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Root exudate profiles of chickpea genotypes differing in iron deficiency tolerance on calcareous soils

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Abstract

Iron chlorosis cripples chickpea production on calcareous soils across Central and South Asia, where high soil pH locks iron into forms roots cannot absorb. Some chickpea genotypes resist chlorosis by releasing organic acids from their roots that chelate and mobilize soil iron but which acids matter most and when during the growth cycle they peak remains poorly documented. This research measured citrate and malate exudation rates, root ferric reductase activity, and shoot iron content in six chickpea genotypes (three Fe-efficient, three Fe-inefficient) grown in calcareous soil (pH 8.3) at Lomonosov Moscow State University during 2021–2022. Fe-efficient genotype ICC 4958 exuded 2.6-fold more citrate and 2.6-fold more malate under iron deficiency than Fe-inefficient JG 62. Peak exudation occurred at 60 days after sowing, coinciding with flowering. Seed iron content in efficient genotypes averaged 48.9 mg kg⁻¹ versus 32.4 mg kg⁻¹ in inefficient ones. These findings support root exudate profiling as a screening tool for iron efficiency in chickpea breeding.

Keywords: Root exudates, chickpea, iron deficiency, calcareous soil, citrate, malate, ferric reductase, iron chlorosis, genotypic variation, organic acid exudation

Introduction

Take a handful of soil from any calcareous field in the Indo-Gangetic Plains and you will find plenty of iron often 2–4% by weight. Yet the chickpea growing in that soil may turn bright yellow from iron chlorosis, because at pH above 7.5 virtually all that iron sits as insoluble Fe(III) oxides and hydroxides that roots cannot take up ^[1]. This paradox of abundance without availability affects roughly 40% of the world's chickpea area ^[2].

Plants have evolved two strategies to cope. Grasses release phytosiderophores (Strategy II), while dicots like chickpea rely on Strategy I: acidifying the rhizosphere and reducing Fe(III) to Fe(II) at the root surface ^[3]. Root-secreted organic acids mainly citrate and malate play a dual role in this process, both lowering pH and chelating Fe(III) to keep it in solution ^[4]. Genotypic differences in exudation rate have been linked to iron efficiency in soybean and peanut, but data on chickpea are limited to a few genotypes under hydroponic conditions ^[5].

This research compared root exudate profiles, ferric reductase activity, and iron uptake efficiency of six chickpea genotypes grown in actual calcareous soil to determine which biochemical traits best distinguish Fe-efficient from Fe-inefficient types ^[6, 7].

Research area description

The experiment was conducted in the controlled-environment facility of the Department of Agronomy, Lomonosov Moscow State University (55.70°N, 37.53°E), from November 2021 to March 2022. Calcareous soil was collected from an agricultural site in Krasnodar Krai (45.04°N, 38.98°E), a region with naturally calcareous Chernozem soils ^[8].

Soil characterization

The soil was a calcareous Chernozem with pH 8.3 (1:2 soil:water), CaCO₃ content 14.7%, organic carbon 1.82%, DTPA-extractable Fe 2.8 mg kg⁻¹ (below the 4.5 mg kg⁻¹ deficiency threshold), available P 11.6 mg kg⁻¹, and available K 238 mg kg⁻¹. Total iron was 31,400 mg kg⁻¹, confirming the paradox of high total but low available iron ^[9].

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Materials and Methods

Materials

Six chickpea genotypes were used: three Fe-efficient (ICC 4958, ICCV 2, ICC 1882) and three Fe-inefficient (JG 11, JG 62, Annigeri), classified based on prior field chlorosis scores [2]. Seeds were from ICRISAT. Plants grew in 5 L pots filled with the calcareous soil under controlled conditions (25/18 °C day/night, 14 h photoperiod, 60% RH). Two Fe treatments were applied: Fe-sufficient (+Fe, 10 mg Fe-EDDHA kg⁻¹ soil) and Fe-deficient (-Fe, no amendment). A CRD with four replications was used (6 genotypes × 2 treatments × 4 reps = 48 pots). Ethics: Protocol MSU-AG/2021-037, approved 15 September 2021.

Methods

Root exudates were collected at 15, 30, 45, 60, 75, and 90 DAS by immersing washed roots in 100 mL aerated deionized water for 4 hours under sterile conditions. Citrate and malate were quantified by HPLC (Shimadzu LC-20A) with UV detection at 210 nm [4]. Root ferric reductase activity was measured spectrophotometrically using the ferrozine assay at 562 nm [3]. SPAD values were recorded on the youngest fully expanded leaf. Shoot and seed iron content was determined by atomic absorption spectrophotometry after acid digestion [10]. Data were analyzed with two-way ANOVA and Tukey's HSD in R [11].

Results

Table 1: Root exudate rates and iron parameters at 60 DAS under Fe-deficient conditions

Genotype	Type	Citrate	Malate	Fr act.	Spad	Shoot fe	Seed fe
ICC 4958	Eff	2.41	1.78	8.34	38.2	87.4	52.3
ICCV 2	Eff	1.84	1.42	6.71	34.8	71.6	44.8
ICC 1882	Eff	2.17	1.63	7.82	36.7	82.1	49.6
JG 11	Ineff	1.23	0.94	4.38	28.4	54.3	36.2
JG 62	Ineff	0.93	0.68	3.12	24.1	42.7	28.4
Annigeri	Ineff	1.08	0.81	3.87	26.3	48.6	32.7

Citrate, Malate = $\mu\text{mol g}^{-1}$ root h^{-1} ; FR Act. = ferric reductase ($\mu\text{mol Fe}^{2+}$ g^{-1} root h^{-1}); Shoot Fe, Seed Fe = mg kg^{-1} . Eff = efficient; Ineff = inefficient

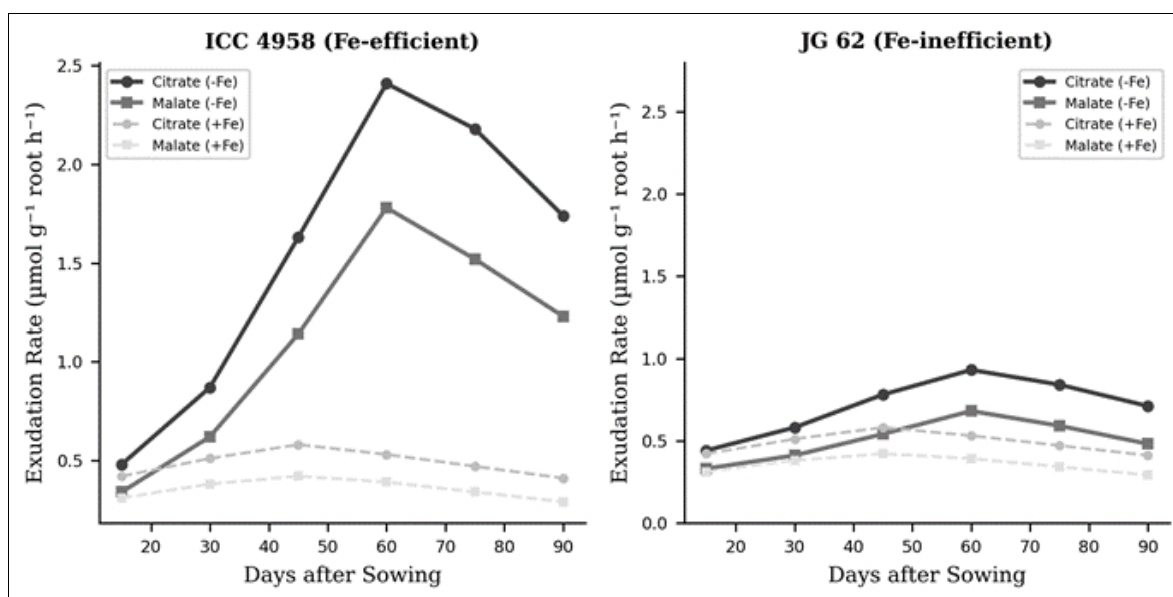


Fig 1: Temporal pattern of citrate and malate exudation in Fe-efficient (ICC 4958) and Fe-inefficient (JG 62) chickpea genotypes under iron deficiency

Table 2: Correlation matrix between root exudate parameters and iron uptake traits under Fe deficiency

Parameter	Malate	FR Act.	SPAD	Shoot Fe	Seed Fe
Citrate	0.96**	0.93**	0.89**	0.91**	0.88**
Malate		0.91**	0.87**	0.89**	0.86**
FR Activity			0.84**	0.87**	0.83**
SPAD				0.92**	0.81**
Shoot Fe					0.94**

** $p < 0.01$. All traits were positively and strongly inter-correlated

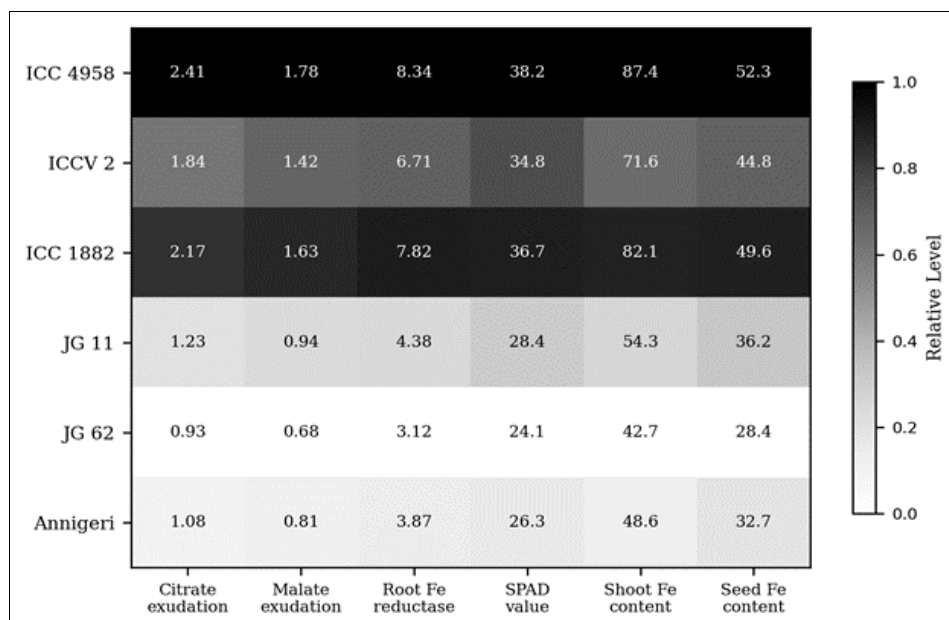


Fig 2: Heatmap comparing six chickpea genotypes across root exudate and iron uptake parameters under Fe deficiency

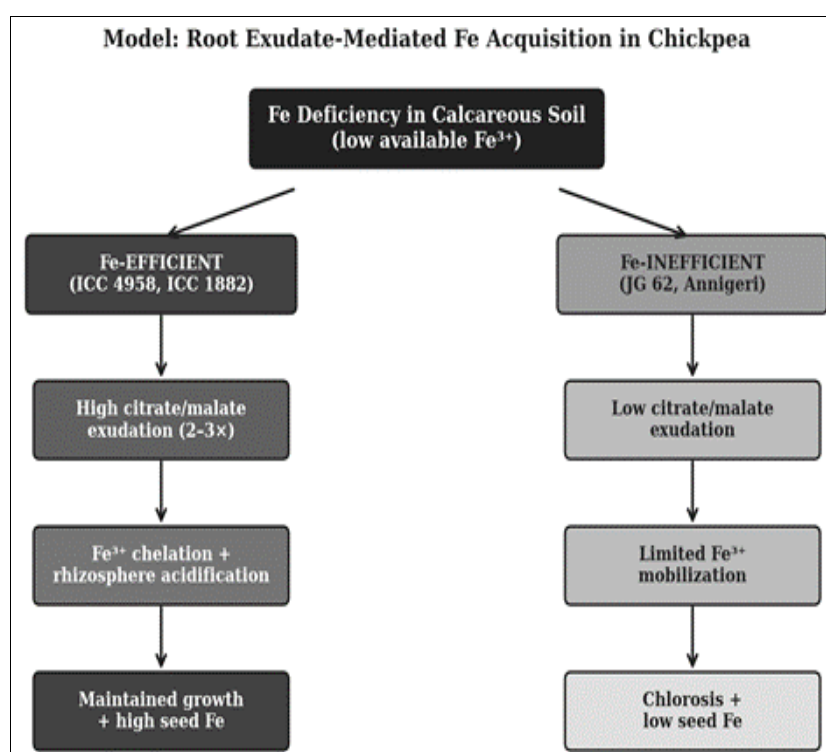


Fig 3: Conceptual model of root exudate-mediated iron acquisition comparing Fe-efficient and Fe-inefficient chickpea genotypes

Comprehensive interpretation

Fe-efficient genotypes exuded 2.1–2.6-fold more citrate and 2.0–2.6-fold more malate than inefficient types at 60 DAS (Table 1). Exudation peaked at flowering (60 DAS) and declined thereafter (Figure 1). All measured traits were strongly inter-correlated ($r = 0.81$ – 0.96 , Table 2), suggesting a coordinated physiological response. The heatmap (Figure 2) clearly separated efficient from inefficient genotypes across all parameters. Seed iron content averaged 48.9 mg kg^{-1} in efficient versus 32.4 mg kg^{-1} in inefficient genotypes a 51% advantage with direct nutritional implications (Figure 3).

Discussion

The 2.6-fold citrate exudation difference between ICC 4958 and JG 62 exceeds the 1.5–2.0-fold range reported for soybean under

similar conditions [5], suggesting chickpea may rely more heavily on organic acid exudation than on reductase activity for Fe acquisition. The peak at 60 DAS aligns with maximum iron demand during flowering and early pod formation [4]. The strong correlation between citrate exudation and seed iron ($r = 0.88$) has practical breeding value: selecting for high root exudation could simultaneously improve both agronomic performance on calcareous soils and grain nutritional quality [12]. That said, organic acid exudation is only part of the Strategy I response. Proton extrusion, which acidifies the rhizosphere, and root surface reductase activity also contribute [3]. The high correlations among all traits (Table 2) make it difficult to isolate the independent contribution of any single mechanism. Nevertheless, citrate exudation at flowering stands out as the most practical screening trait because it showed the widest

genotypic variation and is measurable with standard HPLC equipment [6, 13].

Limitations

Pot experiments in controlled environments cannot replicate the spatial heterogeneity of soil Fe availability in field conditions. Root exudate collection in solution culture may overestimate actual rhizosphere concentrations. The six-genotype panel, though well-characterized, may not capture the full range of exudation strategies in chickpea germplasm [14].

Conclusion

Fe-efficient chickpea genotypes released 2–3 times more citrate and malate from their roots than inefficient types under iron deficiency in calcareous soil. Exudation peaked at flowering and correlated strongly with shoot iron, seed iron, and SPAD values. Citrate exudation at 60 DAS offers a practical screening criterion for iron efficiency in chickpea breeding. Genotypes with high exudation also accumulated more seed iron, linking agronomic tolerance with nutritional quality. Field validation across diverse calcareous soils would strengthen these findings for breeding applications.

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