



## Evaluation of Nutrient Supply, Kinetics, and Prediction of Iron (Fe) Uptake in Iron-deficient Soil using a Mechanistic Model

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### Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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### ABSTRACT

A pot culture experiment in the factorial completely randomized design (FRCD) was formulated with the three levels of iron (Fe) @ 0, 20 and 40 mg kg<sup>-1</sup> in the iron-deficient *Typic Ustochrept* sandy loam soil at Anand Agricultural University, Gujarat, India to evaluate and predict the iron uptake, nutrient efficiency and its depletion of in the rhizosphere of efficient and in-efficient chick pea cultivars using mechanistic model NST 3.0. The current investigation was carried out with the four chickpea cultivars namely, Fe-efficient (GG-1 and GAG-735) and Fe-inefficient (ICCC-4 and GJG-305). Plant observations were recorded during three different growth stages viz., 20 DAG, 40 DAG and maturity, respectively. The shoot weight and shoot length of Fe-inefficient varieties (ICCC-4 and GJG-305) well responded to the application of Fe as compared to Fe-efficient varieties (GG-1 and GAG-735) with 20 mg Fe Kg<sup>-1</sup> application through FeSO<sub>4</sub>. Lower dose of 20 mg Fe kg<sup>-1</sup> was found equally effective in increasing root length and root radius. Root radius ( $r_0$ ) and initial soil solution concentration of Fe ( $C_{Li}$ ) were found most sensitive parameters influencing Fe uptake, which was followed by maximum net influx ( $I_{max}$ ). In no Fe treatment, increasing  $r_0$ ,  $C_{Li}$  by a factor of 2.0 times individually caused increase in Fe uptake by 1.60, 1.45 times, 1.36, 1.53 times, 1.16, 1.15 times and 1.05, 1.25 times, respectively in GG-1, GAG-735, ICC-4 and GJG-305 varieties of chickpea. While, increasing  $I_{max}$  and  $K_m$  separately by a factor of 2.0 Fe uptake altered in proportions by 1.03, 0.57

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times, 0.93, 0.57 times, 0.73, 0.54 times and 0.69 and 0.48 times, respectively in GG-1, GAG-735, ICC-4 and GJG-305 varieties of chickpea. The ICC-4 instead of GG-1 and GAG-735 could be rational choice to grow on Fe deficient soil to get with dense Fe content. On the other hand, Fe-inefficient varieties had 2 times of higher mean Fe-influx at 40 mg Fe kg<sup>-1</sup> application than Fe-Efficient varieties.

**Keywords:** Fe-uptake; chickpea; Fe influx; NST 3.0; root growth rate.

## 1. INTRODUCTION

Soil is the main of source and supplier of nutrients including macro and micronutrients to the plants in a systematic way. Availability and mobility of metal nutrients in soil are mainly controlled by various factors such as soil pH, organic matter, clay content, nutrient reserve, calcium carbonate content and cation exchange capacity (CEC) [1]. The soil-plant transfer of metals is a part of chemical element cycling in nature [2]. Generally, crop species differs widely in nutrient uptake especially metal nutrient like iron (Fe) and acquisition efficiency, which means that some crops perform better than others when grown on low iron soil. This can be due to differences in root architecture (*i.e.*, root length, root radius, root surface area, root hair density, and rate of shoot growth) [3]. Iron is primarily absorbed by plants, and it solubilizes Fe<sup>3+</sup> and then reduces it to Fe<sup>2+</sup> for absorption or transport into the root. Strategy II systems are characterized by an iron deficiency-induced release of specific Fe<sup>3+</sup>-chelating compounds "phyto siderophores" [4] and a high affinity uptake system (transport protein) for Fe<sup>3+</sup> phyto siderophores [5]. The physiological properties of roots, such as root exudation and uptake kinetics, have also been identified as causes of differential Fe efficiency. Chickpea often releases substantial quantities of organic acids [6]. The large variation found in uptake kinetics parameters of plant genotypes can be screened by growing them on nutrient stress condition of that concerned element [7]. Bennett [8] reported that reduction of Fe<sup>+3</sup> by root ferric chelate reductase further enhances iron solubility, since Fe<sup>+2</sup> is more soluble than Fe<sup>+3</sup>. Reduction also prepares iron for uptake by IRT1 types (*i.e.* Ferrous transporters), which move Fe<sup>+2</sup> across the root epidermal area to the plasma membrane. Uptake kinetics describes the relationship between the nutrient influx and concentration at the root surface. A high influx at a low concentration can be obtained by a high maximum influx ( $I_{max}$ ) and/or a small Michaelis-Menten constant ( $K_m$ ), which enable a plant to grow in soils containing only sparingly soluble

compounds. Thus, a number of factors may contribute to differences in Fe efficiency among plants. The differences between the cultivars in terms of Fe efficiency may be explained by the variation in the internal requirement or in the uptake efficiency. The latter depends on the size of the root system and efficiency of uptake of each root segment (influx).

Generally, for prediction of concentrations of heavy metals in plants due to uptake from soil or soil solution, mechanistic, empiric and mathematical models [9]. Chaney [10] reported that dicotyledonous plants might enhance their capacity for iron uptake, in response to a developing deficiency, by increasing their ability to reduce ferric chelates at the root surface. The relationship between metal concentrations in various soils and plants is often described by a transferring ability of plant and mobilizing power of nutrient involved. Krauss [11] used Freundlich-type functions to predict Cd, Cu, Pb, and Zn concentrations in wheat grain and leaf. Multiple regression analysis was used successfully by Ivezic [12] to predict metal concentrations in wheat grain in uncontaminated soil. Nutrient availability in soil and acquisition by plants interact at the soil root interface and thus it is useful to evaluate the rate and amount of nutrient that are actually taken up by plants [13]. The processes interact in various ways which makes it difficult to determine the importance of individual factors by measurement and to know their role in plants growing in soil. The availability of mineral nutrient in soil is the result of interactions between two complex phenomena: supply of nutrients in soil and the ability of plant to acquire nutrients. Both soil and plant parameters are therefore, important for plant nutrition point of view.

Adequate supply of nutrients to plant roots is not merely a function of total amount of a particular nutrient in the soil, but also depends on the rate of replenishment of nutrient from the soil solution. Since, roots absorb nutrients in dissolved state only; the soil solution is the immediate source of plant nutrients. The nutrient bound to soil solid

phase is virtually unavailable to plants. Even the nutrients dissolved in soil solution are not very mobile because they are entrapped in the water filled fraction of the tortuous pore system of the soil. Hence nutrient transport through the soils is often limited to low rates and at short distances. Therefore, a root system must develop in a way that will give access to the soil nutrients and is the major factor distinguishing nutrient efficient from inefficient varieties.

When contribution of root hair was included in nutrient uptake model calculations, it gave better prediction of uptake. In the last decade, nutrient uptake models have been validated for simulation of K, P, and Mn uptake by different crops [14-17]. These models are based on ion transport from soil to roots by means mass flow and diffusion and nutrient uptake following Michaelis-Menten kinetics. The present investigation was planned to find reasons for differences in Fe efficiency of four chickpea cultivars by studying different plant and soil parameters. A recent version of mechanistic model (NST 3.0) of nutrient uptake from soil was used to simulate Fe uptake and to evaluate the significance of each soil and plant parameter in the system through sensitivity analysis of the rhizosphere of chickpea, systematics changes in each parameter from 0.5 to 2.0 times of its initial uptake value were calculated by simulation of Fe uptake model, keeping all other parameters constant.

## 2. MATERIALS AND METHODS

A greenhouse pot experiment was conducted in the Micronutrient Project (ICAR), Anand Agricultural University, Gujarat, India (22° 11" N, 73° 27" E) to study and explain differences in Fe efficiency of different cultivars of chickpea and to determine soil and plant parameters required for nutrient uptake model calculations. Iron-deficient loamy sand soil (*Typic Ustochrept*, DTPA extractable Fe- 4.63 mg kg<sup>-1</sup>, pH 7.5, electrical conductance 0.15 dS m<sup>-1</sup>, and organic carbon 3.9 g kg<sup>-1</sup>) was used. Chickpea cultivars from the preliminary experiment of NAIP component-IV, Fe-efficient (GG-1 and GAG-735) and Fe-inefficient (ICCC-4 and GJG-305) were grown in earthen pots containing 8.0, 8.0 and 15.0 kg of soil kept for three different harvest period, 20 DAG, 40 DAG and maturity respectively, treated with 0, 20, and 40 mg Fe kg<sup>-1</sup> soil as given through FeSO<sub>4</sub>.7H<sub>2</sub>O. A basal dose of 20 kg N ha<sup>-1</sup> as CO (NH<sub>2</sub>)<sub>2</sub>, 40 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> soil as KH<sub>2</sub>PO<sub>4</sub> was applied to soil in all pots.

Treatments were replicated three times for three harvests in a factorial completely randomized design. 8, 8 and 4 plants were kept for 20 DAG, 40 DAG and maturity stages, respectively.

Mean minimum and maximum air temperatures were 8.34°C and 37.6°C, respectively, during the growth period of the crop. Soil moisture was maintained at 38 % (v/v) by weighing the pots and replenishing the water loss daily. Pots without plants were used to estimate moisture loss through evaporation. Transpiration was calculated by subtracting the amount of water evaporated from the amount of water lost from the pots with plants during the growth period. Plant samples after each harvest were washed with dilute HCl and distilled water and dried at 70 C to constant weight. Shoot dry weight was recorded. The dried samples were ground in a stainless steel Willey mill and digested in HNO<sub>3</sub> and HClO<sub>4</sub> (3:1 ratio). Digests were analysed for micronutrient content (Fe, Zn, Mn and Cu). Roots were carefully separated from soil by washing and floating over sieves, and foreign material was removed by hand. The roots were then kept between two filter papers to remove surface water and fresh root weight was recorded. Root length was measured and recorded by using the advanced version of *Winrhizo* software and different root related parameters were analysed as per the formula given in the Table 1.

Sensitivity analysis was performed to evaluate the effect of each soil and plant parameter on Fe influx and Fe uptake, while considering that all the parameters are independent of one another. Simulation of Fe uptake was done by varying each parameter between 0.5 and 2.0 times from its measured value. While each parameter was changed, the remaining parameters were held constant at initial values.

## 3. RESULTS AND DISCUSSION

### 3.1 Plant Parameters

Application of Fe resulted in a significant increase in fresh and dry weight of shoot in different growth stages which confirmed that the soil used was deficient in Fe (Table 2). The average improvement in shoot dry weight was found highest in ICCC-4 which was developed 5.35 times, GJG-305 developed 4.04 times in control condition than efficient varieties GG-1 (3.37 times) and GAG-735 (2.84 times). Increasing level of Fe application also increased the shoot production of inefficient varieties which

may be due to more utilization on applied Fe as these varieties required comparatively more Fe for better performance at higher level of Fe application. In case of efficient varieties, lower response was noticed; it may be due to their lower requirement of Fe, which indicated that these varieties are Fe tolerant varieties under stress conditions which were accompanied with Khoshgoftarmanesh [18]. In control condition, varieties GG-1 was 1.42, 4.16 times and ICC-4 were 1.58, 7.22 times higher root development at second and maturity stage, respectively. Our findings are in agreement with Yaqoob [19] who suggested that stress at pre flowering stage (early stage) of crop period being harmful and detrimental for screening chickpea under nutrient deficient conditions.

### 3.2 Root Parameters

The cultivar ICC-4 had a smaller root length, but it showed good shoot and root growth rate. The ICC-4 and GJG-305 had a high mean root radius which might be due to more dispersion effect of roots than other varieties. Decrease in root length was noticed in chickpea and it is attributed to reduce partitioning of biomass towards root [20]. Again the mean root radius decreased with age of plant, this might be due to increase in lateral and secondary root growth besides basal root length at later stage of soybean crop growth [21-22]. The average distance between neighbouring roots was decreased by 9, 34 and 19, 45 per cent by efficient and inefficient varieties, respectively in second and maturity growth stages. This may be probably due to dense growth of roots with advancement in time. The GG-1 and GAG-735 had a more surface area over ICC-4 and GJG-305; because of former both varieties had more root length than latter. With application of Fe, slight increase in surface area was noticed which could be due to some increment in root length. The root surface area increased from 1.64 to 5.37 and 1.91 to 6.83 times in efficient and inefficient varieties, respectively in second and maturity growth stage. This could be mainly attributed to good and dispersed growth of root with advancement of age of the concerned crop.

### 3.3 Iron Content and Uptake

The highest Fe content was found at 40 DAG and showed decreased in further increase in growth stage. After stem elongation (growth stage increases), Fe concentration fell, it may be

due to rapid structural growth and dilution effect of concerned element. Similar results were reported by Hebborn [23] in case of Mn in barley crop and Edwards and Barber [24] in case of P. Therefore, the results also indicated that concentration of Fe in plant tissues is not being considered as a reliable parameter for distinguishing of genotypes [25]. However, it was noticed that at maturity stage efficient accumulates lower Fe content than inefficient varieties; the observation is in agreement with that observed by Graham [26]. They have reported that efficient genotypes do not necessarily have higher concentration in their shoot or edible parts than inefficient genotypes. In general, the higher shoot Fe content in inefficient varieties may be due to it had high water influx rate, which cause high movement of nutrient from soil to plant by transpiration pull. Even though ICC-4 and GJG-305 had a smaller root length, which accumulated comparatively higher Fe mainly due to topsoil foraging, which is a component of Fe-efficient nutrient acquisition of inefficient crops [27]. It might also be due to dispersion of adventitious roots over shallow basal roots [28].

Plants possess a number of transport mechanisms to control the acquisition, partitioning and the deposition of the micronutrients viz. Zn, Fe, Mn and Cu. This control is important because the plants must obtain adequate levels of these micronutrients for both vegetative and reproductive tissues and these control processes vary temporally and spatially within a given plant. As results indicated that Fe uptake was increased with increase in Fe levels due to the fact that dry shoot weight and Fe content of shoot increased with increase in Fe level (Table 3). Fe uptake in inefficient varieties (ICC-4 and GJG-305) increased by 1.37, 1.26 times, 1.24, 1.20 times and 1.27, 1.31 times over the efficient varieties at Fe 20 and 40 mg kg<sup>-1</sup> respectively in all the growth stages. These results are in agreement with Cakmak [29] who reported similar results in Zn-efficient rye and Zn-inefficient durum wheat under Zn deficient soil condition. The lower uptake of Fe was recorded in efficient varieties as compared to inefficient varieties even with the application of Fe which clearly indicated that the requirement of Fe by efficient varieties was comparatively lower than inefficient varieties.

**Table 1. Plant parameters for uptake model calculations**

<b>Parameters</b>	<b>Formulae and Description</b>
<b>Minimum Net Influx</b>	$I_n = \frac{U_2 - U_1}{RL_2 - RL_1} \times \frac{\ln(RL_2/RL_1)}{t_2 - t_1}$ $I_n$ = net influx (mol cm <sup>-1</sup> s <sup>-1</sup> ) $U$ = Fe content in shoots, (mol plant <sup>-1</sup> ) $RL$ = root length (cm) $t$ = time of harvest (s)
<b>Mean root radius</b>	$r_0 = \frac{\sqrt{FRW}}{\pi \cdot RL}$ $FRW$ = fresh root weight (g) $RL$ = root length (cm) $r_0$ = root radius (cm)
<b>Mean half distance between neighbouring roots (<math>r_1</math>)</b>	$r_1 = \frac{\sqrt{Vs}}{\pi \cdot RL}$ $Vs$ = soil volume (cm <sup>3</sup> ) $RL$ = root length (cm) $r_1$ = mean half distance between neighbouring roots (cm)
<b>Root surface area</b>	$RSA = 2 \pi r_0 RL$ $r_0$ = root radius (cm) $RL$ = root length (cm)
<b>Water influx (<math>V_0</math>)</b>	$V_0 = \frac{T_2 - T_1}{RA_2 - RA_1} \times \frac{\ln(RA_2/RA_1)}{t_2 - t_1}$ $T$ = transpiration (cm <sup>3</sup> ) $RA$ = root surface area (cm <sup>2</sup> ) $t$ = time of harvest (s) $V_0$ = water influx (cm <sup>3</sup> cm <sup>-2</sup> s <sup>-1</sup> )
<b>Relative root growth rate</b>	$K = \frac{\ln RL_2/RL_1}{t_2 - t_1}$ $k$ = relative root growth rate (s <sup>-1</sup> ) $RL$ = root length (cm) $t$ = time of harvest (s)
<b>Relative shoot growth rate</b>	$RSR = \frac{\ln SDW_2/SDW_1}{t_2 - t_1}$ $SDW$ = shoot dry weight (g)

Parameters	Formulae and Description
Shoot Demand on root	$T = \text{time of harvest (s)}$ $= \frac{W_2 - W_1}{RL_2 - RL_1} \times \frac{\ln(RL_2/RL_1)}{t_2 - t_1}$ <p>W= dry matter yield of shoot (g)                      RL= root length (cm)                      T=time (s)</p>
Fe uptake kinetics	$I_n = \frac{I_{max}(C_{L0} - C_{Lmin})}{k_m + C_{L0} - C_{Lmin}}$ <p><math>I_{max}</math> = Maximum net influx, which is calculated by following formula: <math>I_{max} = I_n * k_m / C_{Li}</math>  <math>C_{Lmin}</math> =Minimum Soil solution concentration. <math>C_{Lmin}</math> is the soil solution concentration at which net influx equals to zero  <math>k_m</math> = Michaelis –Menten constant. <math>K_m</math> is the difference between <math>C_{Lmin}</math> and the concentration at which influx is the half the <math>I_{max}</math>.</p>
Diffusion coefficient	<p><math>D_L</math> = Diffusion coefficient of Fe in water at 25<sup>0</sup> C, cm<sup>-2</sup> s<sup>-1</sup> value. Parsons [34], <math>\Theta</math> = The volumetric water content of the soil, cm<sup>3</sup> cm<sup>-3</sup>. This is cc of water in cm<sup>-3</sup> of soil (volume of water/ volume of soil)                      i.e. volume of water in pot/ vol. of soil in pot at field capacity. f = impedance factor was calculated from the formula of Barraclough and Tinker (1981) <math>f = 1.58 \Theta - 0.17</math></p>

Table 2. Effect of Fe application on dry matter yield, root length, root radius and mean distance between roots of chickpea cultivars at different growth stages

Parameters	Fe Dose (mg kg <sup>-1</sup> )	Fe-Efficient									Fe-Inefficient			Parameter	CD @ 5%
		GG-1			GAG-735			ICCC-4			GJG-305				
		20 DAG	40 DAG	Maturity	20 DAG	40 DAG	Maturity	20 DAG	40 DAG	Maturity	20 DAG	40 DAG	Maturity		
Shoot dry matter (g plant <sup>-1</sup> )	0	0.25	0.71	3.55	0.24	0.81	3.86	0.20	1.07	3.30	0.23	0.93	3.64	Fe	0.11
	20	0.24	0.77	3.71	0.29	0.94	4.06	0.24	1.12	3.96	0.25	1.15	3.73	Stage	0.11
	40	0.20	0.84	3.39	0.27	1.16	3.92	0.26	1.31	4.10	0.27	1.15	3.73	Variety	0.13
Root length (cm)	0	214.5	305.5	892.5	192.7	203.9	984.3	116.7	184.5	843.4	124.2	169.3	768.3	Fe	16.10
	20	232.2	326.4	786.7	202.2	228.9	1002.7	132.8	191.0	861.4	127.3	233.2	759.6	Stage	16.10
	40	240.8	316.7	842.7	211.7	235.8	1040.7	137.5	197.2	879.0	196.5	269.4	792.3	Variety	18.59
Root radius (10 <sup>-2</sup> cm)	0	2.50	3.17	3.43	2.77	3.63	3.33	3.53	4.33	3.23	3.33	3.43	3.87	Fe	NS
	20	2.70	3.00	3.70	2.93	4.10	3.23	3.20	4.60	3.77	3.20	3.47	3.70	Stage	0.10
	40	2.57	3.23	3.53	2.67	4.77	3.07	3.33	4.97	4.00	2.63	3.65	3.63	Variety	0.11
Distance between Roots (cm)	0	2.97	2.49	1.99	3.14	3.04	1.89	4.03	3.20	2.05	3.90	3.34	2.15	Fe	0.05
	20	2.85	2.41	2.12	3.06	2.88	1.88	3.78	3.15	2.03	3.86	2.87	2.16	Stage	0.05
	40	2.80	2.46	2.06	2.99	2.83	1.85	3.70	3.09	2.01	3.10	2.65	2.11	Variety	0.06

**Table 3. Effect of Fe application on root surface area, Fe content, Fe uptake and soil DTPA-Fe of chickpea cultivars at different growth stages**

Parameters	Fe Levels (mg kg <sup>-1</sup> )	Fe-Efficient									Fe-Inefficient				Parameter	CD @ 5%
		GG-1			GAG-735			ICCC-4			GJG-305					
		20 DAG	40 DAG	Maturity	20 DAG	40 DAG	Maturity	20 DAG	40 DAG	Maturity	20 DAG	40 DAG	Maturity			
Root surface area	0	33.9	60.3	193.16	33.55	46.13	204.20	25.85	50.30	171.83	25.88	52.98	186.12	Fe	3.67	
	20	39.0	61.7	183.29	37.10	58.69	201.87	26.83	54.85	201.95	25.66	49.96	175.61	stage	3.67	
	40	38.8	63.7	187.49	35.21	66.29	199.88	28.89	51.59	220.99	33.19	58.30	180.37	Var.	4.23	
Fe Content	0	136.7	359.0	345.2	166.5	348.6	278.0	132.6	347.0	276.2	144.6	328.2	317.0	Fe	10.4	
	20	178.0	390.8	297.3	241.7	390.7	232.5	197.6	349.0	335.0	205.4	381.5	349.0	stage	10.7	
	40	186.5	414.5	287.0	257.3	409.6	274.0	209.5	463.3	383.0	209.0	423.5	338.1	Var.	NS	
Fe uptake	0	59.8	502.2	2538.0	70.2	503.7	1744.0	48.7	646.2	1622.0	60.7	543.2	2440.2	Fe	104.2	
	20	76.3	536.2	2607.9	124.1	657.7	1791.6	82.7	699.4	2391.2	67.1	785.1	2806.1	stage	104.2	
	40	68.1	619.9	2571.7	102.9	772.6	1898.9	75.7	876.5	2832.0	62.4	603.6	2490.1	Var.	120.3	
Soil DTPA-Fe	0	3.7	3.4	3.82	3.69	5.09	4.11	3.25	3.85	4.13	3.29	3.84	4.53	Fe	0.26	
	20	4.5	4.4	4.96	4.88	5.08	4.93	4.45	4.59	4.85	4.49	5.02	5.89	stage	0.26	
	40	5.7	5.1	6.04	5.65	5.31	5.85	5.09	5.93	5.40	5.57	5.76	6.53	Var.	0.31	

**Table 4. Effect of Fe application on shoot growth rate, root growth rate, iron influx, seed yield and chickpea cultivars at different growth stage**

Parameter	Fe applied (mg kg <sup>-1</sup> )	Fe-Efficient				Fe-Inefficient		Parameter	CD @ 5%
		GG-1	GAG-735	ICCC-4	GJG-305				
Shoot growth rate (10 <sup>-7</sup> s <sup>-1</sup> )	0	6.18	7.10	8.66	7.99	Fe	0.68		
	20	6.88	7.20	9.05	11.37	Variety	0.79		
	40	8.31	8.56	10.26	8.23	Fe X V	1.31		
Root growth rate (10 <sup>-7</sup> s <sup>-1</sup> )	0	2.04	1.43	2.66	1.79	Fe	NS		
	20	2.27	2.06	2.43	2.80	Variety	0.46		
	40	2.20	1.79	2.59	1.98	Fe X V	NS		
Iron influx (10 <sup>-5</sup> nmol plant <sup>-1</sup> s <sup>-1</sup> )	0	1.00	1.27	2.33	1.92	Fe	0.20		
	20	1.27	1.44	2.24	2.44	Variety	0.24		
	40	1.16	1.74	3.53	2.51	Fe X V	0.41		
Seed yield (g pot <sup>-1</sup> )	0	11.99	14.30	9.59	9.03	Fe	0.97		
	20	12.39	16.76	15.05	9.27	Variety	1.12		
	40	12.13	14.99	12.51	9.24	Fe X V	1.93		
Seed Fe content (mg kg <sup>-1</sup> )	0	71.17	63.00	67.17	39.17	Fe	3.47		
	20	72.33	65.00	76.67	40.50	Variety	4.00		
	40	73.67	66.33	95.67	57.00	Fe X V	3.93		

Table 5. Plant and soil parameters used in NST 3.0 for nutrient uptake model calculations

Levels of Fe (mg kg <sup>-1</sup> soil)	GG-1			GAG-735			ICCC-4			GJG-305		
	0	20	40	0	20	40	0	20	40	0	20	40
<b>Plant parameters</b>												
$I_{\max}$ (10 <sup>-3</sup> nmol cm <sup>-2</sup> s <sup>-1</sup> )	1.30	1.25	1.50	1.66	1.88	2.26	3.32	3.19	5.03	2.73	3.47	3.57
$K_m$ (10 <sup>3</sup> nmol cm <sup>-3</sup> )	7.84	7.84	7.84	7.84	7.84	7.84	8.55	8.55	8.55	8.55	8.55	8.55
$C_{\min}$ (nmol cm <sup>-3</sup> )	0	0	0	0	0	0	0	0	0	0	0	0
$r_o$ (10 <sup>-2</sup> cm)	3.17	3.73	3.23	3.63	4.1	4.46	4.33	4.60	4.37	3.63	3.8	3.65
$V_o$ (10 <sup>-6</sup> cm s <sup>-1</sup> )	16.9	18.7	18.5	16.8	19.7	18.9	24.1	25.5	23.7	17.8	26.4	22.0
$r_1$ (cm)	2.49	2.41	2.46	3.04	2.88	2.83	3.20	3.15	3.09	3.34	2.87	2.65
$K$ (d <sup>-1</sup> )	0.0176	0.0170	0.0133	0.0038	0.0063	0.0680	0.230	0.0181	0.0180	0.0155	0.0299	0.0157
$RL_o$ (cm)	214.5	232.2	240.8	192.7	202.2	211.7	116.7	132.8	137.5	124.2	127.3	196.5
<b>Soil parameters</b>												
$C_{Li}$ (nmol cm <sup>-3</sup> )	0.600	0.775	0.825	0.600	0.775	0.825	0.600	0.775	0.825	0.600	0.775	0.825
$\Theta$	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26
$f$	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24
$b$	139.0	258.7	345.7	139.0	258.7	345.7	139.0	258.7	345.7	139.0	258.7	345.7
$D_L$ (10 <sup>-6</sup> cm <sup>2</sup> s <sup>-1</sup> )	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1

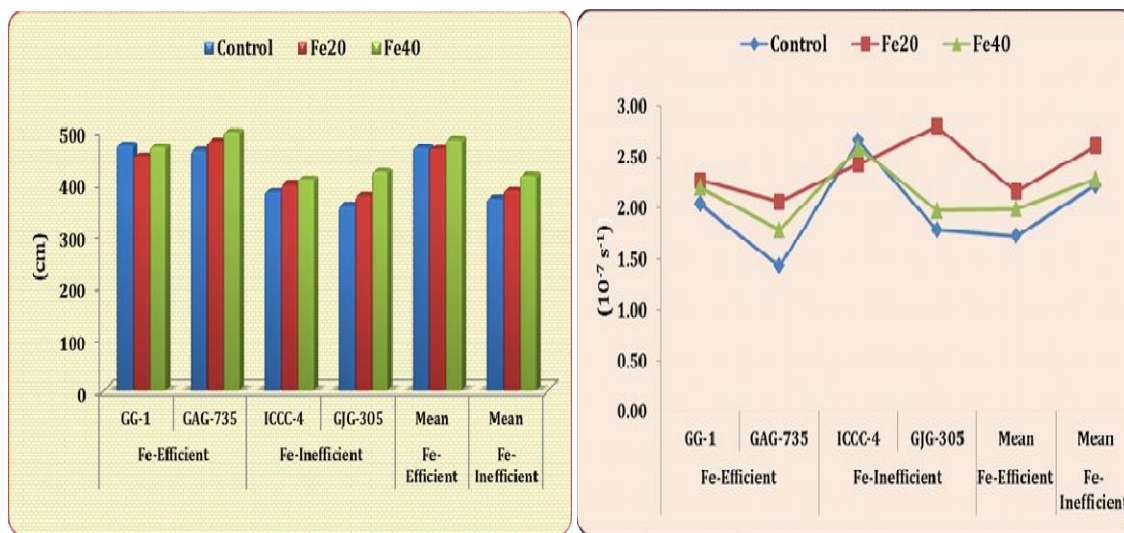


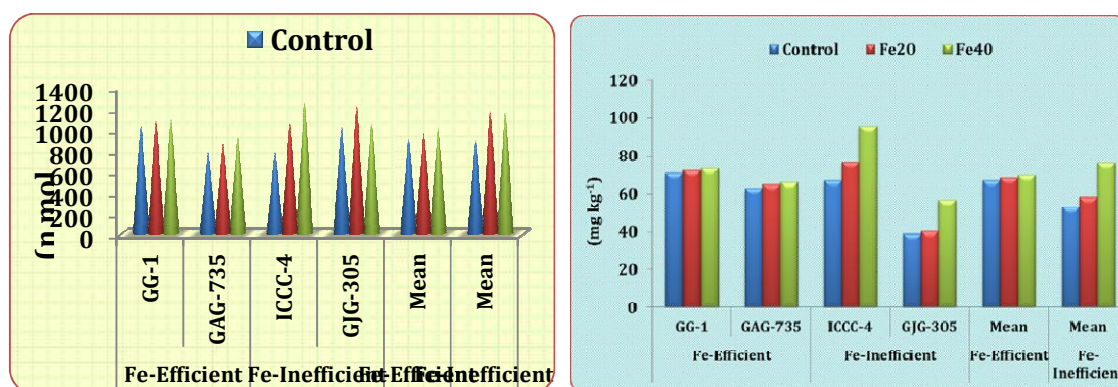
### 3.4 Nutrient Influx and Prediction

Even though ICC-4 and GJG-305 had a smaller root length, ICC-4 and GJG-305 had a high relative root growth rate than others (Table 4). It indicated that the latter varieties had better root development ability in nutrient stress condition than former. Similar results were also observed by Sadana [17] in raya that although the raya had smaller root length, it had a higher Mn influx. Further increase in Fe application at 40 mg kg<sup>-1</sup> root growth rate comparatively decreased because of very low response to higher level of Fe application. The ICC-4 and GJG-305 had the highest shoot growth rate because of these varieties showed better capacity of shoot production than root production. Increasing level of Fe significantly increased shoot growth rate only in inefficient group which was because of better performance to Fe application by inefficient genotypes. The results are agreement with those reported by Lata [30] who demonstrated that Mn efficient wheat variety registered response to Mn application. The highest relative shoot growth might have been reflected in terms of Fe influx characteristic.

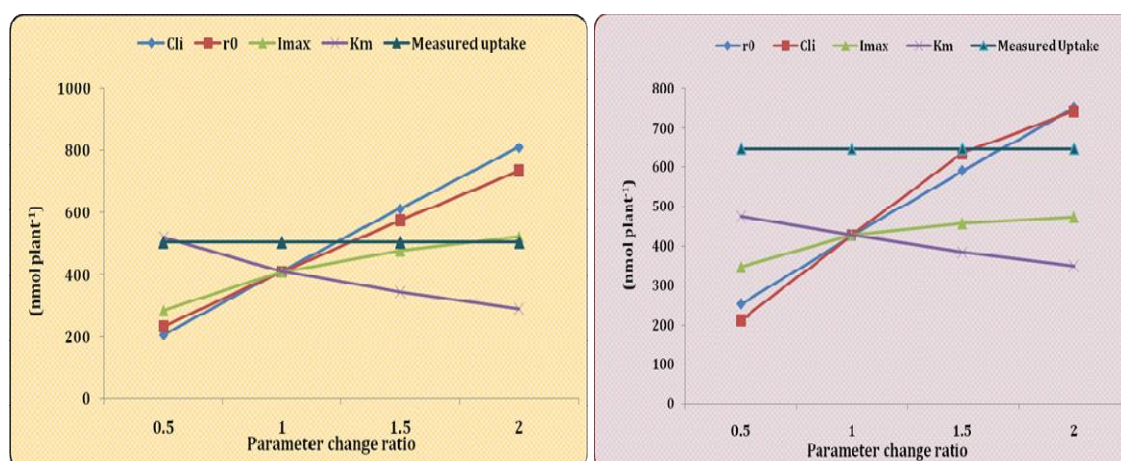
The varieties ICC-4 and GJG-305 had a higher Fe influx  $2.70 \times 10^{-5}$  nmol plant<sup>-1</sup> and  $2.29 \times 10^{-5}$  nmol plant<sup>-1</sup>, respectively which revealed that these two varieties had a more shoot surface area, higher shoot demand, higher shoot and root growth rate. It indicated that higher shoot and root growth rate are directly involved in Fe-influx in chickpea plants. Results also clearly indicated that a change in DTPA-Fe content of

soil after harvest was observed due to Fe application. The increase in DTPA Fe was by 25% and 50% with 20 and 40 mg Fe kg<sup>-1</sup> application, respectively. Application of Fe registered a significant increase in mean seed yield of chickpea by 10 and 3 per cent in efficient varieties and 30 and 16 per cent inefficient varieties with 20 and 40 mg Fe kg<sup>-1</sup> application, respectively over control condition. The variation in grain yield may be due to genotypic variation for Fe stress tolerance between the genotypes as reported by Marcar and Graham [31]. But in case of seed yield, overall performance of Fe-efficient varieties was significantly higher than Fe-inefficient varieties. The varietal performance with respect of Fe content of seed was observed as ICC-4>GG-1>GAG-735>GJG-305 (Fig. 1). The results indicated that seed Fe content was found maximum in inefficient variety ICC-4 (80.00 mg kg<sup>-1</sup>) which was 11, 25 and 75 per cent higher than GG-1, GAG-735 and GJG-305, respectively. It was observed that plants grown from seed with high micronutrient (Fe) content achieved higher grain yield and seed content as also reported by Yilmaz [32]. In case of efficient varieties, increasing level of Fe application from 20 to 40 mg Fe kg<sup>-1</sup> increased Fe content of seed by 4 and 1 per cent only over control, respectively. While, inefficient varieties registered increase in seed Fe content by 43 and 30 per cent over control. It would be stated that a large variation found in Fe concentrations or content among genotypes in major germplasm banks is sufficient to justify the possibility of developing micronutrient efficient and inefficient genotypes [33].





**Fig. 1. Root length, Iron influx, Fe uptake and seed Fe content of Fe-efficient and Fe-Inefficient cultivars of chickpea at different Fe levels**



**Fig. 2. Sensitivity analysis of predicted Fe uptake of GG-1 (Fe-Efficient) and ICC-4 (Fe-inefficient variety) at 40 DAG (Fe concentration in soil solution: 0.600 nmol)**

Increasing root radius ( $r_0$ ), initial soil solution concentration (CLi) by a factor of 2.0 times individually caused increase in Fe uptake by 1.60, 1.45 times, 1.36, 1.53 times, 1.16, 1.15 times and 1.05, 1.25 times, respectively in GG-1, GAG-735, ICC-4 and GJG-305 varieties of chickpea (Table 5 & Figs. 1 & 2). While, increasing maximum net influx ( $I_{max}$ ) and Michaelis-Menten constant ( $K_m$ ) separately by a factor of 2.0 in varieties of chickpea resulted in Fe uptake in proportions by 1.03, 0.57 times, 0.93, 0.57 times, 0.73, 0.54 times and 0.69 and 0.48 times, respectively. However, overall prediction of this model indicated that Fe uptake of Fe-efficient varieties influenced by  $r_0$  and CLi was increased by 1.48 and 1.49 times over inefficient varieties of chickpea. To increase the Fe uptake by chickpea, higher values of  $r_0$  and CLi are necessary; while lower value of  $k_m$  is desirable. Under low Fe supply conditions,

increasing initial soil solution concentration of Fe or selecting crop species with thicker roots or with more efficient uptake kinetics would be helpful in overcoming Fe deficiency.

#### 4. CONCLUSION

A wide range of variability of iron (Fe) content was observed in the genotypes of chickpea namely iron efficient and inefficient; efficiency of the cultivar varies with the root architecture, geometry and nutrient acquisition behaviour. The iron- inefficient varieties were well responded to the iron application through sulphates source *i.e.*  $FeSO_4 \cdot 7H_2O$ . Presence of Adventitious roots have several attributes that may enhance iron-acquisition efficiency in chickpea. Fe-inefficient varieties with increased level of Fe application; and suggest that adventitious rooting a useful trait for genetic enhancement of crop in low

fertility soil. The ICC-4 instead of GG-1 and GAG-735 could be a rational choice to grow on Fe deficient soils to get seed with dense Fe content. It is likely that initial seed Fe reserves contribute to a higher performance of ICC-4 variety over other varieties. The application of Fe at 20 mg kg<sup>-1</sup> on Fe deficient soil was found beneficial for better growth and development of Fe-inefficient chickpea varieties besides better Fe content in plant. Root radius ( $r_o$ ) and initial soil solution Fe concentration ( $C_{Li}$ ) were found most sensitive factor/parameters which influenced uptake of Fe in varieties of chickpea. In general, chickpea varieties released with low phytic acid and high micronutrient dense cultivars could be beneficial and involve in alleviate hidden hunger.

## 5. RECOMMENDATION

Growing of Fe-inefficiency chickpea cultivars with external application of iron nutrition through iron sulphate in iron deficient soil could be a beneficial factor to enhance the nutrient reserve in the chick pea grains.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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