were subjected to grinding. Iron and zinc content of pigeonpea seeds samples was determined by using Atomic Absorption Spectrophotometer (AAS).

Procedure of digestion of pigeonpea seeds sample for iron and zinc estimation (Lindsay and Novell, 1978)

From each genotype 0.50 gram of pigeonpea seeds powder was taken in 100 ml conical flask and 10 ml of di-acid mixture (HNO_3: $HClO_4$=2:1) was added to it. Mixture was kept overnight at room temperature (2.00 PM to 11.00 AM). Then the conical flask was placed on sand bath at temperature 180~200 °C for one to two hours. After a few minutes brown fume was evolved. This indicated the starting of digestion process. Finally white fume was seen by clearing the solution. At the bottom of the conical flask about 2-3 ml solution was noticed. After that, heating was stopped and the digested sample was cooled for 20 minutes. Then about 20~30 ml distilled water was added to each conical flask and the solution was filtered into a 50 ml volumetric flask and volume was made up to the mark (50 ml) by adding distilled water. The 50 ml solution was then transferred into a plastic bottle for each genotype for the further use.

Iron and Zinc content determination by AAS

It is based on the principle that atoms of iron and zinc which is normally remain in ground state, under flame condition absorb energy when subjected to radiation is proportional to the specific wavelength. The absorption of radiation is proportional to the concentration of iron and zinc. Iron and zinc content was estimated in the aliquot of seed extract by using Atomic Absorption Spectrophotometer (AAS) at 248.33 nm and 213 nm, respectively. The iron and zinc content were expressed in ppm.

Statistical analysis

Three plants were selected randomly and data on individual mean for each trait was subjected to statistical analysis. The data was analyzed using MSTAT-C software programme. For the analysis of the data the following statistical methods were employed. Critical differences were calculated at 1 and 5 per cent level wherever 'F' test was significant.

Results and Discussion

Fe and Zn biofortification, which aims to enhance Fe and Zn concentration as well as bioavailability of pigeonpea grain, is considered as the more sustainable and economical solution to address human micronutrient deficiency. Genetic biofortification and agronomic biofortification are the two important agricultural tools to improve pigeonpea grain Fe and Zn concentration. However, yield factor, interactions between genotype and environment, lack of sufficient genetic diversity in current cultivars for breeding programme, consumer resistance and safety of genetically modified crops are the main bottlenecks of genetic biofortification. The traditional and efficient strategy of agronomic biofortification, such as Fe and Zn fertilization is, therefore, urgent, essential and rapid solution for improving Fe and Zn concentration in pigeonpea grain to address the ongoing micronutrient deficiency in human beings.

Two methods, including soil amendment and foliar application of Fe and Zn have been extensively used (Cakmak, 2008). Since it has the advantages of low application rates and avoiding losses through soil fixation (Nasri *et al.*, 2011).

Furthermore, foliar applied Fe and Zn caused greater increase in mungbean Fe and Zn concentration than soil application (Sohrabi *et al.,* 2012).

In control plots (without foliar application) wide differences were observed among the genotypes for iron content as indicated by the range value of 23.25 to 80.1 ppm, 24.08 to 80.27 ppm and 23.67 to 79.48 ppm with mean performance of 59.70, 60.30 and 59.99 in 2014, 2015 and pooled mean, respectively.

Similarly, the zinc content of pigeonpea seed varied widely from 5.91 to 37.92, 6.37 to 38.22 and 6.14 to 38.07 ppm with mean performance of 19.95, 20.37 and 20.16 ppm in 2014, 2015 and in pooled mean, respectively (Table 1) (only pooled mean data provided).

Table.1 Iron and zinc content (ppm) of pigeonpea genotypes as influenced by micronutrient spray of Fe @ 0.5 % and Zn @ 0.5 %

Genotypes	Iron (Control)	Iron (Spray)	Zinc (Control)	Zinc (Spray)
NTL-900	42.70	52.60	7.30	12.85
GRG-333	38.54	60.08	6.14	11.86
GRG-2009-1	50.63	57.45	11.00	15.18
GRGB-131	41.41	61.64	16.96	24.83
GRGC-132	50.41	70.13	7.95	15.55
GRG-8793	55.05	64.45	12.40	17.40
AKTE-11-1	43.56	55.18	19.44	25.43
RVKT-261	37.92	53.88	15.33	24.43
IPPF-5M5	67.67	76.62	10.33	16.91
GRG-82	51.88	73.10	7.45	14.43
IPPF-V34	67.99	80.86	31.88	35.96
GRG-2009	75.90	81.52	25.00	30.47
GPHR-08-11	76.30	81.57	27.70	33.69
GRG-13-11	74.92	78.96	33.96	37.62
ICP-11320	72.11	79.83	23.51	34.49
AKT-9913	43.81	61.22	21.00	27.69
BDN-2008-12	70.50	78.63	25.44	32.04
RVK-275	61.62	76.70	15.55	24.22
BDN-2008-1	66.04	74.14	17.37	23.69
TDRG-33	23.67	31.32	25.72	31.81
PT-04-307	60.64	71.57	18.08	26.25
GRG-K1	44.71	57.23	30.77	37.55
PRIL-B-136	65.12	77.21	16.17	23.74
PRIL-B-155	75.10	81.85	17.16	24.14
PRIL-B-165	71.12	81.71	16.94	24.22
AGL-1666	70.83	80.40	17.49	24.89
AGL-1919	64.06	71.71	18.31	24.70
AGL-2013	55.64	73.68	16.07	22.05
AGL-1643	64.43	77.54	10.46	18.05
MARUTI	66.62	76.58	11.88	20.31
WRP-1	57.41	73.17	13.43	19.14
TS-3R	60.51	71.20	12.39	18.54
RVKT-260	50.53	66.21	10.19	15.88
BRG-10-02	54.89	71.26	11.56	17.90
BRG-11-01	52.56	70.36	11.91	17.61
PT-257	63.14	73.14	13.20	17.51
SKNP-1005	38.95	57.71	23.64	29.75
WRGE-97	43.81	55.56	12.72	18.70
GRG-140	60.32	68.70	14.44	19.59
ICP-8793	48.10	62.93	11.89	17.04

ICP-11320	48.32	57.87	26.37	31.33
CORG-9701	73.45	81.54	24.18	30.20
ICP-16317	75.37	82.50	18.73	23.64
ICP-14944	71.26	81.49	16.02	24.31
ICP-14840	72.28	79.96	18.75	24.46
ICP-14471	46.86	61.56	17.48	25.66
AGL-1603	74.10	79.39	24.74	30.49
AGL-2249	42.33	56.98	30.94	35.81
AGL-1632	70.25	78.07	36.53	42.92
GRG-160	38.36	55.08	38.07	45.00
RVK-275-R-I	76.04	80.84	34.60	41.27
ICPL 14001	77.82	85.29	35.77	41.87
ICPL 99050	73.83	79.04	32.71	38.97
ICPL 20116	68.44	79.09	33.91	40.05
ICPL 20136	77.05	82.32	28.97	33.48
ICPL 96053	37.33	56.94	32.96	39.79
ICPL 96061	79.48	86.50	23.51	30.70
ICPL 99044	75.84	80.93	27.14	31.34
ICPL 85051	68.55	74.93	23.58	29.71
GRPH-1	57.83	74.61	22.65	30.89
GRPH-2	69.45	77.53	34.70	39.48
MARUTI	66.64	76.58	11.85	20.31
WRP-1	57.43	73.17	13.40	19.14
TS-3R	60.53	71.20	12.36	18.54
Mean	**59.99**		**20.16**	
Minimum	23.67		6.14	
Maximum	79.48		38.07	
S.Em±	1.15	1.28	0.87	1.00
CD @ 5%	3.22	3.59	2.44	2.81

Table.2 Influence of micronutrient spray of Fe @ 0.5 % on iron content of pigeonpea genotypes

Genotypes	**Pooled mean (C)**	**Pooled mean (S)**	**Percent increase over control**
ICP-14840	74.92	78.96	5.39
GNV-SW-L105	38.54	60.08	55.89
S.E.D	1.15	1.28	
CD @ 5%	**3.22**	**3.59**	

Table.3 Influence of micronutrient spray of Ze @ 0.5 % on zinc content of pigeonpea genotypes

Genotypes	Pooled mean (C)	Pooled mean (S)	Percent increase over control
GRG-13-11	33.96	37.62	10.78
GRGC-132	7.95	15.55	95.60
S.E.D	0.87	1.00	
CD @ 5 %	**2.44**	**2.81**	

An attempt has been made to enrich Fe and Zn content of pigeonpea through foliar spray of Fe @ 0.5 per cent and Zn @ 0.5 per cent at pod setting stage. Results revealed compared to control that, foliar application of Fe @ 0.5 per cent and Zn @ 0.5 per cent at the time of pod setting stage significantly increased Fe concentration of pigeonpea by 71.29 per cent and Zn by 26.52 per cent. Fe concentration in pigeonpea increased from 38.54 to 60.08 ppm and Zn from 38.07 to 45.00 ppm. Among the genotypes, ICPL 96061 in both the years for iron and GRG-160 Zn showed good response to foliar application. Several authors *viz*., Vijayata *et al*., (2013), Fang *et al*., (2008) and Wei *et al*., (2012) also observed good response to foliar application of Fe and Zn for enriching these two elements in rice and mungbean and also observed good response to foliar application of Fe and Zn for enriching these two elements in pigeonpea (Table 1, 2 and 3).

Increase in mineral element in grains through foliar application might be due to easy penetration of Fe and Zn through leaves either by transportation or via stomatal pathway. Moreover, time of foliar application may differentially influence the grain Fe and Zn concentration. It is now well established fact that, foliar application after flowering stage distinctly increases the grain Fe and Zn concentration. Plant growth and metabolism, seed morphology, as the tissue distribution of Fe in mungbean seeds is under genetic control (Ariza-Nieto *et al*., 2007 and Sohrabi *et al*., 2012). This result is in close agreement with Thalooth *et al*., (2006), Fang *et al*., (2008), Khoshgoftarmanesh *et al*., (2010) and Nasri *et al*., (2011), who observed that foliar application of either $ZnSO_4$ or Zn-chelates can increase grain Zn concentration in plants with adequate Zn mobility in the phloem. The response of grain Fe and Zn concentration to foliar fertilization is cultivar dependent. Similar study rice genotypes also differed greatly in their response to foliar fertilization in increasing grain Fe and Zn concentration (Wissuwa *et al*., 2008 and Wu *et al*., 2010).

The response of grain Fe and Zn concentration to foliar fertilization was cultivar dependent. Thus, impact of foliar Fe and Zn application on pod setting stage Fe and Zn can be maximized by selecting genotypes with higher ability in pod setting stage absorption and seed deposition of foliar spray.

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