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Article

Agronomic Approach to Iron Biofortification in Chickpea

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Abstract: Iron (Fe) deficiency specifically is the most common nutritional disorder due to insufficient absorbable Fe. Biofortification is a process of enriching the nutrient content of staple crops and is considered as a sustainable and cost-effective strategy to address micronutrient deficiency problems especially in the less developed countries. Chickpea (*Cicer arietinum* L.) is a staple food in many developing countries worldwide and is an excellent source of micronutrients. Biofortification of chickpea is a possible solution to address Fe deficiency problem. Chickpea biofortification experiment was conducted under field conditions to evaluate the effects of different doses of Fe fertilizer (0 kg ha⁻¹, 10 kg ha⁻¹ and 30 kg ha⁻¹ of Fe-EDDHA) on Fe content in seeds of 18 chickpea cultivars. The experiment was designed as a factorial combination of 18 chickpea cultivars and 3 different doses in a randomized complete block design with 4 replications at two locations in Saskatchewan in 2015 and 2016. Fe concentration in seeds across 18 different chickpea cultivars increased with Fe fertilization. Fe concentration in X05TH20-2 and CDC Frontier cultivars increased from 57 ppm to 59 ppm and 56 ppm to 58 ppm, respectively, after adding Fe fertilizer in both location in 2015 and 2016.

Keywords: biofortification; chickpea; iron deficiency; Fe-EDDHA; fertilization

1. Introduction

Fe is a key micronutrient for most living organisms to conduct ubiquitous metabolic processes involving electron transfer. Examples of such metabolic processes include DNA synthesis, oxygen transport, cellular respiration, and photosynthesis. Moreover, Fe is vital as a co-factor in numerous heme-complexes, for example, hemoglobin, catalase and DNA helicases [1-3]. Although Fe in many arable lands is relatively abundant (range concentrations of 20-40 g kg⁻¹), the low amount of the available form has resulted in Fe deficiency that limits plant growth [4]. Since Fe is highly reactive to oxygen, formation of insoluble oxidized Fe (III) restricts Fe uptake by roots especially in high pH and high HCO₃⁻ calcareous soils. In high pH and well aerated soils, the total conc. of Fe in soil solution was around 10⁻¹⁰M, which is 10⁻⁴- 10⁻⁵ folds less than the required amount for optimum plant growth [5,6]. In terms of Fe uptake from soil, plants are divided into two categories: Strategy I in nongraminaceous plants and Strategy II in graminaceous plants [6]. Strategy I plant species acquires Fe after the reduction of Fe (III) chelates at the root surface followed by absorption of Fe (II) ions throughout the plasma membrane [7]. Since one-third of the world's arable land is too alkaline for optimum plants growth, many studies have focused on how plants acclimatized with Fe deficiency [8]. In addition, limited uptake of Fe was mostly observed in Strategy I plant species that depend on ferric reductase for transferring Fe [9,3]. Consequently, this condition decreased plant productivity and led to low quality including low Fe content in seeds that ultimately result in public health problem. Fe in plant-based diets is a non-heme Fe that is less bioavailable than heme Fe. As such, the prevalence of Fe deficiency induced anemia often occurred in populations where total calorie intake came from monotonous plant-based diets [1].

Biofortification is a long-term food-based approach to alleviate micronutrient deficiency. It is a strategy of producing staple food crops with increasing concentration of bioavailable micronutrients in the edible parts, that is considered more sustainable and economical [10,11]. To increase bioavailable Fe in seeds, Fe biofortification strategy can be implemented that includes agronomy, plant breeding and genetic engineering approaches [12]. Agronomic approach could be a rapid solution to boost Fe content in plants. Furthermore, agronomic biofortification can be integrated with other breeding-based biofortification methods [13]. Agronomic biofortification, which is also known as ferti-fortification, involves the use of fertilizer either to soil and/or to foliage to increase the bioavailability of nutrients in edible parts of plant [14,15]. Adding micronutrients in soil is a functional strategy to enhance nutritional status in plant [16-18]. However, there are several factors that control the increase of bioavailable nutrient into seeds. For example, source of fertilizer, time and application method of fertilizer, and quantity of fertilizer [19,20]. As Fe can be rapidly converted into unavailable form when it is applied to high pH soil, the application of inorganic fertilizer such as ferrous sulphate (FeSO_4) could be ineffective [3]. Synthetic Fe chelates, in which Fe is combined with an organic chemical to form a chelate makes the Fe in a form accessible to plants. Moreover, Fe-chelates is soluble for a longer period than inorganic Fe. In this context, the application of chelated Fe fertilizers, for example, Fe-DTPA, Fe-EDTA and Fe-EDDHA can be effective in high pH soils. Among the three, Fe-EDDHA is the most effective Fe fertilizer than others [21].

Chickpea (*Cicer arietinum* L.) is a staple food crop in many African and Asian countries where the incidence of Fe deficiency is common [1,22,23]. Based on global production, chickpea is the second most important pulse after common bean [24]. Globally, chickpea production has increased gradually mostly (96%) in developing countries [24]. Like production, the highest chickpea consumption rate occurred in the South Asia and Middle East-North Africa at 4.25 kg person⁻¹ and 2.11 kg person⁻¹ year⁻¹, respectively [25]. The growth of chickpea consumption has also increased in developed countries. In USA, the consumption of chickpea has increased almost double from 199.6 g in 2010 to 322.1 g per person per year in 2014 [26]. Chickpea is consumed in a variety of ways, for example, green pods, immature seeds and young leaves as vegetables, whereas as the primary commodity, chickpea is consumed as dried mature seeds, as whole, hulled or flour form [27,28]. Nutritionally chickpea is a rich source of protein (20-22%) along with micronutrients including Fe (3.0-14.3 mg 100 g⁻¹) [29,30]. Therefore, Fe biofortification of chickpea to produce seeds with increased Fe concentration can mitigate Fe deficiency in populations with poor Fe intake [22]. Several studies have reported that agronomic approach could result in higher micronutrients in edible parts of different crops. Examples of successful agronomic biofortification include zinc (Zn) fertilizer to wheat and selenium (Se) to maize [31, 32]. Moreover, in chickpea, soil application of Zn fertilizer increased grain Zn content and Zn yield compared to control [33]. In addition, foliar application of Zn and Fe fertilizer also increased grain Zn yield in chickpea and leaves, stems and grains Fe content in mung bean [33, 34]. Foliar application of Se fertilizer increased Se concentration in pea and common bean seeds [35, 36]. In addition, Fe and Zn concentration in grain of cowpea increased after applying Zn-EDTA in potting compost [37]. Combined application of Zn-DTPA and ZnSO_4 also increased Fe and Zn content in bean using hydroponic system [38]. Fe biofortification in cowpea also showed that combined application of ferrous sulfate and ferrous chelate in potting compost increased Fe content of cowpea seeds compared to control [39]. In terms of bioavailability, several authors reported that Zn and Se fertilization increased their bioavailability in human diets [40-42].

To date, there is no report on the agronomic approach for Fe biofortification in chickpea. Given the importance of soil-Fe concentration, soil pH and HCO_3^- level, we hypothesised that the application of Fe fertilizer through soil increased Fe concentration in seeds. The main objectives of this study were: 1) to evaluate the effects of soil applied Fe fertilizer on Fe concentration in the seeds; and 2) to find out correlation between the Fe concentration and yield and correlation between the Fe concentration and Fe yield.

2. Materials and Methods

2.1. Description of location and year

The study was conducted at two locations, Elrose and Moose Jaw of Saskatchewan, Canada during the growing seasons from May to September in 2015 and 2016. The geographical coordinates of Elrose and Moose Jaw are 51.2006° N, 108.0329° W and 50.3916° N, 105.5349° W, respectively. The soil texture at both locations is clay loam to clay. The soil climatic zone of Elrose and Moose Jaw are dark brown and brown soil zones, respectively

2.2. Soil sample analysis

Soil sample analysis was done to examine the physico-chemical characteristics along with the concentrations of several micronutrients including Fe. Soils from both locations are calcareous (pH > 7.0) with the Fe status was above the marginal level (Figure 1).

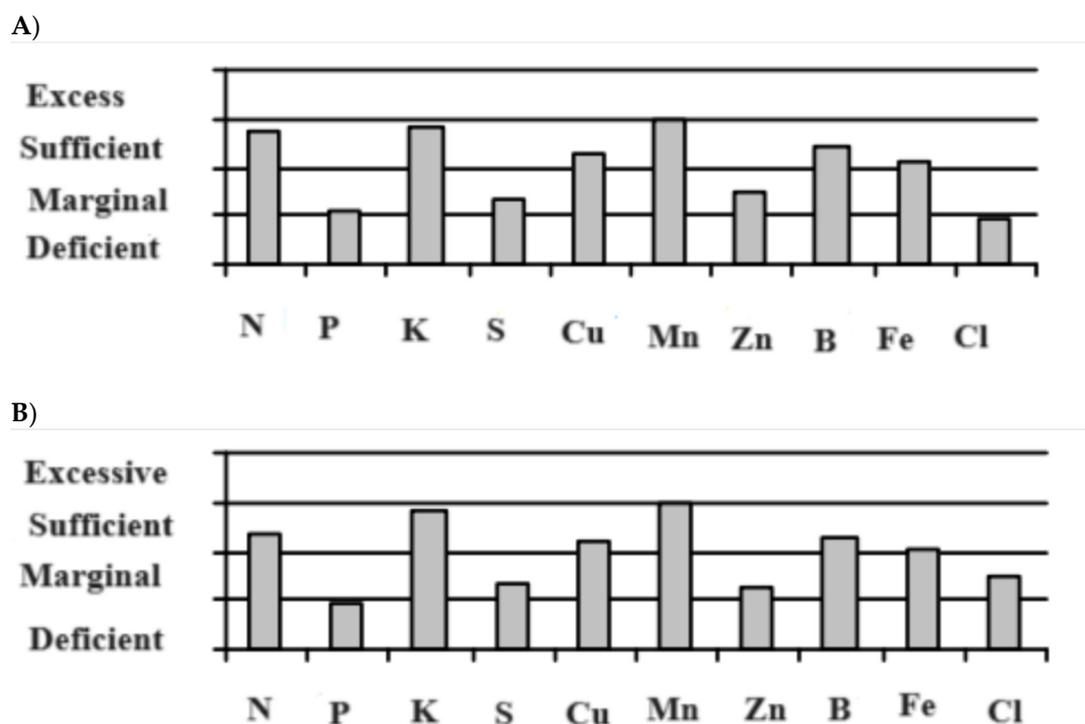


Figure 1. The status of Fe and other macro and micronutrients at the experimental sites at A) Elrose and B) Moose Jaw, SK.

Ten to twelve soil samples were collected diagonally from two layers (0-6" and 6-12") from each location for physico chemical analyses before and after Fe fertilization. Samples were air dried at 35° C for five days and a wooden roller was used to ground the soils. The ground soil samples were analyzed for different nutrients and chemical properties. The summary of soil properties prior to fertilization and after fertilization in 2015 and 2016 showed in

Table 1. Summary of soil properties prior to fertilization and after fertilization in 2015 and 2016.

	Before Fertilization								After Fertilization							
	Location				Location				Location				Location			
	Elrose		Moose Jaw		Elrose		Moose Jaw		Elrose		Moose Jaw		Elrose		Moose Jaw	
	2015		2016		2015		2016		2015		2016		2015		2016	
	Depth (inches)		Depth (inches)		Depth (inches)		Depth (inches)		Depth (inches)		Depth (inches)		Depth (inches)			
Soil Properties	0-6	6-12	0-6	6-12	0-6	6-12	0-6	6-12	0-6	6-12	0-6	6-12	0-6	6-12	0-6	6-12
pH	7.5	7.9	7.9	8.2	7.9	8.1	7.5	8.2	7.2	7.6	7.9	8.2	7.4	8.1	7.7	8.2
N (mg kg ⁻¹)	13.0	10.0	6.5	3.5	8.6	8.1	12.4	7.6	8.8	4.8	8.1	5.4	4.4	1.7	6.6	1.0
P (mg kg ⁻¹)	13.0	3.5	9.5	2.0	3.6	2.0	14.4	3.9	19.3	7.5	10.8	2.0	11.9	2.0	13.2	2.1
K (mg kg ⁻¹)	270	255	270	255	699	501	756	614	1120	856	849	420	932	582	885	507
Fe (mg kg ⁻¹)	20.5	11.4	13.3	9.4	19.8	19.8	18.3	17.0	22.1	23.0	16.7	13.8	20.7	21.3	22.1	19.7

2.3. Plant Materials

Eighteen chickpea cultivars and advanced breeding lines were used in this experiment (Table 2). All the cultivars were obtained from the chickpea breeding program at the Crop Development Centre, University of Saskatchewan.

Table 2. Description of eighteen cultivars used in the soil applied Fe fertilization.

Entry	Cultivars	Types	1000 Seed Weight(g) (Avg. of 2015 and 2016)
1.	1173-1	Kabuli	349
2.	1460-2	Desi	203
3.	AB06-156-2	Kabuli	376
4.	Amit	Kabuli	252
5.	CA05-75-45	Kabuli	265
6.	CDC Alma	Kabuli	293
7.	CDC Cabri	Desi	286
8.	CDC Consul	Desi	299
9.	CDC Corinne	Desi	255
10.	CDC Cory	Desi	266
11.	CDC Frontier	Kabuli	309
12.	CDC Leader	Kabuli	356
13.	CDC Luna	Kabuli	297
14.	CDC Orion	Kabuli	360
15.	CDC Palmer	Kabuli	393
16.	CDC Vanguard	Desi	219
17.	X05TH20-2	Kabuli	371
18.	X05TH47-3	Kabuli	351

2.4. Fe Fertilizer and Application

Synthetic Fe (III)-chelates (Fe-EDDHA) was used as soil applied fertilizer as chelating Fe fertilizer is more effective and stable in high pH soil (Table 3).

Table 3. Fe (III)-chelates and their stability level on high pH soil in order to correct Fe deficiency.

Fe fertilizer	Stability	Fe deficiency
Fe-EDTA, Fe-DTPA and Fe-HEDTA	Low	Limited/no results
Fe-EDDHA	High	Effective

Source: (Lucena, 2006) [43].

Eighteen chickpea cultivars (Table 2) and three rates of Fe fertilizer (Fe-EDDHA) were used in the experiment. Fe fertilizer was applied at three rates: S0 (control: no Fe application), S1 (10 kg ha⁻¹) and S2 (30 kg ha⁻¹) of Fe (Fe-EDDHA) solution with 6% actual Fe and 38.7% EDDHA. The application of Fe fertilizer was done by spraying method by using nozzle sprayer to the soil immediately after planting. Each low dose plot (S1) was applied with 9 g of Fe-EDDHA solution, whereas high dose plot (S2) with 27 g. The range of solution preparation for regular agricultural practice was 0.05 to 0.1 g/ml of H₂O. The solution of Fe-EDDHA with the rate of 38.7% EDDHA was prepared with 0.07 g of Fe-EDDHA per ml of H₂O. By this protocol, 128 ml and 385 ml of Fe-EDDHA solution were sprayed in the S1 and S2 plots, respectively. The timing of spraying in each row of S1 plot was 6 seconds, whereas in each S2 plot was 18 seconds.

2.5. Experimental Design

To evaluate the interaction effects of different doses of Fe fertilizer and chickpea cultivars, the experiment was arranged as a 18x3 factorial randomized complete block design (RCBD) with four replications at each location and year. Each experimental plot was 4.2 m² and total number of plots was 216 at each location and year. The plot consisted of 3 rows with 0.31m intra-row spacing. Seeding rate was 180 seeds per plot. The experiment was seeded using plot seeder on the 29th of April and 1st May 2015 at Elrose and Moose Jaw, respectively, and 3rd May and 29th April 2016 at Elrose and Moose Jaw, respectively. General crop management practices were done following the recommendation for chickpea crop in the area. No herbicide was used to manage weeds. When required, weeding at the experimental site was done manually. In 2016, due to wet condition in the second half of the growing season, both locations were infected by the ascochyta blight disease.

2.6. Data collection

2.6.1. Agronomic traits

Data were taken for the following agronomic characteristics: germination (%), node number, days to flowering, days to maturity, plant height (cm), disease score, 1000-seed weight and seed yield (converted to kg ha⁻¹). Germination % was determined by counting the total plant number at each plot and then was converted into %. Node number and plant height were recorded by randomly selected five plants from the middle row of each plot. Days to flowering was calculated from the seeding date until 50% of the plants within a plot had flowered. Like days to flowering, days to maturity was counted when 50% of the plants have changed color. At maturity, five individual plants from each plot were taken randomly to determine the biomass (dry weight). The reaction to ascochyta blight disease was scored at late podding stage (before maturity) by using 0-9 scale [44]. The detail rating scale for ascochyta blight on chickpea is given in Table 4.

Table 4. Disease rating scale (0-9) of ascochyta blight in chickpea.

Rating	Symptoms
0	No symptoms
1	Few, very small (<2mm ²) lesions on leaves and/or stems, <2% plant area affected (PAA)
2	Very small (<2mm ²) lesions, 2-5% PAA
3	Many small lesions (#2-5mm ²), 5-10% PAA
4	Many small lesions, few large (>5 mm ²) lesions, 10-25% PAA
5	Many large lesions, 25-50% PAA
6	Lesions coalescing, 50-75% PAA
7	Lesions coalescing with stem girdling, 75-90% PAA
8	Stem girdling or breakage, >90% PAA
9	Plant dead

Source: Chongo et al.2004 [44].

The 1000-seed weight was determined by randomly taking 200 seeds that were counted by using seed counter followed by weighing with digital balance. The weight of 200 seeds was then converted into 1000-seed weight. Seed yield of each plot was recorded in gram per plot and then converted into kg ha⁻¹. Fe yield was calculated by multiplying seed Fe concentration with seed yield and then converted into g ha⁻¹.

2.6.2. Seed Fe analysis

Fe concentrations (µg g⁻¹) in mature seeds of eighteen different cultivars with three doses of Fe fertilizer were measured by flame atomic absorption spectrometry (F-AAS, Nova 300, Analytik Jena AG, Jena, Germany) at the analytical laboratory at the Department of Plant Sciences, University of Saskatchewan. Before Fe concentration analysis, the Vulcan digester (Vulcan 84, Questron

Technology, Ontario, CA, USA) was used to digest the samples. The procedures for digestion and Fe concentration analysis were described previously [45,30]. After harvest, mature seeds were cleaned to get rid of any soil particles using air pressure. Cleaned seeds were grounded by using a cyclone sample mill (UDY Corporation, Fort Collins, CO, USA). One-half gram of powdered sample was used for determining seed Fe concentration with three replications.

2.7. Statistical analysis

Statistical analysis was done following PROC MIXED procedure of the SAS version 8.0 (SAS institute Inc., Cary, NC, USA) for a factorial randomized complete block design. Initially, the analysis was conducted for each location and year followed by combined analysis across locations and years. The location and year and their interactions were considered as random effects, whereas the effect of cultivar and Fe fertilizer dose were considered fixed. Least Significant Difference (LSD) test was used for separating mean values.

3. Results

The effects of soil-applied Fe fertilizer across locations, years, their interaction (location*year), cultivar, dose and their interaction (cultivar* dose) along with all the two, three, and four-ways interactions on different parameters of chickpea cultivars are presented in Table 5.

Table 5. Analysis of variance and F values of the effect of location (LOC), year (YEAR), replication (REP), cultivar (CUL), dose (DOS) and the interaction of LOC*YEAR, LOC*CUL, LOC*DOS, CUL*YEAR, CUL*DOS, DOS*YEAR, LOC*YEAR*CUL, LOC*YEAR*DOS, CUL*YEAR*DOS, CUL*LOC*DOS, and LOC*YEAR*CUL*DOS on germination (%), node no., days to flowering (50%), days to maturity (50%), plant height (cm), biomass (g), 1000 seed weight (g), yield (kg ha⁻¹), seed Fe (µg g⁻¹) and Fe yield (g ha⁻¹) of eighteen chickpea cultivars with three doses of Fe-EDDHA over four replications at both sites.

Sources of Variation	df	Germination	Node Number	Days to Flowering	Days to Maturity	Plant Height	Biomass	1000 Seed Weight	Yield	Seed Fe Conc
LOC	1	68.3**	3.6 ^{ns}	1232**	536**	766**	101**	0.6 ^{ns}	1254**	1248**
YEAR	1	227**	93.8**	131**	12804**	5701**	0.8 ^{ns}	1385**	1367**	884**
REP	3	0.9 ^{ns}	3.0*	3.2*	1.9 ^{ns}	4.7*	2.4*	7.8**	22.0**	12.4**
CUL	17	4.3**	9.9**	12.2**	8.2**	17.5**	8.1**	116**	35.1**	19.3**
DOS	2	1.3 ^{ns}	0.7 ^{ns}	1.8 ^{ns}	2.7 ^{ns}	0.3 ^{ns}	61.7**	1.7 ^{ns}	0.0 ^{ns}	14.1**
LOC*YEAR	1	0.9 ^{ns}	19.0**	395**	968**	157**	1292**	99.0**	1866**	88.9**
LOC*CUL	17	1.4 ^{ns}	1.4 ^{ns}	2.1*	2.4*	1.9*	6.0**	3.1**	5.9**	2.5*
LOC*DOS	2	1.6 ^{ns}	1.4 ^{ns}	4.3*	15.8**	2.0 ^{ns}	25.5**	3.4*	3.6*	0.9 ^{ns}
CUL*YEAR	17	6.9**	3.5**	6.8**	6.1**	3.2**	9.4**	17.0**	7.7**	11.0**
CUL*DOS	34	1.2 ^{ns}	1.2 ^{ns}	0.6 ^{ns}	0.7 ^{ns}	0.5 ^{ns}	1.4 ^{ns}	0.7 ^{ns}	1.6*	0.6 ^{ns}
DOS*YEAR	2	2.5 ^{ns}	0.2 ^{ns}	1.4 ^{ns}	1.5 ^{ns}	2.6 ^{ns}	2.4 ^{ns}	2.0 ^{ns}	1.3 ^{ns}	4.1*
LOC*YEAR*CUL	17	1.5 ^{ns}	3.1**	6.0**	2.4*	2.6*	3.2**	4.2**	12.6**	2.0*
LOC*YEAR*DOS	2	2.0 ^{ns}	0.4 ^{ns}	0.5 ^{ns}	11.3**	0.6 ^{ns}	32.1**	1.5 ^{ns}	1.3 ^{ns}	1.4 ^{ns}
CUL*YEAR*DOS	34	1.1 ^{ns}	1.6*	0.7 ^{ns}	0.8 ^{ns}	0.7 ^{ns}	1.0 ^{ns}	0.8 ^{ns}	2.4**	0.7 ^{ns}
CUL*LOC*DOS	34	0.9 ^{ns}	0.8 ^{ns}	0.6 ^{ns}	0.7 ^{ns}	0.7 ^{ns}	1.1 ^{ns}	0.8 ^{ns}	1.7*	0.9 ^{ns}
LOC*YEAR*CUL*DOS	34	1.1 ^{ns}	1.3 ^{ns}	0.9 ^{ns}	0.8 ^{ns}	0.5 ^{ns}	1.9*	0.8 ^{ns}	1.5*	1.2 ^{ns}

* = significant at 5%, ** = significant at 1%, ns= nonsignificant, df = degrees of freedom.

Across the sixteen different factors, cultivar (CUL) and the interaction of cultivar and location (CUL*LOC) were highly significant ($p < 0.01$) for all parameters. Furthermore, the effect of location (LOC), year (YEAR), replication (REP), the interactions of location and year (LOC*YEAR), location and cultivar (LOC*CUL), and location, year and cultivar (LOC*YEAR*CUL) were also significant on most of the parameters. However, the effects of Fe fertilizer were significant only on plant biomass and seed Fe concentration. The interaction effects between cultivar and fertilizer dose (CUL*DOS), dose and year (DOS*YEAR), location, year, and dose (LOC*YEAR*DOS), cultivar, year, and dose (CUL*YEAR*DOS), cultivar, location, and dose (CUL*LOC*DOS) and location, year, cultivar, and

dose (LOC*YEAR*CUL*DOS) on most of the parameters were not significant. However, the interaction effects of location and dose (LOC*DOS) were significant on most of the parameters, except germination, node number, plant height, and seed Fe concentration (Table 5).

The effects of soil-applied Fe fertilizer across locations, cultivar, dose and their interaction(location*cultivar), (location*dose), (cultivar*dose), and (location*cultivar*dose) on disease ascochyta blight score of chickpea cultivars in 2016 are presented in Table 6.

Table 6. Analysis of variance and F values of the effect of location (LOC), replication (REP), cultivar (CUL), dose (DOS) and the interaction of LOC*CUL, LOC*DOS, CUL*DOS, and CUL*LOC*DOS on disease score of eighteen chickpea cultivars with three doses of Fe-EDDHA over four replications at both sites in 2016.

Sources of Variation	df	Disease score
LOC	1	62.4**
REP	3	37.7**
CUL	17	8.9**
DOS	2	2.1 ^{ns}
LOC*CUL	17	0.6 ^{ns}
LOC*DOS	2	13.2**
CUL*DOS	34	0.5 ^{ns}
LOC*CUL*DOS	34	0.7 ^{ns}

* = significant at 5%, ** = significant at 1%, ns= nonsignificant, df = degrees of freedom.

Across the eight different factors, location (LOC), cultivar (CUL), replication (REP) and the interaction of location and dose (LOC*DOS) were highly significant ($p < 0.01$) for ascochyta blight disease score. However, the effect of Fe fertilizer on the disease severity was not significant. The interaction effects between location and cultivar (LOC*CUL), cultivar and dose (CUL*DOS) and cultivar, location, and dose (CUL*LOC*DOS) on disease were also not significant (Table 6).

3.1. Biomass

The biomass data were based on the mean dry weight of randomly harvested five plants per plot. The mean biomass (g) of each of eighteen chickpea cultivars with three Fe fertilizer doses (0 kg ha⁻¹, 10 kg ha⁻¹, and 30 kg ha⁻¹) at both locations in 2015 and 2016 are presented in Table 7.

Table 7. The mean biomass (g plant⁻¹) of eighteen chickpea cultivars with three Fe fertilizer doses (0 kg ha⁻¹, 10 kg ha⁻¹, and 30 kg ha⁻¹) at Elrose and Moose Jaw in 2015 and 2016.

Cultivars	Elrose						Moose Jaw						Culti vars Mea n
	2015			2016			2015			2016			
	0 kg ha ⁻¹	10 kg ha ⁻¹	30 kg ha ⁻¹	0 kg ha ⁻¹	10 kg ha ⁻¹	30 kg ha ⁻¹	0 kg ha ⁻¹	10 kg ha ⁻¹	30 kg ha ⁻¹	0 kg ha ⁻¹	10 kg ha ⁻¹	30 kg ha ⁻¹	
1173-1	199	208	255	109	119	127	89	125	99	157	166	182	153
1460-2	175	232	244	112	116	124	118	89	105	171	257	171	159
AB06- 156-2	149	280	295	101	117	146	149	127	157	198	201	207	177
Amit	195	220	244	126	136	160	118	125	148	216	246	247	182
CA05-75-	179	198	263	125	128	149	87	108	139	173	189	263	167

CDC	183	170	208	82	92	100	102	119	85	162	121	113	
Alma													128
CDC	166	260	329	129	144	161	81	73	111	127	178	153	
Cabri													159
CDC	116	215	223	133	139	143	83	125	85	176	207	261	
Consul													159
CDC	167	226	260	147	148	170	129	138	134	196	217	219	
Corinne													179
CDC	167	250	262	160	162	174	109	143	123	229	245	301	
Cory													194
CDC	238	240	263	136	161	169	136	137	121	243	264	248	
Frontier													196
CDC	195	222	235	137	141	145	94	82	86	205	216	259	
Leader													168
CDC	150	248	304	115	134	130	102	90	93	100	164	165	
Luna													150
CDC	207	316	389	130	134	148	105	109	106	191	191	197	
Orion													185
CDC	166	170	215	119	132	141	113	112	95	222	229	266	
Palmer													165
CDC	201	259	261	127	129	157	157	127	149	132	238	141	
Vanguar d													173
X05TH20 -2	270	302	325	117	124	147	122	122	114	172	172	178	180
X05TH47 -3	160	166	258	129	156	186	131	134	111	210	214	223	173
Dose Mean	182	232	268	124	134	149	112	116	115	182	206	211	169
LSD0.05	51.9	72.9	58.1	30.0	29.6	39.8	40.9	37.7	36.4	55.9	53.9	71.7	48.2

LSD = Least Significant Difference.

The effects of locations, cultivars, and their interactions were significant on biomass (Table 5). Moreover, the main effect of Fe fertilizer on biomass was highly significant ($p < 0.01$). The highest biomass was obtained from cultivar CDC Frontier (196 g per plant) followed by CDC Cory (194 g per plant), whereas the lowest was found by CDC Alma (128 g per plant). The highest mean of biomass (268 g) was obtained with 30 kg ha⁻¹ of Fe fertilizer at Elrose in 2015. Biomass of the cultivars grown with no Fe fertilizer was significantly lower than the other two doses. However, some cultivars at Moose Jaw in 2015 obtained the highest biomass with no Fe fertilizer compared to the other two doses. For instance, cultivar CDC Luna had the highest biomass (102 g per plant) at 0 kg ha⁻¹ of Fe fertilizer compared to the other two doses (Table 7).

3.2. Seed Fe

The mean seed Fe concentrations (mg kg⁻¹) of eighteen cultivars with three Fe fertilizer doses (0 kg ha⁻¹, 10 kg ha⁻¹, and 30 kg ha⁻¹) at both locations in 2015 and 2016 are presented in Table 8.

Table 8. Seed Fe concentrations (mg kg⁻¹) of eighteen chickpea cultivars with three Fe fertilizer doses (0 kg ha⁻¹, 10 kg ha⁻¹, and 30 kg ha⁻¹) at Elrose and Moose Jaw in 2015 and 2016.

Cultivars	Elrose						Moose Jaw						Culti var Mea n
	2015			2016			2015			2016			
	0 kg ha ⁻¹	10 kg ha ⁻¹	30 kg ha ⁻¹	0 kg ha ⁻¹	10 kg ha ⁻¹	30 kg ha ⁻¹	0 kg ha ⁻¹	10 kg ha ⁻¹	30 kg ha ⁻¹	0 kg ha ⁻¹	10 kg ha ⁻¹	30 kg ha ⁻¹	
1173-1	43	48	49	61	63	67	38	37	41	46	46	52	49
1460-2	47	52	54	56	60	61	39	39	44	45	44	50	49
AB06-													
156-2	51	52	57	67	70	71	41	43	41	47	49	55	54
Amit	49	50	48	59	65	72	40	41	43	46	49	51	51
CA05-75-													
45	48	43	49	58	57	59	35	34	34	49	44	51	47
CDC													
Alma	43	50	50	65	72	80	38	39	39	48	50	54	52
CDC													
Cabri	46	46	54	57	57	60	37	40	40	47	48	51	49
CDC													
Consul	52	52	54	57	63	57	42	47	43	41	44	47	50
CDC													
Corinne	46	50	50	55	55	60	41	39	41	41	41	43	47
CDC													
Cory	47	48	51	56	60	64	42	44	45	42	45	48	49
CDC													
Frontier	57	55	55	66	73	76	43	45	46	49	52	57	56
CDC													
Leader	48	47	45	60	60	62	38	37	39	43	46	53	48
CDC													
Luna	48	47	48	78	73	80	38	39	37	49	56	56	54
CDC													
Orion	46	53	52	70	68	71	39	37	40	47	52	54	52
CDC													
Palmer	48	48	48	64	63	69	37	40	39	46	44	48	50
CDC													
Vanguar													
d	44	47	48	53	49	57	40	39	47	41	44	48	46
X05TH20													
-2	55	57	58	75	72	76	49	50	46	50	51	54	58
X05TH47													
-3	53	50	56	72	67	70	46	49	48	49	52	51	55
Dose													
Mean	48	50	51	63	64	67	40	41	42	46	48	51	51

LSD0.05	7.0	11.4	7.6	9.6	11.6	9.3	4.9	4.8	5.5	7.1	5.5	6.8	7.6
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LSD = Least Significant Difference.

The main effects of locations, years, and cultivars as well as their interactions were highly significant ($p < 0.01$) on seed Fe concentrations (Table 5). Moreover, significant differences were found among doses. Seed Fe concentrations obtained from Elrose in the year 2015 and 2016 were significantly higher than from Moose Jaw. The highest Fe concentration in seed was observed for cultivar X05TH20-2 (58 mg kg⁻¹) followed by CDC Frontier (56 mg kg⁻¹). Furthermore, the highest dose means (67 mg kg⁻¹) in seed Fe concentration was obtained at 30 kg ha⁻¹ of Fe fertilizer at Elrose in 2016 compared to all other doses at both locations in 2015 and at Moose Jaw in 2016. Seed Fe concentrations obtained from no Fe fertilizer was the lowest compared to the other two doses, except for cultivar X05TH47-3 at Elrose in 2016. The lowest dose means (40 mg kg⁻¹) in seed Fe concentration was observed under no Fe fertilizer treatment at Moose Jaw in 2015 compared to both locations in 2016 and Elrose in 2015 with other two doses (Table 8).

3.3. Fe Yield

The mean of Fe yield (g ha⁻¹) of eighteen chickpea cultivars with three Fe fertilizer doses (0 kg ha⁻¹, 10 kg ha⁻¹, and 30 kg ha⁻¹) at both locations in 2015 and 2016 are presented in Table 9.

Table 9. The mean of Fe yield (g ha⁻¹) of eighteen chickpea cultivars with three different doses (0 kg ha⁻¹, 10 kg ha⁻¹, and 30 kg ha⁻¹) of Fe-EDDHA at Elrose and Moose Jaw in 2015 and 2016.

Cultivars	Elrose						Moose Jaw						Mean
	2015			2016			2015			2016			
	0 kg ha ⁻¹	10 kg ha ⁻¹	30 kg ha ⁻¹	0 kg ha ⁻¹	10 kg ha ⁻¹	30 kg ha ⁻¹	0 kg ha ⁻¹	10 kg ha ⁻¹	30 kg ha ⁻¹	0 kg ha ⁻¹	10 kg ha ⁻¹	30 kg ha ⁻¹	
1173-1	311	347	316	129	116	31	86	83	111	141	131	170	164
1460-2	340	337	369	137	125	126	113	91	113	171	132	184	187
AB06-													
156-2	396	410	447	137	170	125	93	104	104	145	98	199	202
Amit	306	337	335	132	113	174	93	86	89	132	150	150	175
CA05-75-													
45	305	250	311	160	132	138	74	59	61	112	171	126	158
CDC													
Alma	234	250	247	62	65	63	89	79	109	.	.	.	133
CDC													
Cabri	291	326	319	284	172	187	74	99	90	152	72	107	181
CDC													
Consul	430	405	419	172	171	135	132	127	129	196	253	291	238
CDC													
Corinne	405	438	434	174	248	211	122	110	100	149	182	189	230
CDC													
Cory	376	351	374	262	264	155	122	144	159	127	203	141	223
CDC													
Frontier	443	433	427	130	150	314	140	131	131	78	62	75	209

CDC													
Leader	359	420	367	152	135	69	98	104	104	190	156	165	193
CDC													
Luna	282	294	291	149	112	141	100	107	110	.	.	.	136
CDC													
Orion	267	379	315	139	96	185	106	96	104	106	89	81	164
CDC													
Palmer	416	342	422	190	206	204	97	95	98	173	170	300	226
CDC													
Vanguard													
d	272	372	288	168	151	156	105	95	94	108	97	104	167
X05TH20													
-2	263	334	306	109	97	147	85	92	83	104	98	109	152
X05TH47													
-3	346	367	387	133	182	113	103	141	123	198	153	209	205
Dose													
Mean	335	355	354	157	150	148	102	102	106	135	131	155	186
LSD0.05	73.4	90.8	69.6	73.0	98.8	136.2	24.8	24.5	34.4	57.3	59.6	65.7	67.3

LSD = Least Significant Difference There were significant differences in Fe yield among cultivars and doses. Fe yield obtained from Elrose in 2015 was significantly higher than other location and year. The highest cultivars mean for Fe yield was obtained from CDC Consul (238 g ha⁻¹) followed by CDC Corinne (230 g ha⁻¹). Furthermore, Fe fertilizer with a dose of 10 kg ha⁻¹ and 30 kg ha⁻¹ yielded the highest Fe yield of 355 g ha⁻¹ and 354 g ha⁻¹, respectively, at Elrose in 2015 compared to the Fe yield at both locations in 2016 and at Moose Jaw in 2015. Fe yield obtained from plants with no application of Fe fertilizer was the lowest than the other two doses for most cultivars. However, some cultivars were able to produce the highest Fe yield with low dose (0 kg ha⁻¹) of Fe fertilizer compared to the other two doses. For instance, cultivar 1173-1 had the highest Fe yield (129 g ha⁻¹) at 0 kg ha⁻¹ of Fe fertilizer (Table 9).

4. Discussion

The application of synthetic Fe chelates on chickpeas across two years and two locations in Saskatchewan had significant effects ($p < 0.01$) on biomass and seed Fe concentration (Table 5). The chelate Fe fertilizer at 10 kg ha⁻¹ and 30 kg ha⁻¹ improved the seed Fe concentration across cultivars and environments. In 2015 and 2016, Elrose yielded higher levels of seed Fe concentration compared to Moose Jaw. Moreover, the highest seed Fe concentration (58.5 mg kg⁻¹) was obtained from CDC Frontier with 30 kg ha⁻¹ dose of chelate Fe fertilizer, whereas the lowest (44.5 mg kg⁻¹) was obtained from CDC Vanguard with no fertilizer application. At 30 kg ha⁻¹ Fe fertilizer, seed Fe concentration of CDC Frontier at Elrose and Moose Jaw in 2016 increased by 15% and 16%, respectively, compared to control. However, the seed Fe concentrations at different doses across environments only gained 5-11% increase compared to control. This suggests that the application of chelated Fe, which is the dominant form of Fe in the alkaline soil, provided readily available Fe to the roots of chickpea plants. Consequently, Fe concentration in seeds increased compared to control. Our findings are in agreement with findings of Moraghan et al. [46] who reported that application of Fe-EDDHA increased seed Fe concentration in common bean. Moreover, the highest seed Fe concentration (65 mg kg⁻¹) was observed at Elrose in 2016 compared to the rest of the environments (Table 8). The cultivars X05TH20-2 (58 mg kg⁻¹) and CDC Frontier (56 mg kg⁻¹) had the highest mean of seed Fe concentration compared to the rest of the cultivars, whereas CDC Vanguard had the lowest

concentration (46 mg kg⁻¹). The variability in Fe concentrations was mostly attributed to cultivars across locations and years. Similar findings were previously reported in chickpea [30].

Elrose location produced higher Fe yield compared to Moose Jaw in 2015 and 2016. The highest Fe yield (447 g ha⁻¹) was found on AB06-156-2 cultivar at 30 kg ha⁻¹ of chelate Fe fertilizer. At 30 kg ha⁻¹ fertilizer rate, Fe yield of AB06-156-2 cultivar at Elrose in 2015 increased by 12% compared to control. The overall Fe yield increased varying from 4 to 19% in parallel with the Fe fertilizer doses across environments (Table 9). Kumar et al. [47] reported that the application of varying levels of Fe fertilizer up to 10 kg ha⁻¹ significantly increased Fe concentration in chickpea grain over control. Similar findings were also reported by Sharma et al. [48] who observed that the application of chelated Fe fertilizer improved the Fe content in seeds of pigeon pea.

Locations, cultivars, and their interaction significantly affected biomass. The highest biomass (228 g per five plants) was observed at Elrose in 2015 compared to the rest of the environments. These findings are similar to those reported by Kumawat et al. [49] and Sahu et al. [50] who observed that soil-applied Fe fertilizer increased biomass yield in chickpea. Similarly, in cowpea, Mahriya and Meena [51] reported that the application of Fe fertilizer improved biomass, which is consistent with our findings. Furthermore, the results are also similar to the findings of Bansal and Chahal [52] who reported that application of 25 µg g⁻¹ Fe in mung bean grown in an alkaline soil significantly increased biomass and Fe content, which is in agreement with our findings. However, previous studies done in chickpea and soybean reported that application of Fe-EDDHA did not result in significant increase in biomass, which contrasts with our findings [53,54,55].

The present study also showed that ascochyta blight disease affected the yield in both locations in 2016 (Table S1.). The correlation analysis showed that ascochyta blight and yield were highly correlated ($r = 0.75$; $p < 0.01$) at Moose Jaw in 2016 (Table S2.). Due to ascochyta blight, Fe was most likely distributed to relatively a smaller number of plants that ultimately increased seed Fe concentration level at Moose Jaw location in 2016 compared to 2015. These findings suggested that the magnitude of the effects of ascochyta blight on seed Fe concentration depended on the cultivars and environments.

Other characteristics such as thousand seed weight, and seed yield varied significantly ($p < 0.01$) among locations, years, cultivars, and their interactions in the two-year experiment (Table 5). For thousand seed weight, both locations in 2015 produced larger seed size compared to 2016. This is mostly attributed to ascochyta blight disease that affected the plants in both locations in 2016 (Table S1). As a result, thousand seed weight decreased. Moreover, the highest location means of thousand seed weight (363 g) was observed at Elrose in 2015, whereas the lowest (250 g) was found at Elrose in 2016 (Table S3.). The highest grain yield (6904 kg ha⁻¹) was observed at Elrose in 2015, whereas the lowest (2421 kg ha⁻¹) was found at Elrose in 2016. CDC Corinne had the highest mean yield (4832 kg ha⁻¹), whereas CDC Alma had the lowest yield (2137 kg ha⁻¹) (Table S4.). Mevada et al. [56] also reported that the application of Fe chelates increased grain yield significantly over control in urdbean. Similar findings were also observed by Kumar et al. [47] and Sahu et al. [50] who reported that the application of Fe fertilizer increased grain yield of chickpea by 17.3%. Furthermore, thousand seed weight and seed yield were higher in resistant cultivars such as CDC Corinne, CDC Consul, CDC Leader, and CDC Frontier compared to the susceptible cultivars CDC Alma and CDC Luna. The variations in thousand seed weight and seed yield among cultivars could be due to the differences in their genetic constitution, physiology, and the cultivar response to various environmental conditions. Similar observations were also made by many authors in previous studies in chickpea and common bean [57,30].

The effects of Fe fertilizer on germination, node number, days to flowering, days to maturity, and plant height were not significant (Table 1). However, locations, years, cultivars and their interactions significantly affected germination, node number, days to flowering, days to maturity, and plant height. Germination at both locations in 2015 was higher than in 2016. For instance, germination at Elrose in 2015 was 10% and 4% higher than in Elrose and Moose Jaw in 2016. However, node number, days to flowering, days to maturity, and plant height were higher at both locations in 2016 than 2015. The variations in the above-mentioned characteristics were mostly due to ascochyta

blight infestation, cultivar response to various environmental conditions, genetics, and physiology. Current findings were consistent with previous studies in mung bean, chickpeas, common bean that showed application of Fe fertilizer did not improve vegetative growth attributes [30,47,57,58,59]. However, previous studies in cowpea, black gram, and pea showed that growth characteristics were increased with the application of Fe fertilizer, which are in contrast with our findings [60-62].

5. Conclusions

Chickpea is a daily staple in many developing countries where people often affected by Fe deficiency and Fe deficiency related anemia. Improvement of Fe concentration in seeds of chickpea along with increasing consumption is one of the major strategies to correct Fe deficiency. The present study demonstrated that chickpea contained Fe at 3.4-8.0 mg 100 g⁻¹ concentration. One of the major findings from this study is chelate Fe fertilizer 30 kg ha⁻¹ increased the seed Fe concentration. Results from soil-applied Fe fertilizer showed that the highest seed Fe concentration was obtained from X05TH20-2 and CDC Frontier cultivars (58 and 56 mg Fe kg⁻¹ seed, respectively). Therefore, Fe biofortified in seeds of those two cultivars can provide approximately 6 mg Fe 100⁻¹ g seeds. Thus, a 58 and 83 g serving of Fe biofortified chickpea seeds can provide adequate amount (50%) of Fe for children at the age groups 1-3 (7 mg Fe day⁻¹) and 4-8 (10 mg day⁻¹) years, respectively. Moreover, at the age group 19-50 years, 67 and 150 g of Fe biofortified chickpea seeds can provide adequate amount (50%) of Fe for men (8 mg Fe day⁻¹) and women (18 mg Fe day⁻¹).

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. **Supplementary Table S1.** Disease score (0-9) of eighteen chickpea cultivars with three different doses of Fe-EDDHA at Elrose and Moose Jaw in 2016. **Supplementary Table S2.** Correlation between ascochyta blight score and yield as well as Fe yield of eighteen chickpea cultivars with three different doses of Fe-EDDHA at Elrose and Moose Jaw in 2016. **Supplementary Table S3.** Thousand seed weight (g) of eighteen chickpea cultivars with three different doses of Fe-EDDHA at Elrose and Moose Jaw in the year 2015 and 2016. **Supplementary Table S4.** Average yield (kg ha⁻¹) of eighteen chickpea cultivars across all doses of Fe-EDDHA at Elrose and Moose Jaw in the year 2015 and 2016

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