J Bangladesh Agril Univ 20(4): 362-372, 2022 https://doi.org/10.5455/JBAU.120912

ISSN 1810-3030 (Print) 2408-8684 (Online)

Journal of Bangladesh Agricultural University

Journal home page: http://baures.bau.edu.bd/jbau

Research Article

Agronomic Approaches to Biofortify Iron in Tomato

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ARTICLE INFO

Article history
Received: 15 Oct 2022
Accepted: 06 Dec 2022
Published: 31 Dec 2022

Keywords
Agronomic approaches, Biofortification, Fe, Fertilizer, Tomato

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OPEN ACCESS

Abstract

The study was conducted to biofortify Fe in tomato fruit. Seven tomato varieties were tested in this study to screen out the potential variety for biofortification. Based on Fe concentrations of tomato fruit, BARI Tomato-14 was selected as test crop. Six different methods for Fe application viz. 100% as seedling priming, 50% in soil + 50% as foliar spray at seedling stage, 50% as seedling priming + 50% as foliar spray, 100% as foliar spray at seedling stage, 100% as foliar spray at flowering stage and 100% as foliar spray at fruiting stage @ 4 kg Fe ha^{-1}. Iron was applied for 3 times at 7 day interval at all growth stages. Experiments were laid out in a completely randomized design (CRD) with 3 replications. Different application methods of Fe significantly influenced the growth, yield contributing characteristics, yield, biochemical constituents, nutrient concentrations and their uptake by BARI Tomato-14. The highest values of most of the studied parameters including Fe content and uptake were recorded from the application of 50% Fe as seedling priming + 50% Fe as foliar spray. Significant and positive correlations among the growth and yield contributing parameters due to the application of Fe were observed. Interestingly the highest vitamin-C, protein, lycopene, N, K and Ca contents and uptake were observed in 50% as seedling priming + 50% as foliar spray of Fe @ 4 kg ha^{-1}. Except few most of the studied nutrients were negatively correlated with Fe. Iron was biofortified by 66.28 µg g^{-1} in BARI Tomato-14. Results suggest that application of 2 kg Fe ha^{-1} as seedling priming + 2 kg Fe ha^{-1} as foliar spray at seedling stage for 3 times at 7 days interval along with the recommended doses of NPK fertilizers in soil can be practiced for Fe biofortification in tomato.

Introduction

The malnutrition of minerals (Fe, Zn) and vitamin A are major food-related primary health problem among populations of the developing world including Bangladesh where there is an extremely reliance on cereal-based diets and limited access to fruits and vegetables. About 800 million people around the world suffer from hunger, but even more suffer from micronutrients malnutrition, also called "hidden hunger", particularly in the developing countries (WHO, 2006). One such approach to combat the issue of micronutrients malnutrition is through biofortification. Biofortification is a process of adding nutrients to food crops. Currently, agronomic, conventional, and transgenic biofortification are three common approaches. Agronomic biofortification can provide temporary micronutrients increase through fertilizers in a wide range of crops. Agronomic biofortification increasing Fe concentration during agricultural approaches is a widely applied strategy to reduce the prevalence of Fe deficiency problem in human populations (Aciksoz et al., 2014; Naz et al., 2015). Among the vegetables, tomato (*Lycopersicon esculentum* L.) is one of the most popular and highly consumed vegetables in the world (Osman et al., 2019). It has important nutrients and antioxidants which plays a significant role in human diet, especially for vegetarian diet. It is also a rich source of minerals and vitamins (Bhowmik et al., 2012*).* Anemia is one of the common health problems by the reason of Fe deficiency, especially about 40-45% of preschool-aged children are anemic, where more than half of the Fe in the human body is bound to hemoglobin (Grillet et al., 2014). The recommended human dietary Fe varies between 8-18 mg day^{-1} depending on the age, body weight, gender and pregnancy (Anonymous, 2019). Iron malnutrition due to its inadequate presence in daily diet (Clemens, 2014) and deficiencies of this nutrition is a reason of serious health problem on world population especially in developing countries like Bangladesh (Zou

Cite This Article
Rabbi, R.H.M., Chowdhury, M.A.H. and Saha, B.K. 2022. Agronomic Approaches to Biofortify Iron in Tomato. *Journal of Bangladesh Agricultural University*, 20(4): 362-372. https://doi.org/10.5455/JBAU.120912

et al., 2019). Although Fe is one of the most abundant metals in the earth's crust, its availability to plant roots is very low. Usually Fe remains as deficient in plant because of insoluble Fe (III) in soil for plant growth. Plants mainly acquire Fe from the rhizosphere (Morrissey and Guerinot, 2009). In soils that are aerobic or of higher pH, Fe is readily oxidized, and is predominately in the form of insoluble ferric oxides. At lower pH, the ferric Fe is freed from the oxide, and become more available for uptake by roots (Kobayashi et al., 2019). After entering the epidermis, Fe is likely bound by unknown chelators or chaperones, due to its potential reactivity. Fe moves symplastically through the interconnected cytoplasm of the root, perhaps diffusing along the concentration gradient. At the pericycle, Fe is effluxed into the xylem, and moves towards the shoot through the transpiration stream (Morrissey and Guerinot, 2009). Tomato usually contains low amount of Fe which cause dietary Fe deficiency for animals and humans. While Fe is required in small amounts, it is critical for sustaining plants and animals (including humans) in part due to its role on the structure and function of a wide range of macromolecules as many metabolic pathways are activated by iron, and it is a prosthetic group constituent of many enzymes (Rout and Sahoo, 2015). The micronutrient Fe is needed for optimum growth and yield of tomato (Olowolaju et al., 2021). Iron is also involved in photosynthesis and respiration, and can directly, or indirectly, affects crop performance (Krohling et al., 2016). Earlier several studies have reported that high and low concentrations of Fe positively affected growth and yield of tomato (Sakya and Sulandjari, 2019). Usually vegetables have low phytic acid and high ascorbate content as well as phenolic and carotenoids which increased the availability of Fe (Giordano et al., 2019). Fe also plays a role in nitrogen fixation, energy transfer and metabolism of plant. Application of fertilizers to increase the Fe in edible parts of vegetables are suggested by Prasad et al. (2015). Alshaal and El-Ramady (2017) stated that foliar application is the quickest and easy method of nutrient application for fortification of micronutrients (Fe, Zn, Cu etc.) in plants. Many studies reported that Fe application on both through leaves and soil had a positive effect on growth and yield (Sakya, 2019). Application of Fe fertilizer through leaves improve the efficiency of Fe in plants, because the distance and length of Fe transportation also affect the efficiency of Fe application. Moreover, the application through leaves is a more efficient in providing nutrients for plants than application through soil, particularly when soil conditions are not suitable for Fe availability (Vasconcelos and Grusak, 2014; Olowolaju et. al., 2021). Therefore, these studies were designed to find out a suitable tomato variety to biofortify Fe through different application methods of Fe fertilizer.

Materials and Methods

Two sequential experiments viz; i) screening of tomato variety ii) choosing the best application method to biofortify Fe in tomato, were conducted in the laboratory of Department of Agricultural Chemistry, Bangladesh Agricultural University (BAU) and at the farmer's field, Kewatkhali, Mymensingh Sadar, Bangladesh in two consecutive years (September 2019 to April 2021). The chemical analyses of soil and tomato fruits were done in the laboratory of the Department of Agricultural Chemistry, Department of Biochemistry and Professor Mohammad Hossain Central Laboratory, Bangladesh Agricultural University, Mymensingh.

Experimental location

Geographically the experimental site was located at 24'75'N Latitude and 90'50'E Longitude at an elevation of 18 m above the sea level. The site belongs to the Non calcareous Dark Floodplain soil under the Agro-ecological Zone of Old Brahmaputra Floodplain (AEZ-9) (FAO and UNDP, 1988). The climate of the experimental area was under the sub-tropical climate zone, which is characterized by moderate to high temperature, heavy rainfall, high humidity and relatively long day during *Kharif* (April to September) and scanty rainfall, low humidity, low temperature and short-day period during *Rabi* season (October to March). The prevailing meteorological information during the study period regarding average temperature (17.80-28.06 ^{0}C and 18.06-29.06 ^{0}C), highest rainfall (18 mm and 17.1 mm), relative humidity (71.97-83.58 % and 66.41-83.00 %) and sunshine (144.60-229.1 hrs and 134.3-229.10 hrs) respectively, in October 2019 to March 2020 and October 2020 to March 2021 have been presented in Supporting Information (SI) Table S1 & S2.

Experimental set up

The soil used in the experiments was collected from Genetics field laboratory of Bangladesh Agricultural University, Mymensingh. The surface soils of 0-15 cm depth were collected on 23 November 2019. The soil was analyzed primarily for its physical and chemical properties according to Olsen et al., 1954; Black, 1965; Jackson, 1973; Page et al., 1982; Ghosh et al., 1983; McLaren et al., 1984. The morphological, physical and chemical properties of the initial soil have been presented in Table 1. Each plastic pot (30 cm in height with 24.5 cm diameter at the top and 20 cm diameter at the bottom) was filled with 10 kg of processed soil leaving 2 cm empty from the top and labelled with proper tagging.

Table 1. Morphological, physical and chemical characteristics of the soil

A. Morphological characteristics	
Location	Bangladesh Agricultural University, Mymensingh
Soil tract	Old Brahmaputra Alluvium
Soil series	Sonatola
Topography	Medium high land
General soil type	Non-calcareous Dark Grey Deposits
Parent material	Brahmaputra River-borne Floodplain
Agro-ecological zone	AEZ-9 (Old Brahmaputra Alluvium)
Soil color	Dark grey
Drainage	Moderate
Flood level	Above flood level
B. Physical characteristics	**0-15 cm depth**
Textural class	Silt loam
Sand (%)	17.54
Silt (%)	61.81
Clay (%)	20.65
C. Chemical characteristics	**0-15 cm depth**
pH	6.18
Total N (%)	0.10
Organic carbon (%)	0.61
Organic matter (%)	1.06
Available soil P (mg kg^{-1} soil)	19.25
Exchangeable soil K (cmol $100g^{-1}$ soil)	0.16
Available S (mg kg^{-1})	8.36
Available Zn (mg kg^{-1})	0.94
Available B (mg kg^{-1})	0.26
Available Fe(mg kg^{-1})	25.9
Available Cu (mg kg^{-1})	4.58
Available Mn (mg kg^{-1})	6.35

Screening of tomato variety for Fe biofortification

Seven different high yielding, popular and commonly grown tomato varieties were selected for the screening study. The varieties were BARI Tomato-2, BARI Tomato-14, BARI Tomato-15, BARI Tomato-17, BINA Tomato-6, BINA Tomato-7 and BARI Hybrid Tomato-5. The seeds of these tomato varieties were collected from Bangladesh Agricultural Research Institute (BARI), Joydevpur, Gazipur, Bangladesh and Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, Bangladesh. These varieties were then tested in a pot trial experiment with three replications following Completely Randomized Design (CRD) at the net house of Department of Agricultural Chemistry, Bangladesh Agricultural University (BAU), in order to screen out the suitable variety for Fe biofortification. Proper care and ideal management for tomato cultivation was maintained till the final harvesting of fruits. The collected fruits of different varieties were then analyzed for Fe concentrations in the laboratory of Department of Agricultural Chemistry, BAU. The extracts of tomato fruits were prepared through wet oxidation method defined earlier by Singh et al. (1999). Whatman No. 1 filter paper was used to filter the extract before the chemical analyses. Iron concentration was determined using AAS method (Atomic Absorption Spectrophotometry) (Stammer and Mallarino, 2018). Among the varieties, the lowest concentration of Fe was found in BARI Tomato-14. Therefore, this variety was selected for the Fe biofortification.

Test crop

The seeds of BARI Tomato-14 were collected from Horticulture Division, BARI. This variety is usually cultivated as a winter variety, which is high yielding and disease resistant. It requires 60 to 70 days to mature. It can be grown in any types of soil, but sandy loam is the best for proper growth and development of plant.

Effects of different Fe application methods on BARI Tomato-14

Six different strategies of Fe application viz. 100% Fe as seedling priming, 50% Fe in soil + 50% as foliar spray, 50% Fe as seedling priming + 50% as foliar spray, 100% Fe as foliar spray at seedling stage, 100% Fe as foliar spray at flowering stage and 100% Fe as foliar spray at fruiting stage @ 4 kg ha^{-1} for 3 times at 7 days interval were applied. The collected soils from 0-15 cm depth were pulverized and inert materials, visible insect pest residues were removed. Iron was applied as $FeSO_4.7H_2O$. Every plot received an equal amount of other nutrients like, 120 kg N ha^{-1}, 100 kg P ha^{-1}, 90 kg K ha^{-1} and 30 kg S ha^{-1}, respectively from urea, triple super phosphate, muriate of potash and gypsum. Well decomposed cow dung was applied @ 3 ton ha^{-1} and mixed uniformly with soil at 7 days before seedling transplanting. Urea was applied in two different instalments, one half at the time of pot preparation and rest at 30 days after transplanting. Healthy and uniform sized 30 days old seedlings were uprooted separately from the seed bed to minimize damage to the roots. Three seedlings were transplanted in each experimental pots in the afternoon. Intercultural operations were performed as and when necessary, throughout the growth period of the crop. The plants were supported by bamboo sticks to prevent lodging. Weeding and irrigation were done as and when necessary. Pot-wise growth, yield, biochemical and chemical constituents were analyzed.

Determination of biochemical and mineral nutrients of BARI Tomato-14 fruit

Fresh fruit samples from every pot were chemically analyzed for biochemical components viz; vitamin C, protein and lycopene content according to (Ranganna, 1994; Sharma and Le Maguer, 1996). Plant and fruit extracts were prepared by wet oxidation method as described by Singh et al. (1999). The extract was filtered through filter paper (Whatman No. 1) before the chemical analyses. The extract was used for the analysis of P, K, Ca, Mg, S, Zn, Fe, Cu and Mn contents following standard methods (Page et al., 1982; Tandon, 1995; Nielsen, 2010; Paul et al., 2017; Stammer and Mallarino, 2018). The uptake of nutrients was calculated from its concentrations multiplied by fruit dry weight using the following formula of Sultana et al. (2021).

$$\text{Uptake (mg pot}^{-1}\text{)} = \frac{\text{Nutrient concentration (\%)}}{100} \times \text{Fruit dry weight (mg pot}^{-1}\text{)}$$

Statistical analyses

Collected data on the plant growth, yield attributes, yield, biochemical and nutrient concentrations were tabulated and analyzed using statistical software Minitab 2017 Version 17.0 (Minitab Inc, USA). The means for all the treatments were calculated and analysis of variance (ANOVA) for all the parameters under consideration were performed and Tukey's range test to determine the significant difference among the treatments.

Results

Screening of different varieties of tomato for biofortification

The experimental data revealed in the Table 2, showed that the tomato varieties contained significantly different Fe in the tomato fruit. The range of Fe content varied from 43.75 to 62.35 µg g^{-1}. Where the highest Fe content (62.35 µg g^{-1}**)** was recorded in BINA Tomato-6 and the lowest Fe content (43.75 µg g^{-1}) was in BARI Tomato-14. Based on the Fe content data of the studied varieties, BARI Tomato-14 was selected for biofortification due to its very low Fe content.

Table 2. Iron concentration of the fruits of different tomato varieties

Varieties	Iron (µg g^{-1})
BARI Tomato-2	53.18c
BARI Tomato-14	43.75e
BARI Tomato-15	60.47ab
BARI Tomato-17	59.19ab
BINA Tomato-6	62.35a
BINA Tomato-7	58.55b
BARI Hybrid Tomato-5	49.21d
SE ±	0.81
Level of significance	**
CV (%)	4.89

SE± = Standard error of means, CV= Co-efficient of variation ** indicates significant at 1% level of probability

Effects of different Fe application methods on Fe biofortification in BARI Tomato-14

Growth and yield contributing characteristics

The result of the study showed that the plant height varied significantly among the different methods of Fe application and the advancement of growth period (Table S3). While, different methods of Fe application had a significant influence (except 15 DAT) on number of branches $plant^{-1}$ and number of leaves $plant^{-1}$ (Table S4 & S5). From the study, it was also perceived that different methods of Fe application had a significant influence (except 15 and 30 DAT) on number of clusters of flowers $plant^{-1}$ (Table S6). Moreover, the number of fruits $plant^{-1}$ was significantly influenced (except 15, 30 and 90 DAT) by the different methods of Fe application (Table S7). The plant height and number of branches $plant^{-1}$ increased slowly up to 30 DAT and then increased promptly up to 75 DAT and then remained almost same till the final harvest at 90 DAT (Figure 1A & 1B). The number of leaves $plant^{-1}$ was increased gradually with a similar pattern for all the Fe application strategies up to 90 DAT (Figure 1C). From the observations, it was clear that flowering started within one month of transplanting (Figure 1D). The number of cluster of flower $plant^{-1}$ and number of fruits $plant^{-1}$ was increased abruptly up to 75 DAT and then severely decreased (Figure 1D & 2A). The plant height, number of branches $plant^{-1}$ and number of leaves $plant^{-1}$ ranged from 61.31 to 88.25 cm, 5.66 to 7.66 and 29.33 to 43.66 at 90 DAT across the methods. The tallest plant (88.25 cm) and the highest number of branches $plant^{-1}$ (7.66) at 90 DAT was obtained from the 100% as foliar spray (at seedling stage) of Fe application method. The highest number of leaves plant^{-1} at 60, 75 and 90 DAT was recorded at 50% as seedling priming + 50% as foliar Fe application method. The highest number of flower cluster plant^{-1} (8.00) and the highest number of fruits plant^{-1} (16.0) at 75 DAT was observed in 50% as seedling priming + 50% as foliar Fe application method. The highest number of flower cluster plant^{-1} was statistically similar with all other Fe application methods except 50% Fe in soil + 50% Fe as foliar. On the other hand, the highest number of fruits plant^{-1} was statistically similar with all other Fe application methods. Whereas, the lowest plant height, number of branches plant^{-1} and number of leaves plant^{-1} were obtained from 100% foliar spray (Fruiting stage) at 90 DAT. On the other hand, the lowest number of flower cluster plant^{-1} and the lowest number of fruits plant^{-1} at 75 DAT were also recorded in 100% Fe as seedling priming.

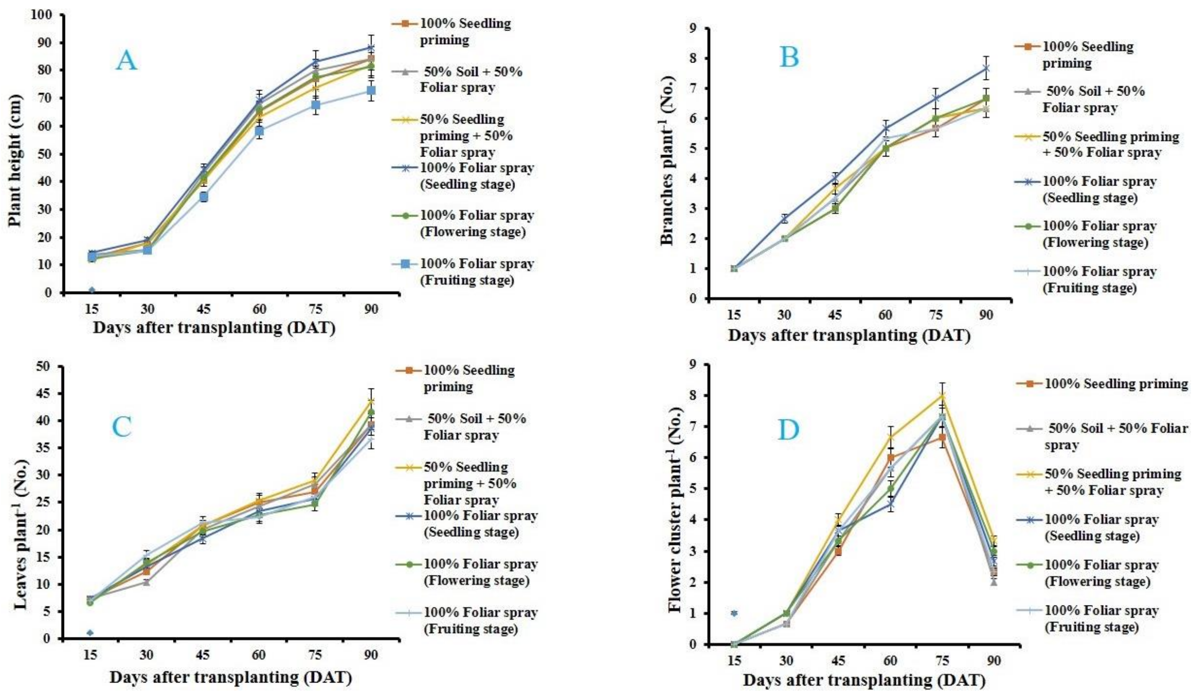

Figure 1. Effects of different Fe application methods on plant height (A), number of branches plant^{-1} (B), number of leaves plant^{-1} (C) and number of flower cluster plant^{-1} (D) of BARI Tomato-14

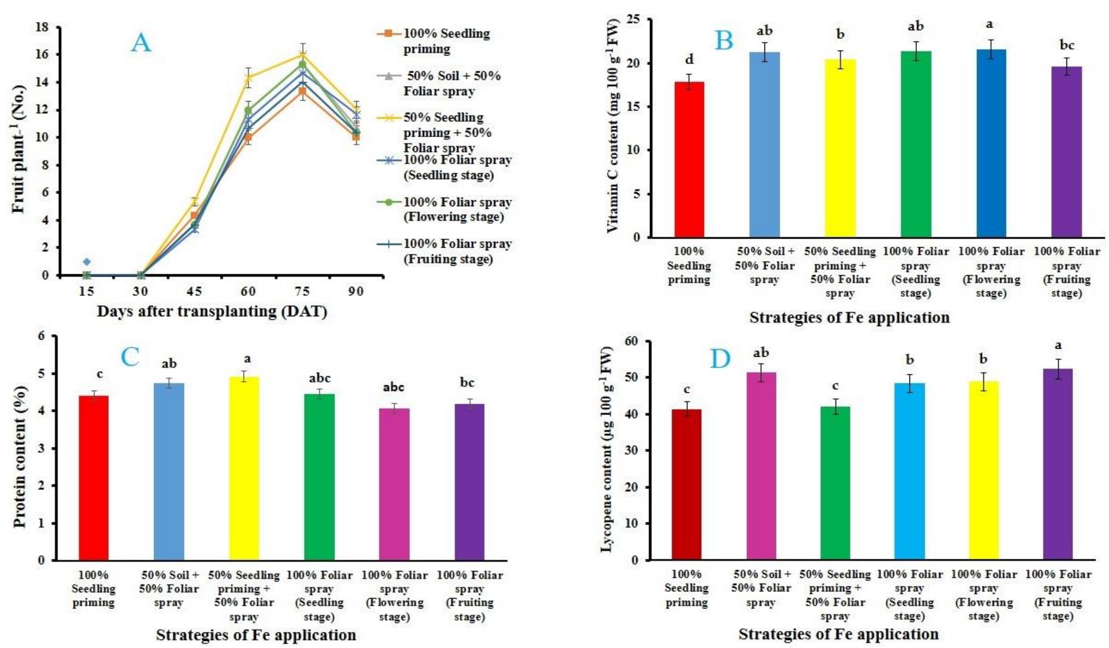

Figure 2. Effects of different Fe application methods on fruit plant^{-1} (A), vitamin-C (B), protein content (C) and lycopene content (C) in BARI Tomato-14

Fruit diameter and yield

Fruit diameter and fresh yield showed significant variation in response of different Fe application methods (Table 3). The highest fruit diameter (36.23 mm) and fruit fresh yield (1580.30 g) were found in 50% as seedling priming + 50% as foliar Fe application method and the lowest fruit diameter (27.50 mm) and fresh yield (1340.40 g) were recorded in 100% Fe as seedling priming.

Table 3. Effects of different Fe application methods on the fruit diameter (mm), fruit fresh weight (g pot^{-1}) and fruit dry weight (g pot^{-1}) by the fruits of BARI Tomato-14

Methods	Fruit diameter (mm)	Fruit fresh weight (g pot^{-1})	Fruit dry weight (g pot^{-1})
100% Seedling priming	29.38cd	1405.3d	70.28d
50% soil + 50% foliar	32.82b	1421.0cd	71.04cd
50% seedling priming + 50% foliar	36.23a	1580.3a	79.04a
100% foliar spray at seedling stage	33.80ab	1495.0b	74.75b
100% foliar spray at flowering stage	30.96bc	1475.3bc	73.77bc
100% foliar spray at fruiting stage	30.66bcd	1480.8bc	74.04b
Level of significance	**	**	**
SE±	1.53	29.69	1.38
CV (%)	5.96	2.50	2.33

SE± = Standard error of means; CV= Co-efficient of variation; ** indicates significant 1% level of probability

Fruit vitamin C, protein and lycopene content

The results presented in figure 2B-2D showed that vitamin C, protein and lycopene content were significantly influenced with different methods of Fe application. The range of the Vitamin C content varied from 17.82 to 21.56 mg 100 g^{-1}. The highest vitamin C content (21.56 mg 100 g^{-1}) was found in 100% foliar spray at flowering stage of Fe application method. While the highest lycopene content (52.36 µg 100 g^{-1}) was recorded in 100% foliar spray at fruiting stage. On the other hand, the highest protein content (4.92%) was found in 50% as seedling priming + 50% as foliar Fe application method. Whereas, the lowest vitamin C (17.82 mg 100 g^{-1}), protein (4.06 %) and lycopene content (41.44 µg 100 g^{-1}) were found in 100% Fe as seedling priming.

Fruit dry matter yield

Different methods of Fe application found to employ a significant effect over the control showed in table 3 & figure 3. The maximum value (79.04 g pot^{-1}) of fruit dry matter was noted under 50% as seedling priming + 50% as foliar Fe application strategy whereas, the minimum value for fruit dry matter yield (67.02 g pot^{-1}) was noted under 100% Fe as seedling priming.

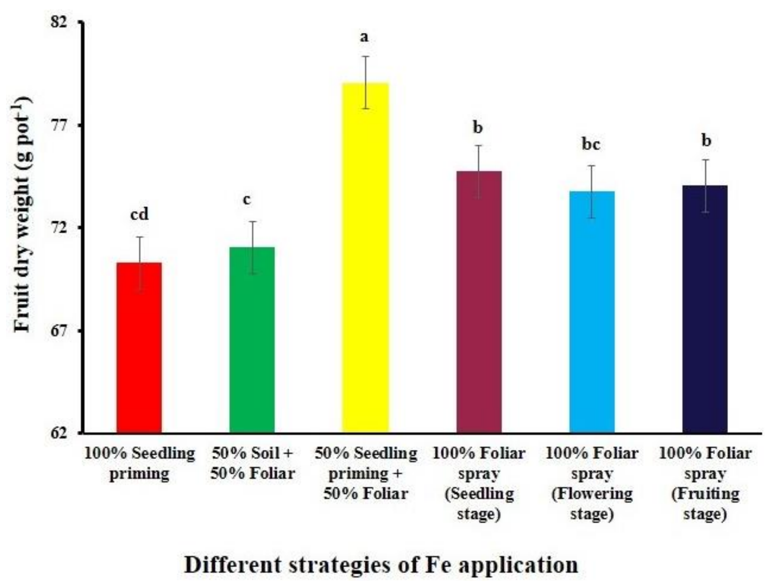

Figure 3. Effects of different Fe application methods on fruit dry weight of BARI Tomato-14

Fruit nutrient concentrations and uptake by BARI Tomato-14

The result of the study revealed that different Fe application methods significantly influenced the N, P, K, Ca, Mg, S, Zn and Mn concentrations and their uptake by BARI Tomato 14 (Table 4 & 5). The highest N, K and Ca concentrations and uptake by BARI Tomato-14 were observed in 50% as seedling priming + 50% as foliar Fe application method (Table 4). Where the highest N concentration (0.84%) was statistically similar with 50% in soil + 50% as foliar Fe application method. The highest K concentration (0.46%) was statistically similar with 100% foliar spray at flowering stage. The highest Ca concentration (0.41%) was statistically similar with 100% foliar spray at fruiting stage. On the other hand, the lowest N, K and Ca concentrations were observed in 100% foliar spray at flowering stage, 100% foliar spray at fruiting stage and 100% seedling priming, respectively. Moreover, the highest P, Mg, Zn, Mn and Cu concentrations and uptake by BARI Tomato 14 were observed in 100% seedling priming (Table 4 & 5). While, the lowest P, Mg, Zn, Mn and Cu concentrations and uptake by BARI Tomato 14 were observed in 50% in soil + 50% as foliar Fe application method (2 kg Fe ha^{-1} in soil and 2 kg Fe ha^{-1} as foliar spray) (Table 4). The highest S concentration (0.25%) and uptake (178.07 mg) were found at 50% in soil + 50% as foliar Fe application method (2 kg Fe ha^{-1} in soil and 2 kg Fe ha^{-1} as foliar spray) while the lowest S concentration (0.13%) and uptake (86.720 mg) were found in 50% as seedling priming + 50% as foliar Fe application method. The highest Zn concentration was statistically similar with 50% as seedling priming + 50% as foliar Fe application method and 100% as foliar spray at fruiting stage Fe application method.

Table 4. Effects of different strategies of Fe application on N, P, K, Ca, Mg and S concentration (%) and their uptake (mg pot^{-1}) by the fruit of BARI Tomato-14

Methods	Nitrogen		Phosphorus		Potassium		Calcium		Magnesium		Sulphur	
	Conc.	Uptake	Conc.	Uptake	Conc.	Uptake	Conc.	Uptake	Conc.	Uptake	Conc.	Uptake
100% Seedling priming	0.75abc	547.65c	0.49a	357.00a	0.43bc	362.13b	0.32de	252.07c	0.48a	357.79a	0.16cd	137.81b
50% Soil + 50% Foliar	0.81ab	626.65b	0.37d	241.13g	0.41bc	310.45d	0.36bcd	203.57d	0.31d	268.21f	0.25a	178.07a
50% Seedling priming + 50% Foliar	0.84a	686.55a	0.37d	286.27e	0.46a	388.48a	0.41a	296.16a	0.40c	344.08c	0.14d	107.44e
100% Foliar spray at seedling stage	0.75abc	525.95e	0.38d	275.27f	0.41bc	298.03e	0.37abc	265.21b	0.45bc	320.52d	0.19b	133.49bc
100% Foliar spray at flowering stage	0.69c	509.44f	0.44c	347.83bc	0.43ab	346.67c	0.33cde	260.89b	0.42c	304.11e	0.17bc	123.09d
100% Foliar spray at fruiting stage	0.71bc	534.06d	0.45bc	338.82cd	0.40c	308.71d	0.40ab	264.09b	0.34d	221.17g	0.17bc	128.40cd
Level of significance	**	**	**	**	**	**	**	**	**	**	**	**
SE±	0.31	2.06	0.01	2.63	0.01	2.75	0.02	3.18	0.02	3.91	0.01	2.55
CV (%)	8.73	0.46	4.97	1.00	3.56	1.05	6.97	1.57	6.61	1.54	8.38	2.44

SE± = Standard error of means; CV= Co-efficient of variation; ** indicates significant 1% level of probability

Iron concentration and uptake

Different methods of Fe application had a significant effect on Fe concentration and uptake by BARI tomato-14 showed in Table 5. The range of Fe concentration and uptake varied from 42.22 to 110.03 µg g^{-1} and 2.82 to 8.69 mg pot^{-1}, respectively. The highest Fe concentration (110.03 µg g^{-1}) and uptake (8.69 mg) by BARI tomato-14 fruit were observed in 50% as seedling priming + 50% as foliar Fe application method. The highest Fe concentration was statistically similar with all other Fe application methods except 100% Fe as seedling priming and 100% as foliar spray at seedling stage Fe application method. While the lowest Fe concentration (42.22 µg g^{-1}) and uptake (2.82 mg) were observed in 100% Fe as seedling priming.

Table 5. Effects of different methods of Fe application on Zn, Fe, Mn and Cu concentration (µg g^{-1}) and their uptake (mg pot^{-1}) by the fruit of BARI Tomato-14

Methods	Zinc		Iron		Manganese		Copper	
	Conc.	Uptake	Conc.	Uptake	Conc.	Uptake	Conc.	Uptake
100% Seedling priming	21.33a	1.59a	79.330b	5.65e	26.66a	0.94a	12.63	1.99a
50% Soil + 50% Foliar	13.27d	0.89f	104.61a	7.42c	20.33b	0.68e	10.19	1.36f
50% Seedling priming + 50% Foliar	20.15abc	1.48b	110.03a	8.69a	27.34a	0.80d	11.28	1.94bc
100% Foliar spray at seedling stage	19.26bc	1.34cd	80.460b	5.86d	24.05ab	0.82d	11.68	1.69e
100% Foliar spray at flowering stage	17.13c	1.27e	99.220a	7.42c	26.11a	0.91bc	12.42	1.92c
100% Foliar spray at fruiting stage	20.44abc	1.53ab	105.46a	7.81b	25.33ab	0.88c	11.95	1.88d
Level of significance	**	**	**	**	**	**	NS	**
SE±	1.73	0.02	6.75	0.05	2.69	0.03	1.68	0.02
CV (%)	11.03	2.04	9.31	0.84	12.99	2.78	17.10	1.78

SE±= Standard error of means, CV= Co-efficient of variation, ** indicates significant at 1% level of probability

Discussion

Selection of tomato varieties from screening

The result of the study depicted that among the different studied varieties, Fe content was the lowest in BARI Tomato-14. BARI Tomato-14 is one of the most popular variety to the marginal growers of Bangladesh. Moreover, the yield and disease resistance of this variety is comparatively good enough. Therefore, this BARI Tomato-14 variety was selected for Fe biofortification based on its disease resistance, popularity among the local growers and lower Fe content. An earlier study of Al-Mahmud et al. (2018) in rice showed consistent reason for variety selection.

Effects of different Fe application methods on Fe biofortification in BARI Tomato-14

Growth and yield contributing characteristics

Tomato plant grown in pot with different application methods of Fe responded differently regarding plant height, number of branches plant^{-1}, number of leaves plant^{-1}, number of flower cluster plant^{-1}, and number of fruits plant^{-1}, fruit diameter, fruit fresh weight and fruit dry weight. The highest plant height and number of branches plant^{-1} was obtained in 100% as foliar spray at seedling stage of Fe application method but the number of leaves plant^{-1}, number of flower cluster plant^{-1} and number of fruits plant^{-1} were obtained in 50% as seedling priming + 50% as foliar Fe application method. It was also observed that the average fruit diameter, fresh fruits weight and fruit dry weight were the highest in 50% as seedling priming + 50% as foliar Fe application method whereas, the lowest were observed in 100% Fe as seedling priming. The results indicated that 3 times Fe application through foliage at seedling stage @ 4 kg ha^{-1} and seedling priming @ 2 kg ha^{-1} with 3 times foliar Fe application @ 2 kg ha^{-1} resulted in better plant growth and yield contributing characteristics. The highest parameters in the above mentioned methods of Fe application might be due to the foliar application of Fe, which probably helped to achieve favourable results on growth parameters, yield components and yield quality of BARI tomato-14. As, it was reported that Fe has significant effect on the synthesis of growth regulators and also in chlorophyll synthesis, respiration, chloroplast development and therefore, improves the performance of photosystems (Rawashdeh and Florin, 2015). Moreover, application of Fe fertilizer through foliage might improve the efficiency of Fe in plants, because the distance and length of Fe transportation occurred also influence the efficiency of Fe application. Consequently, the Fe application through foliage is a more efficient method in providing nutrients for plants growth and development. Similar result was obtained in tomato by Olowolaju et al. (2021) and in soybean by Vasconcelos and Grusak, (2014). The highest diameter of tomato obtained in that mentioned method of Fe application might be due to the fact that, foliar application of Fe may resulted in rapid absorption of water and metabolites into fruit xylem and phloem. GutiéRrez-Ruelas et al. (2021) observed a similar result for Fe application.

Fruit yield of BARI Tomato-14

Yield of tomato fruit positively correlated with all other physical characteristics mentioned above having the correlation coefficients (r) of 0.9502**, 0.9327**, 0.9021**, 0.9508**, 0.9246**and 0.9077**, respectively. As the maximum number of fruits plant^{-1} and average fruit diameter plant^{-1} were found in 50% as seedling priming + 50% as foliar Fe application method, in the same way, the highest yield of tomato fruit was also obtained from 50% as seedling priming + 50% as foliar Fe application method and this might be due to availability of foliar sprayed Fe, which is an influential element in the oxidation process of plant that releases energy from sugars and starches and therefore, responsible for the yield of fruit (Eskandari, 2011; Havlin et al., 2014). On the other hand, priming the seedlings into the Fe solution overnight might exert better seedling performance and provides faster uptake of Fe which may accelerate the yield of BARI tomato-14. Reis et al., (2018) observed similar result in a previous study of seed priming on wheat. Moreover, foliar application might also ease the Fe-salt solutions taken up via the cuticle or stomata of leaves of plant thus, transportation of Fe in the plant part become easier to employ the beneficial effects of Fe on plant which ultimately offer higher yield. A quite similar result was obtained in a study of Sakya and Sulandjari, (2019).

Biochemical composition of BARI Tomato-14

Vitamin C, protein and lycopene contents are the most important biochemical components present in tomato. The result of the study reflected that, the highest vitamin C and protein content were obtained from 100% Fe as foliar spray at flowering stage and 50% as seedling priming + 50% as foliar spray Fe application method, respectively. On the other hand, the highest lycopene content was observed in 100% Fe as foliar spray at fruiting stage. This might be probably due to the fact, as Fe is an important co-factor of many enzymes, including those involved in the biosynthetic pathway of vitamin-C and lycopene. Therefore, the foliar application of Fe at both the flowering and fruiting stage might have positive effects on vitamin C and lycopene, respectively. Moreover, foliar application of Fe at flowering stage might helped to increase the activity of ascorbic acid oxidase enzyme which is responsible for vitamin C content in BARI tomato-14. Similar result were observed in the study of Batra et al. (2006). Savitha et al. (2010) conducted a study in chilli, where the best effect on vitamin C and protein content were observed through the foliar application of Fe. Furthermore, application of Fe through foliage increase the lycopene content compare to other treatment was also observed by a previous study (Wala et al., 2022).

Fruit nutrient contents and uptake of BARI Tomato-14

The experimental results outreached that Fe application with different application method to tomato plant greatly influenced different nutrient contents and uptake. The macro and micronutrients like N, P, K, Ca, Mg, S, Zn, Cu and Mn concentrations and uptake were observed in this study. From this study, the highest N, K, and Ca concentration and uptake in tomato fruit were observed at 50% as seedling priming + 50% as foliar Fe application method. The highest N, K, and Ca concentration might be due to the biological response of the tomato seedling kept in the Fe solution overnight as well as through foliage Fe application which probably positively influenced the uptake and translocation of these nutrient and thus increase concentration. Moreover, abundant available soil Fe might be present in the rhizosphere of plant which perhaps promotes root-to-shoot translocation and better distribution of these above mentioned nutrients in BARI tomato-14. On the hand, presence of sufficient Fe also might induce the uptake of these mentioned nutrients as Fe has direct positive effects on these nutrients uptakes on plant (Ezekiel et al., 2020). The highest P, Mg, Zn, Mn and Cu concentration and uptake in BARI tomato-14 fruits were observed in 100% Fe as seedling priming. This might be due to the reason of not being presence of sufficient amount of Fe in the rhizosphere as well as in the plant. Therefore, comparatively lower Fe uptake might be happened during the seedlings priming, as the lower concentration or absence of Fe enhance the uptake of these above mentioned nutrients. Similar antagonistic relationships were observed in some previous study (Kumari and Sharma, 2006; Wala et al., 2022). Furthermore, the lowest of these nutrients similarly might be present in the methods where the availability of Fe was highest. So therefore, the reason of being sufficient nutrients in the fruits of tomato depend on the presence or absence of Fe in the root zone as well as in the plant uptake system.

Iron concentration and uptake

The maximum Fe concentration and uptake in 50% as seedling priming + 50% as foliar Fe application method probably due to the overnight priming of BARI tomato-14 seedling to the Fe containing solution @ 2 kg ha^{-1} and foliar Fe application 3 times @ 2 kg ha^{-1} which might be facilitated the uptake of Fe nutrient in the plant root and thereby to the shoot at the very early stage of plant life. Moreover foliar spray of Fe to tomato plant also might exerted a direct uptake in the plant body. Similarly, in an earlier study (Guha et al., 2022) noticed that seed priming with Fe containing solution increase the grain Fe concentration of rice. Also foliar fertilization of Fe is one of the most important methods of fertilizer application in agriculture practices in order to increase Fe concentration in crops because foliar nutrients help easy and quick consumption of nutrients by penetrating the stomata or leaf cuticle and enter the cells (Rawashdeh and Florin, 2015). The result of the study is in corroborate with the findings of Asri and Sonmez, (2012). Kazemi, (2013) also found similar result in a study where foliar application of Fe increased the fruit Fe concentration of tomato.

Summary and Conclusion

The result of the present studies revealed that different application strategies of Fe on the growth, yield contributing parameters, yield, biochemical and nutrient contents were found to be significant in BARI Tomato-14. The highest plant height, number of branch plant^{-1}, number of leaves plant^{-1}, number of flower cluster plant^{-1}, number of fruits plant^{-1}, fruit diameter and fruit fresh and dry weight plant^{-1} and yield were recorded in 50% Fe as seedling priming + 50% Fe as foliar spray. On the other hand, the highest vitamin-C, protein and lycopene content were found in 100% Fe as foliar spray at flowering stage, 50% Fe as seedling priming + 50% Fe as foliar spray and 100% Fe as foliar spray at fruiting stage, respectively @ 4 kg ha^{-1}. The highest N, K and Ca contents and uptake were recorded in 50% as seedling priming + 50% as foliar Fe application strategy. Moreover, the highest Fe content and uptake were recorded under the level 50% as seedling priming + 50% as foliar Fe application strategy. Therefore, based on the findings of the experiments, it may be concluded that for efficient production as well as for biofortification of Fe in tomato fruits application of 2 kg Fe ha^{-1} as seedling priming + 2 kg Fe ha^{-1} as foliar spray is desired. Further trials in different locations under various soil and climatic condition is needed to justify the results for the common farmers.

References

Aciksoz, S.B., Ozturk, L., Yazici, A. and Cakmak, I. 2014. Inclusion of urea in a 59 Fe EDTA solution stimulated leaf penetration and translocation of 59 Fe within wheat plants. *Physiologia Plantarum*, 151(3): 348-357. htpps//:doi: 10.1111/ppl.12198

Al Mahmud, A., Jahiruddin, M. and Islam, M. 2018. Screening of Rice Varieties for Zinc Biofortification Potentiality in Calcareous and Non-Calcareous Soils.

Alshaal, T. and El-Ramady, H. 2017. Foliar application: from plant nutrition to biofortification. *Environment, Biodiversity & Soil Security,* 1**:** 71–83. htpps//:doi: 10.21608/JENVBS.2017.1089.1006

Anonymous, 2019. National Institutes of Health: Iron Dietary Supplement Fact Sheet. 2016 (Date of access: 20.11.2019).

Asri, F.O. and Sonmez, S. 2012. Effects of different potassium and iron levels on seasonal changes of nutrient concentrations of tomato plant grown in soilless culture. *African Journal of Agricultural Research,* **7:** 401–412. htpps//:doi: 10.5897/AJAR11.1119

Batra, V.K., Lal, M., Kamboj, O.P., Arora, S.K. and Suthar, M.R. 2006. Effect of foliar application of micronutrients on the quality and shelf life of tomato. *Haryana Journal of Horticultural Sciences,* 35(1/2): 140.

Bhowmik, D., Kumar, K.S., Paswan, S. and Srivastava, S. 2012. Tomato-a natural medicine and its health benefits. *Journal of Pharmacognosy and Phytochemistry,* 1(1): 33-43.

Black, C.A. 1965. Methods of Soil Analysis. Part-II. *American Society of Agronomy.* Madison, Washington, USA. pp. 999-1492.

Cakmak, I., Kalayci, M., Kaya, Y., Torun, A.A., Aydin, N., Wang, Y., Arisoy, Z., Erdem, H., Yazici, A., Gokmen, O., Ozturk, L. and Horst, W.J. 2010. Biofortification and localization of zinc in wheat grain. *Journal of Agricultural and Food Chemistry,* 58: 9092–9102. htpps//:doi.org/10.1021/jf101197h

Clemens, S. 2014. Zn and Fe biofortification: the right chemical environment for human bioavailability. *Plant Science*, 225: 52-57. https://doi.org/10.1016/j.plantsci.2014.05.014

Dasgan, H.Y., Ozturk, L., Abak, K. and Cakmak, I. 2003. Activities of iron-containing enzymes in leaves of two tomato genotypes differing in their resistance to Fe chlorosis. *Journal of Plant Nutrition,* 26: 1997–2007. https://doi.org/10.1081/PLN-120024259

Eskandari, H. 2011. The importance of iron (Fe) in plant Products and Mechanism of Its uptake by plants. *Journal of Appllied Environment and Biological Science,* 1(10): 448-452.

Fageria, N.K., Moraes, M.F., Ferreira, E.P.B. and Knupp, A.M. 2012. Biofortification of trace elements in food crops for human health. *Communications in Soil Science and Plant Analysis,* 43: 556–570. https://doi.org/10.1080/00103624.2012.639431

FAO and UNDP. 1988. Land Resources Appraisal of Bangladesh for Agriculture Development. Report-2. Agro-ecological Regions of Bangladesh. BARC and UNDP, Farmgate, Dhaka. pp. 212-221.

Ghosh, A.B., Bajaj, J.C., Hasan, R. and Singh, D. 1983. Soil and Water Testing Method: A Laboratory Manual. Division of Soil Science and Agricultural Chemistry, IARI, New Delhi, India. pp. 221-226.

Giordano, M., El-Nakhel, C., Pannico, A., Kyriacou, M.C., Stazi, S.T., Pascale, S. and Rouphael, Y. 2019. Iron Biofortification of Red and Green Pigmented Lettuce in Closed Soilless Cultivation Impacts Crop Performance and Modulates Mineral and Bioactive Composition. *Agronomy Journal,* 9(6): 290. https://doi.org/10.3390/agronomy9060290

Grillet, L., Mari, S. and Schmidt, W. 2014. Iron in Seeds Loading Pathways and Subcellular Localization. *Frontiers in Plant Science,* 4: 535. https://doi.org/10.3389/fpls.2013.00535

Guha, T., Mukherjee, A. and Kundu, R. 2022. Nano-scale zero valent iron (nZVI) priming enhances yield, alters mineral distribution and grain nutrient content of Oryza sativa L. cv. Gobindobhog: a field study. *Journal of plant growth regulation*, 41(2): 710-733. https://doi.org/10.1007/s00344-021-10335-0

GutiéRrez-Ruelas, N. J., Palacio-MáRquez, A., SáNchez, E., MuñOz-MáRquez, E., CháVez-Mendoza, C., Ojeda-Barrios, D. L. and Flores-CóRdova, M. A. 2021. Impact of the foliar application of nanoparticles, sulfate and iron chelate on the growth, yield and nitrogen assimilation in green beans. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 49(3): 12437. https://doi:10.15835/nbha49312437

Havlin, J.L., Tisdale, S.L., Nelson, W.L. and Beaton, J.D. 2014. Soil fertility and nutrient management: An introduction to nutrient management. (8th Ed). Pearson (pp. 505), Upper Saddle River, New Jersey. U.S.A.

Jackson, M.L. 1973. Soil Chemical Analysis. Prentice Hall of India Pvt. Ltd., New Delhi, India. 498: 151-154.

Kazemi, M. 2013. Effect of Zn, Fe and their combination treatments on the growth and yield of tomato. *Bulletin of Environment, Pharmacology and Life Sciences*, 3(1): 109–114.

Kobayashi, T., Nozoye, T. and Nishizawa, N. K. 2019. Iron transport and its regulation in plants. *Free Radical Biology and Medicine*, 133: 11-20. https://doi.org/10.1016/j.freeradbiomed.2018.10.439

Krohling, C.A., Eutrópio, F.J., Bertolazi, A.A., Dobbss, L.B., Campostrini, E., Dias, T. and Ramos, A.C. 2016. Ecophysiology of iron homeostasis in plants. *Soil Science and Plant Nutrition,* 62(1): 39-47. https://doi.org/10.1080/00380768.2015.1123116

Kumari, S. and Sharma, S.K. 2011. Effect of micronutrient sprays on tomato (*Lycopersicon esculentum*) seed production. *Indian Journal of Agricultural Science,* 76: 11.

McLaren, R.G., Swift, R.S. and Qwin, B.F. 1984. EDTA extractable copper, zinc, iron and manganese in soils of the Conterbury plains. *New Zealand Journal of Agricultural Research,* 27(2): 207-217. https://doi.org/10.1080/00288233.1984.10430423

Morrissey, J. and Guerinot, M. L. 2009. Iron uptake and transport in plants: the good, the bad, and the ionome. *Chemical reviews*, 109(10): 4553-4567. https://doi.org/10.1021/cr900112r

Naz, S., Yousaf, B., Tahir, M. A., Qadir, A. and Yousaf, A. 2015. Iron and zinc bio-fortification strategies in wheat crop by exogenous application of micronutrients. *Food Science and Quality Management*, 35: 49-54.

Nielsen, S.S. 2010. Complexometric determination of calcium. In Food analysis laboratory manual Springer Boston MA pp. 61-67. https://doi:10.1007/978-1-4419-1463-7_8

Olowolaju, E.D., Okunlola, G.O. and Ayeotan, O.J. 2021. Growth, yield and uptake of some nutrients by tomato as affected by iron concentration, *International Journal of Vegetable Science,* 27(4): 378-387. https://doi.org/10.1080/19315260.2020.1805668

Olsen, R.V., Dean, L.A. and Black, C.A. 1954. Methods of Soil Analysis, Part-2. Wis. 9 966-967.

Osman, I.M., Hussein, M.H., Ali, M.T., Mohammed, S.S., Kabir, M.S. and Halder, B.C. 2019. Effect of boron and zinc on growth, yield and yield contributing traits of tomato. *Journal of Agriculture and Veterinary Science,* 12(2): 25-37. https://doi:10.9790/2380-1202012537

Page, A.L., Miller, R.H. and Keeney, D.R. 1982. Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. American Society of Agronomy. *International Soil Science Society of America*, 1159.

Paul, V., Pandey, R., Ramesh, K.V. and Meena, R.C. 2017. Atomic Absorption Spectroscopy (AAS) for Elemental Analysis of Plant Samples. *Manual of ICAR Sponsored Training Programme for Technical Staff of ICAR Institutes on "Physiological Techniques to Analyze the Impact of Climate Change on Crop Plants,* 84.

Prasad, B.V.G., Smaranika, M., Rahaman, S. and Prerna, B. 2015. Bio-fortification in horticulture crops. *Journal of Agricultural Engineering and Food Technology,* ISSN: 2350-0263, 2(2): 95-99.

Ranganna, S. 1994. Handbook of Analysis of Quality Control for Fruit and Vegetables Products. Tata McGraw-Hill Publishing Co. Ltd., New Delhi, India.

Rawashdeh, H. M. and Florin, S. 2015. Foliar application with iron as a vital factor of wheat crop growth, yield quantity and quality: A Review. *International Journal of Agricultural Policy and Research*, 3(9): 368-376. https://dx.doi.org/10.15739/IJAPR.062

Reis, S., Pavia, I., Carvalho, A., Moutinho-Pereira, J., Correia, C. and Lima-Brito, J. 2018. Seed priming with iron and zinc in bread wheat: effects in germination, mitosis and grain yield. *Protoplasma*, 255(4): 1179-1194. https://doi.org/10.1007/s00709-018-1222-4

Rout, G. R. and Sahoo, S. 2015. Role of iron in plant growth and metabolism. *Reviews in Agricultural Science*, 3: 1-24.

Sakya, A.T. and Sulandjari, T. 2019. Foliar iron application on growth and yield of tomato. In *IOP Conference Series: Earth and Environmental Science,* 250: 012001. https://doi.org/10.1088/1755-1315/250/1/012001

Savitha, H.R., Bidari, B.I., Shashidhara, G.C., Murthy, K.K., Poornima, D.S. and Mohan, K.S. 2010. Effect of Fe-EDTA on growth, yield and quality of red chilli (*Capsicum annuum* L.). *International Journal of Agricultural Sciences,* 6(2): 531-533.

Sharma, S.K. and Le Maguer, M. 1996. Lycopene in tomatoes and tomato pulp fractions. *Italian Journal of Food Science,* 8(2): 107-113.

Singh, D., Chhonkar, P.K. and Pandey, R.N. 1999. Soil Plant Water Analysis: A Method Manual. IARI, New Delhi, India, pp. 72-86.

Stammer, A.J., and Mallarino, A.P. 2018. Plant tissue analysis to assess phosphorus and potassium nutritional status of corn and soybean. *Soil Science Society of America Journal,* 82(1): 260-270. https://doi.org/10.2136/sssaj2017.06.0179

Sultana, T., Chowdhury, A. H., Rahman, A., Saha, B. K., Chowdhury, T., Islam, M. A. and Fancy, R. 2021. Phosphorous use efficiency and its requirement for aloe vera cultivated on silty loam soils. *Communications in Soil Science and Plant Analysis*, 52(3): 268-285. https://doi.org/10.1080/00103624.2020.1862152

Tandon, H.L.S. 1995. Methods of Analysis of Soils, Plants, Water and Fertilizers. Fertilizer Development and Consultation Organization New Delhi, India. p. 445.

Vasconcelos, M. W. and Grusak, M. A. 2014. Morpho-physiological parameters affecting iron deficiency chlorosis in soybean (Glycine max L.). *Plant and soil*, 374: 161-172. https://doi.org/10.1007/s11104-013-1842-6

Wala, M., Skwarek-Fadecka, M., Kołodziejek, J., Mazur, J., Lasoń-Rydel, M. and Krępska, M. 2022. Effect of the Fe-HBED chelate on the nutritional quality of tomato fruits. *Scientia Horticulturae*, 293: 110670. https://doi.org/10.1016/j.scienta.2021.110670

WHO. 2006**.** Guidelines on Food Fortification with Micronutrients Geneva.

Zou, C., Du, Y., Rashid, A., Ram, H., Savasli, E., Pieterse, P.J. and Cakmak, I. 2019. Simultaneous biofortification of wheat with zinc, iodine, selenium, and iron through foliar treatment of a micronutrient cocktail in six countries. *Journal of Agricultural and Food Chemistry,* 67(29): 8096-8106. https://doi.org/10.1021/acs.jafc.9b01829