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ORIGINAL RESEARCH

Genotypic differences in response of durum wheat (*Triticum durum* Desf.) to lime-induced iron chlorosis

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Abstract

Wheat, durum wheat, is the first cereal cultivated and consumed in Tunisia. Because the dominance of calcareous soils in its agroecological systems, known by their low availability of iron (Fe) inducing Fe chlorosis and limiting crop production, its yield remains low. Therefore, the search for tolerant genotypes is always current. In this context, the physiological behavior of six Tunisian genotypes of durum wheat (salim, karim, razek, khiar, inrat100, and maali) cultivated on calcareous and fertile soils for 2 months in a pot experiment was investigated. A greenhouse was used to conduct experiments under natural light. Plant growth, SPAD index, Fe nutrition, Fe distribution, and photosynthesis were monitored and used to evaluate and discriminate their respective physiological responses. On calcareous soil, results revealed reduced plant growth, active Fe, SPAD index, and net photosynthesis. Genotypic differences in the response of wheat to calcareous-induced Fe deficiency were observed and allowed to classify the genotypes Salim and Karim as relatively tolerant. These genotypes expressed Fe translocation capacity (FeT) up to 3 times, Fe use efficiency for photosynthesis (FeUEAn) up to 1.6 times, and chlorophyll use efficiency for photosynthesis (ChlUEAn) up to 3.5 times greater than that expressed by the other genotypes, particularly inrat100 and maali. Thus, the relative tolerance of Salim and Karim is the result of the high ability of Fe uptake and translocation to shoots to support chlorophyll biosynthesis, photosynthesis, and plant growth as well as an important Fe and chlorophyll use efficiency.

KEYWORDS
calcareous soils, Fe deficiency, Fe use efficiency, net photosynthesis, SPAD index, wheat

1 | INTRODUCTION

Iron is quantitatively the fourth element of the earth's crust, after oxygen, silicon, and aluminum. In the crop plants, concentrations ranging from 50 to 150 μg g^{-1} dry matter are critical. Low concentrations induce Fe deficiency responsible of several qualitative and quantitative consequences for agricultural production. These problems are commonly associated with Tunisian calcareous soils where Fe availability is low (Krouma et al., 2008). Indeed, Fe is an essential micronutrient playing a central role in many vital processes such as photosynthesis, respiration, and symbiotic nitrogen fixation (Ferhi

Abbreviations: An, net assimilation; ChlUEAn, chlorophyll use efficiency for photosynthesis; DW, dry weight; ET, evapotranspiration; FeT, Fe translocation; FeUEAn, Fe use efficiency for photosynthesis; SC, stomatal conductance.

et al., 2017; Krouma et al., 2008; Slatni et al., 2009). Typically, around 80% of Fe is found in photosynthetic cells where it is involved in the biosynthesis of cytochromes and other heme molecules, including chlorophyll and electrons transport chain (Briat et al., 2007; Hansch & Mendel, 2009). Thus, Fe deficiency affects the structure, the development, and the function of the entire photosynthetic apparatus. It has been shown that Fe deficiency decreases light-harvesting pigments, particularly chlorophylls (Ferhi et al., 2017), and promotes antenna disconnection in PSII (Morales et al., 2001).

The strategy I plant, represented by dicotyledons and monocots other than grasses, takes up Fe in three steps: First, Fe is solubilized through rhizosphere acidification by proton pump and secretion of phenolic compounds. Second, a specific Fe reductase reduces Fe^{3+} to Fe^{2+} that is finally transported into the epidermis cell by Fe transporters (Kobayashi et al., 2010). Inside the plant, citric acid or nicotinamide chelates Fe^{3+}/Fe^{2+} for further transport via the xylem or the phloem. Fe uptake in strategy II plants, represented by grasses, consists of two steps: In the first step, roots export phytosiderophores (PS) into the rhizosphere to solubilize Fe^{3+} ions. In the second, specific protein transporters transport the Fe^{3+}–PS complex. Ogo et al. (2011) demonstrated that grasses induce Fe acquisition systems when Fe availability is low. Masuda et al. (2017) observed that mugineic acid efficiently solubilizes inorganic Fe^{3+} producing Fe^{3+}–mugineic acid complexes absorbed by specific transporters. However, the amount of PS exuded by the plant depends significantly on the amount of PS synthesized in the root and available for release in the rhizosphere (Singh et al., 2017) and root architecture (Bernards et al., 2002; Oburger et al., 2014). The biosynthesis of phytosiderophore is regulated by the Fe deficiency-induced activities of nicotianamine synthase (NAS) and nicotianamine aminotransferase (NAAT). Bernards et al. (2002) observed a higher release of PS from the apical than the primary root system of maize. Garnica et al. (2018) reported that shoots' Fe status directly modulates root-released PS via signaling pathways involving auxin and other possible effectors in Fe-deficient wheat. Recent, Astolfi et al. (2020) reported that tomato (a strategy I plant) can also produce PS (strategy II) when exposed to both Fe and Sulfur stress, suggesting that the distinction between strategy I and strategy II responses is not so clear. Atencio et al. (2021) identified numerous differentially expressed genes associated with soybean stress tolerant responses including the cell cycle, gene silencing, iron acquisition, and defense.

In Tunisian calcareous soils, Fe exists dominantly under insoluble and unavailable forms for plants. These soils cover no more than 10% of the plant's Fe needs (Mortvedt, 1991). Thus, Fe deficiency symptoms are commonly observed on these soils leading to a significant reduction of yield in the agricultural systems. Wheat is one of the most widely cultivated plants in Tunisia, with 1.078 thousand tons produced in 2020. In the world, wheat feeds 40% of the population and provides 20% of the energy for human nutrition (Gupta et al., 2005). However, the wheat yield remains low and unstable because of the pedoclimatic conditions of which soil fertility is the major constraint. Calcareous soils are among these problematic agricultural agroecosystems that continue to hamper the National production of grasses and other cultivated species. Scientific research has focused on abiotic stresses like drought and salinity. However, few results are published on the interaction between soil quality and plant behavior in Tunisia. In particular, the physiological and biochemical responses of wheat (*Triticum durum* Desf.) to induced Fe deficiency commonly observed in calcareous soils are lucking. Still, research in this respect could help breeders increase yield and grain Fe content and growers to optimize agricultural management. Although some authors have demonstrated the importance of the foliar application of Fe (Yaseen et al., 2013), this chemical approach (by injection, spraying, or directly in the soil) remains a failing strategy because of the rapid transformation of Fe to an insoluble form, the cost of chemical fertilizers, and the need for multiple applications. Nevertheless, this strategy has adverse economic and ecological impacts. Development of sustainable agricultural practices will require crops with improved Fe nutrition in problematic, but overspread, cultivated soils in order to reduce the application of these fertilizers. The identification of the physiological, biochemical, and molecular traits and markers of crop tolerance to Fe deficiency represents useful tools for breeding programs. This allows to identify genotypes with high efficiency of nutrient use in calcareous soils. The present study is part of this approach and consists of investigating the genotypic differences in response of wheat to the commonly observed lime-induced Fe deficiency in calcareous soil. A deep analysis of plant growth, chlorophyll status, photosynthesis, and Fe nutrition and distribution was carried out on six Tunisian genotypes of *T. durum* Desf. provided for us by the National Institute of Field Crops (Kef, Tunisia). The relationships between all these physiological functions are established, and valuable traits for further screening programs are identified.

2 | MATERIALS AND METHODS

2.1 | Plant material and experimental design

Six Tunisian wheat (*T. durum* Desf.) genotypes provided to us by the National Institute of Field Crops (Kef, Tunisia) are used (karim, salim, màali, razek, khiar, and inrat100). Healthy grains of uniform size are germinated directly in 1-L plastic pots (5 grains/pot) containing either fine, mixed fertile soil sampled in the region of Gatrana (Sidi Bouzid, 35°9′52.366″ N 9°40′23.689″ E) or calcareous soil sampled in the region of Faiedh (sidi Bouzid, 35°4′38.536″ N 9°40′32.167″ E). Table 1 summarizes the physicochemical characteristics of used soils. Cultures were conducted in a greenhouse, at the Faculty of Sciences and Techniques of Sidi Bouzid (35°2′7.58″ N 9°29′ 2.18″ E), under natural light with a 16-h photoperiod and a temperature of 25°C/17°C (±2°C, day/night), relative humidity about 75% and using tap water for irrigation.

After 2 weeks, homogenous plantlets from each genotype were maintained individually at one plant per pot. The experimental design was 2 factorials arranged in a completely randomized design with

TABLE 1 Main characteristics of used soils

Parameters	FS	CS
pH	7.9	9
Organic matter (%)	2.26	4.93
Active lime (%)	4.6	17.3
Total carbonates (%)	10.45	33.02
K (%)	1.148	0.632
Mg (%)	0.498	0.831
N (%)	0.62	0.42
C (%)	0.93	1.25
P (%)	0.144	0.224

Abbreviations: CS, calcareous soil sampled in the region of Faiedh (Sidi Bouzid, 35°4′38.536″ N 9°40′32.167″ E) ; FS, fertile soil sampled in the region of Gatrana (Sidi Bouzid (35°9′52.366″ N 9°40′23.689″ E).

10 replications (10 pots containing one plant each). After 4 weeks of culture, nondestructive measurements (SPAD index and gas exchange parameters) were made. Afterwards, plants were harvested, separated into shoots and roots, dried at 60°C for 72 h, and then pulverized into a fine powder for further analysis.

2.2 | SPAD index measurements

Relative leaf chlorophyll concentrations were estimated in vivo using a SPAD-502 (Konica-Minolta, Japan) before gas exchange measurements on the third fully expanded apical leaves. Measurements were made on the 10 plants for each genotype and soil. Results are presented as means of 10 replicates per genotype and soil. Values are expressed as SPAD units.

2.3 | Gas exchange measurement

Gas exchange measurements were made with an LI-6400 (LI-COR, Inc.) portable gas exchange system. Measurements were carried out on the same third fully expanded apical leaves from 10 plants for each genotype and soil. Photosynthesis was induced with saturating light (1000 μmol m^{-2} s^{-1}). This light was fitted to the standard 6 cm^2 clamp on the leaf chamber. Sample pCO_2, flow rate, and temperature were kept constant at 362 mbar, 500 μmol s^{-1}, and 25°C, respectively.

2.4 | Active Fe determination

Fe was extracted from powdered samples digested using the HCl method; 25 mg of fine powder was digested in 5 mL of 1N hydrochloric acid solution for 3 days according to Köseoglu and Açikgöz (1995), then filtered. Fe content was determined by the atomic absorption spectrophotometry method.

2.5 | Statistical analysis

Data and statistical analyses were performed using the software StatPlus Pro. All data are presented as mean ± standard deviation error. Analysis of variance (ANOVA) was performed to determine whether the effects of treatments (fertile soil, calcareous soil) on the respective factor were significant. The significance of differences among treatment means was determined by Fisher's least significant difference (LSD) test at 5%. Treatment means were declared significant when the difference between any two treatments was greater than the LSD value generated from the ANOVA. They are marked by different letters in the figures.

3 | RESULTS

3.1 | Plant morphology and biomass production

Daily monitoring of the plant morphology showed specific Fe chlorosis in young leaves of plants grown on calcareous soil. This chlorosis depends on the genotype and increased gradually with time. It appeared early in inrat100 and maali genotypes after 20 days of cultivation on calcareous soil and successively for the genotypes khiar, razek, karim, and salim. No chlorosis was observed on fertile soil for none of the genotypes. The SPAD index that reflects the chlorophyll level in leaves confirmed these observations. Measurements clearly showed that plants' cultivation on calcareous soil led to a significant reduction of the SPAD index in all genotypes. This decrease is genotype-dependent ranging successively from 17% in salim, 27% in karim and razek, 29% in inrat100, to 39% in maali (Figure 1a).

In association with Fe chlorosis and chlorophyll degradation symptoms, plants growing on fertile soil displayed clearly a better development than those growing on a calcareous soil. The quantification of biomass production demonstrated that, depending on the genotype, plant growth decreased significantly on calcareous soil (Figure 1b). Salim appeared the least affected (−19% of biomass production on calcareous soil compared to fertile soil) and maali the most affected genotype (−49% of biomass production on calcareous soil compared with fertile soil). The other genotypes occupied an intermediate position with a biomass reduction ranging from 32% to 44%.

3.2 | Gas exchange

The measurements made for gas exchange parameters demonstrated that plants' cultivation on calcareous soil significantly inhibited net photosynthetic assimilation (*An*) compared with control plants cultivated on fertile soil. The genotype that demonstrated best plant growth and chlorophyll accumulation was salim. It maintained this performance in photosynthesis. As compared with control ones, plants cultivated on calcareous soil decreased their *An* by 22%, 23%, 36%, 56%, 72%, and 78%, respectively, in salim, karim, razek, khiar, inrat100, and maali. Although it decreased on calcareous soil, this

activity was 1.2 times more important in salim than in karim and razek, 2.0 times in salim than in khiar, 3.5 times in salim than in inrat100, and 4 times in salim than in maali (Figure 2). The evapotranspiration (ET) also decreased significantly in plants cultivated on calcareous soil. This decrease was estimated to 22% in salim, 40% in karim, 32% in razek, 38% in khiar, 29% in inrat100, and 39% in maali (Figure 3). Simultaneously, the stomatal conductance (SC) was significantly affected on calcareous soil. It decreased by 22% in salim and karim, 30% in razek and maali, 47% in khiar, and 34% in inrat100 (Figure 4). Taken together, gas exchange, growth, and chlorophyll results revealed that the genotype that demonstrated better tolerance/adaptation to grow on calcareous soil was able to maintain adequate chlorophyll content and photosynthetic activity.

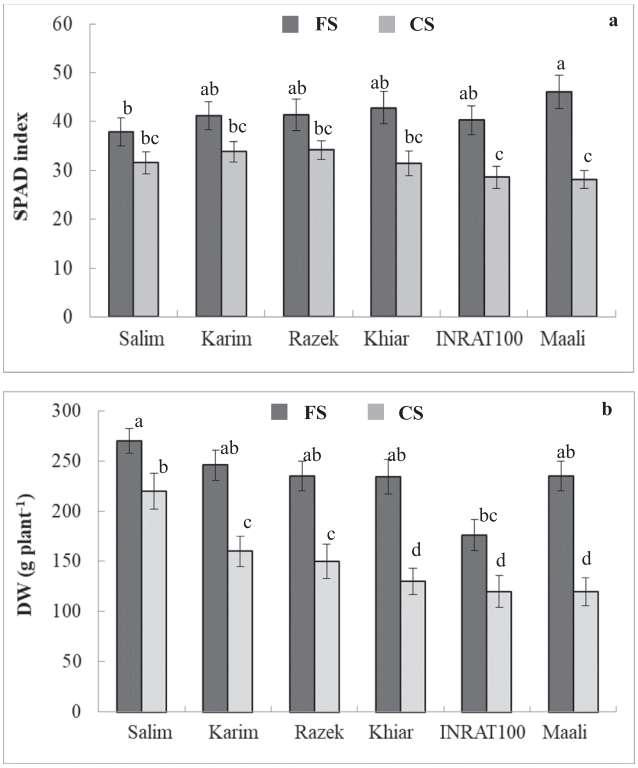

FIGURE 1 Effect of lime-induced iron deficiency on SPAD index (a) and dry weight production (b) in Tunisian wheat (*Triticum durum* Desf.) genotypes. FS: fertile soil, CS: calcareous soil. Within columns, means with the same letter are not significantly different at $\alpha = .05$ according to Fisher's least significant difference. Bars on the columns represent the standard error of the mean ($n = 10$)

3.3 | Fe nutrition and distribution

Analysis of the active physiological form of iron (Fe^{2+}) demonstrated that, compared with fertile soil, the cultivation of wheat plants on calcareous soil led to a drastic decrease in Fe nutrition among all genotypes (Table 2). The genotypic differences previously observed for the above measurements were also expressed for Fe nutrition and distribution. Thus, Shoot Fe concentration decreased on calcareous soil by 19%, 27%, 39%, 55%, 54%, and 57%, respectively, in salim, karim, razek, khiar, inrat100, and maali. The same behavior was observed for the Fe concentration in roots where the decrease ranged from 20% in karim and razek to 52% in inrat100. By comparing Fe distribution in plants cultivated on calcareous soil, concentrations in roots were 2 (in most tolerant genotype, salim) to 4 (in the most sensitive genotype, maali) times more important than in shoots. This result suggests that there is a problem of Fe uptake and distribution in the plant when cultivated on calcareous soil. For this purpose, we calculated the Fe translocation (FeT) expressed as the percentage of Fe translocated to shoots, compared with total Fe accumulated in plants (Table 2). The results demonstrated that FeT decreased in plants cultivated on calcareous compared with fertile soil, by only 8% in salim, demonstrating that the better tolerance of this genotype would be due, at least in part, to its potential to preserve a better ability to allocate Fe to its photosynthetic organs. This decrease ranges from 24% in karim up to 72% in the most sensitive genotype maali.

On the basis of these results, we may suggest that the behavior of wheat genotypes on calcareous soil depends on their ability to uptake and allocate Fe to the shoots. When we represent the shoot growth as a function of its Fe concentration (Figure 5), we obtain a

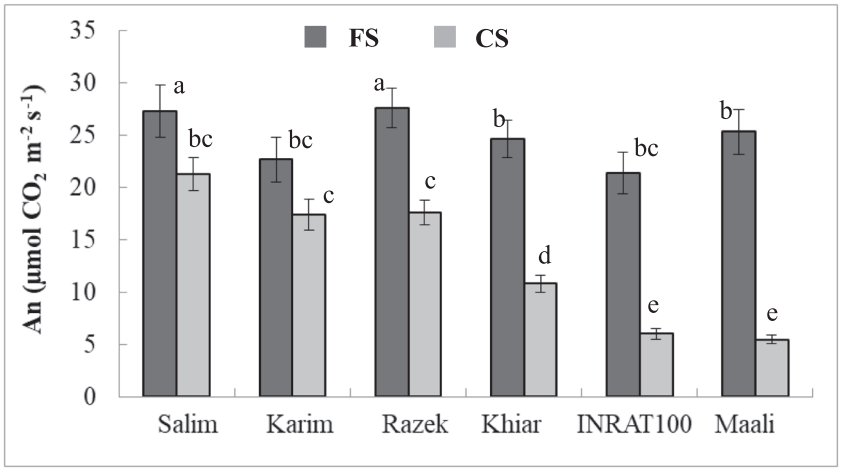

FIGURE 2 Net photosynthesis activity (*An*, µmol CO_2 m^{-2} s^{-1}) in wheat genotypes cultivated on fertile soil (FS) or calcareous soil (CS). Within columns, means with the same letter are not significantly different at $\alpha = .05$ according to Fisher's least significant difference. Bars on the columns represent the standard error of the mean ($n = 10$)

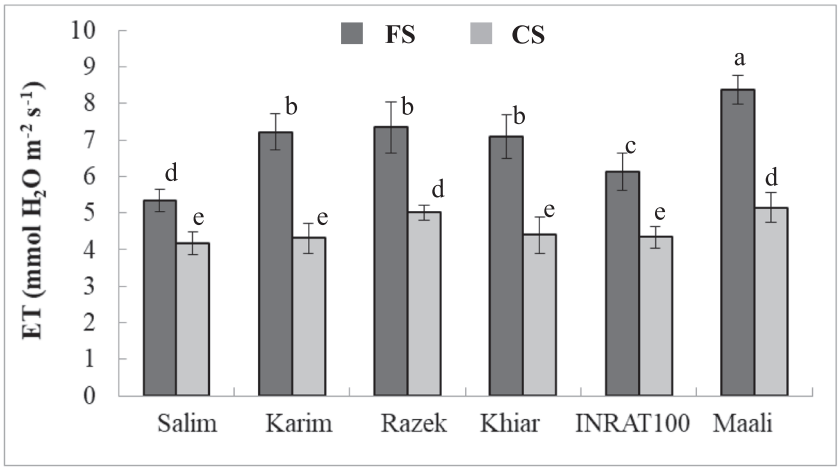

FIGURE 3 Evapotranspiration (ET, mol H_2O m^{-2} s^{-1}) of wheat plants cultivated on fertile soil (FS) or calcareous soil (CS). Within columns, means with the same letter are not significantly different at $\alpha = .05$ according to Fisher's least significant difference. Bars on the columns represent the standard error of the mean ($n = 10$)

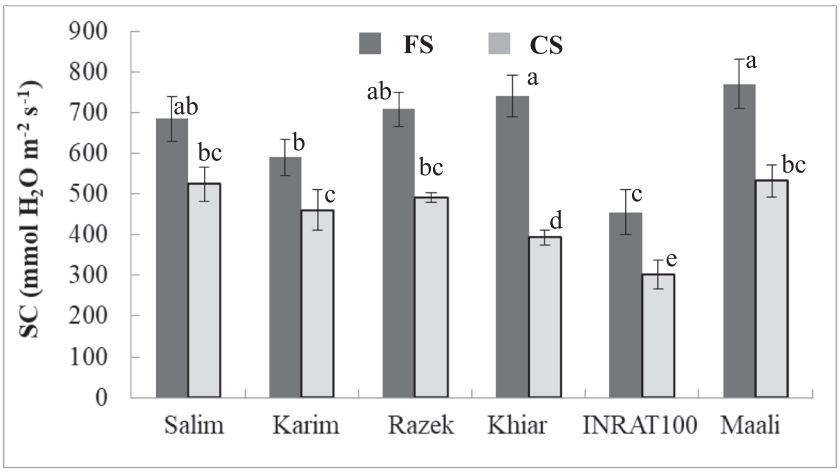

FIGURE 4 Stomatal conductance (SC, mol H_2O m^{-2} s^{-1}) of wheat plants cultivated on fertile soil (FS) or calcareous soil (CS). Within columns, means with the same letter are not significantly different at $\alpha = .05$ according to Fisher's least significant difference. Bars on the columns represent the standard error of the mean ($n = 10$)

TABLE 2 Active iron concentration in the plant organs (µg g−1 DW) and Fe translocation (FeT %) in six Tunisian genotypes of Durum wheat (*Triticum durum* Desf.) cultivated on fertile soil (FS) or calcareous soil (CS)

		Salim	Karim	Razek	Khiar	inrat100	Maali
Fe shoots	FS	$267.3^{a} \pm 21$	$256.4^{b} \pm 19$	$251.9b^{c} \pm 22$	$230.5^{c} \pm 18$	$214^{d} \pm 17$	$206.3^{e} \pm 17$
	CS	$215.6^{d} \pm 21$	$187.6^{f} \pm 18$	$154.7^{g} \pm 15$	$103.7^{h} \pm 11$	$99.4^{h} \pm 10$	$88.1^{i} \pm 9$
Fe roots	FS	$628.4a^{b} \pm 55$	$577.8^{c} \pm 54$	$474^{e} \pm 47$	$638.1^{a} \pm 64$	$501.7^{d} \pm 50$	$607.9^{b} \pm 52$
	CS	$480.5^{e} \pm 44$	$464.6^{ef} \pm 41$	$380.1^{g} \pm 39$	$409.9^{f} \pm 41$	$239.9^{h} \pm 24$	$375.3^{g} \pm 33$
FeT (%)	FS	$38^{ab} \pm 3.1$	$45^{a} \pm 3.8$	$44^{a} \pm 4.2$	$39^{ab} \pm 3.3$	$42^{a} \pm 3.6$	$37ab \pm 2.9$
	CS	$35^{b} \pm 3.1$	$34^{b} \pm 2.8$	$26^{c} \pm 2.2$	$14^{d} \pm 1.2$	$23^{c} \pm 1.7$	$11^{d} \pm 1.3$

Note: Within columns, means with the same letter are not significantly different at $\alpha = .05$ according to Fisher's least significant difference. Standard errors of means of 10 replicates.
Abbreviations: DW, dry weight; FeT, Fe translocation.

close relationship allowing to discriminate genotypes ($R^2 = .99$). The genotype salim that demonstrated a high tolerance on calcareous soil produced the highest biomass with an adequate allocation of Fe to shoots. According to their degree of tolerance, Figure 5 classifies the genotypes as follows (from the least to the most tolerant): maali- khiar- inrat100- razek- karim and salim. Figure 6 represents the relationship between photosynthesis and shoot's Fe-content. It shows a clear interdependence of these two parameters ($R^2 = .80$) confirming the particular tolerance of salim and the clear sensitivity of maali. The other genotypes occupy an intermediate position in the order mentioned above. Furthermore, the calculation of Fe use efficiency for photosynthesis (FeUEAn) demonstrated that the genotype maali expressed the lowest efficiency of Fe use compared with the other genotypes cultivated on calcareous soil (Figure 7a). Similarly, the calculation of chlorophyll use efficiency for photosynthesis (ChlUEAn) confirmed the same behavior as for FeUEAn on

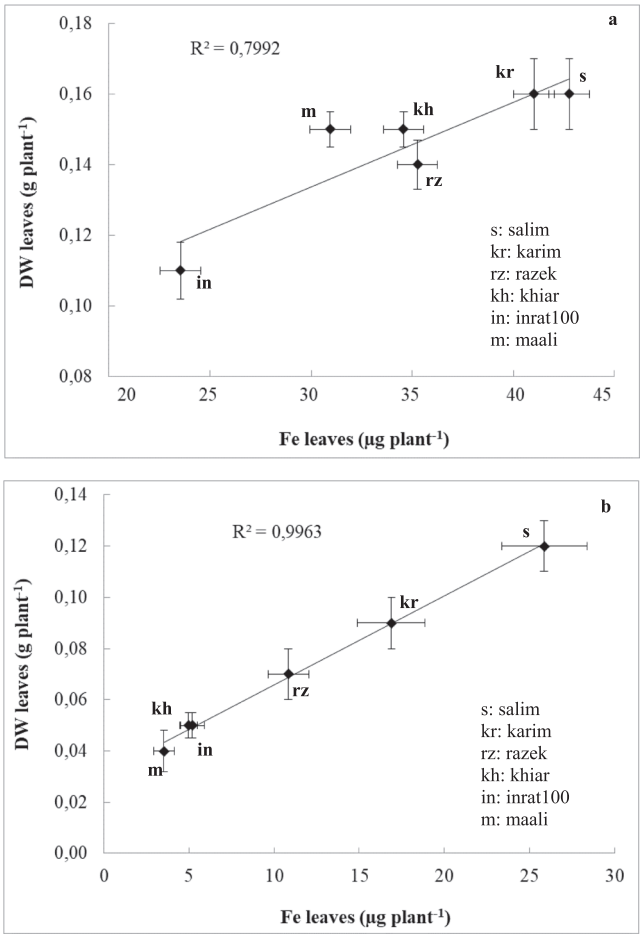

FIGURE 5 Relationship/correlation between plant growth (g plant^{-1}) and shoot Fe (µg plant^{-1}) in wheat genotypes cultivated on fertile soil (a) and calcareous soil (b). Vertical and horizontal bars represent ± standard errors of means of 10 replicates

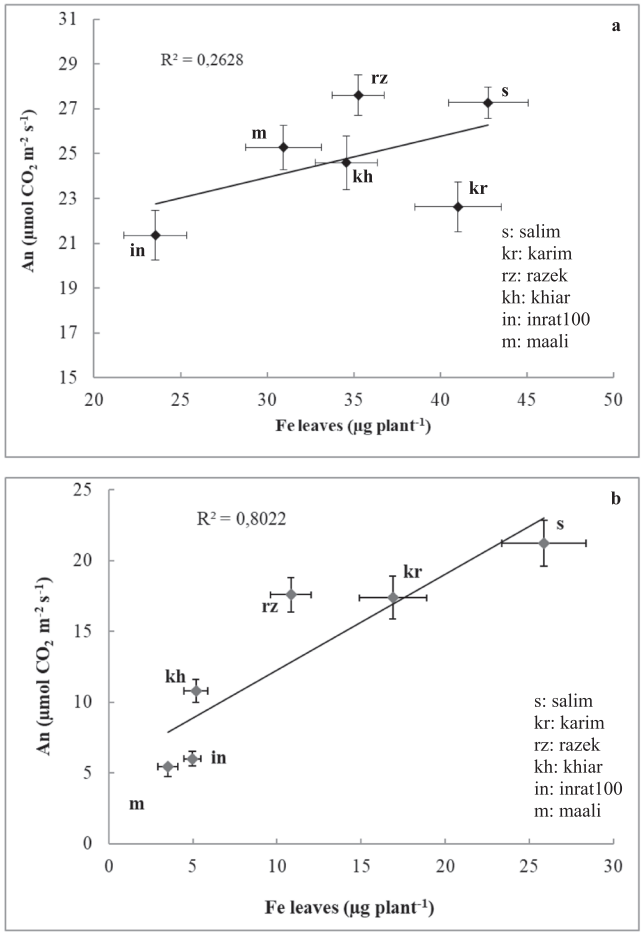

FIGURE 6 Relationship between net photosynthesis (*An*, µmol CO_2 m^{-2} s^{-1}) and shoot Fe (µg plant^{-1}) in wheat genotypes cultivated on fertile soil (a) and calcareous soil (b). Vertical and horizontal bars represent ± standard errors of means of 10 replicates

calcareous soil (Figure 7b). Thus, salim expressed a ChlUEAn 3.2 times more important than that of inrat100 and 3.5 more important than that of maali.

4 | DISCUSSION

Our results demonstrated that the cultivation of Tunisian wheat genotypes on calcareous soil leads to a significant reduction of plant growth, net photosynthesis, and chlorophyll accumulation. However, certain genotypes were more affected than others to lime induced Fe chlorosis with salim as the most tolerant and maali as the most sensitive genotypes. The rest of genotypes behaviors ranged between these two positions. In agreement with our results, several studies showed that subjecting plants to direct Fe deficiency leads to a significant reduction of plant growth and chlorophyll accumulation (Ellsworth et al., 1997; Thoiron et al., 1997). Krouma (2021) showed in pea plants that the first consequence of a limited iron availability is the lack of chlorophyll, then young leaves yellowing. Karimi and Salimi (2021) demonstrated that the leaf chlorosis, electrolyte leakage, Mg and Ca increased significantly when plants are subjected to10 mM sodium bicarbonate, against a drastic decrease of leaf SPAD, N, K, active Fe, total Fe and Zn concentrations.

In pea plants, Ferhi et al. (2017) observed that bicarbonate-induced Fe deficiency decreased plant growth, chlorophyll fluorescence, photosynthesis, SPAD index, and Fe nutrition. In maize, Thoiron et al. (1997) observed Fe chlorosis after 8 days of Fe deprivation. Numerous authors explained this chlorosis by the involvement of iron in the biosynthesis of chlorophylls and carotenoids (Ferhi et al., 2017; Jelali et al., 2011; Rustioni et al., 2017). In sorghum, Luna et al. (2018) showed that after 5 weeks of culture in an alkaline medium (pH 8.7), shoot biomass production significantly decreased in all studied genotypes with a clear genotypic difference. The same authors revealed that subjecting sorghum plants to direct Fe deficiency leads to a significant reduction of plant growth and Fe^{2+} concentration. Celletti et al. (2016) demonstrated in wheat grown on an increasing FeIII (EDTA) concentration that under 75 µM, plant growth and SPAD index decreased concomitantly with the appearance of typical Fe chlorosis. They reported that Fe concentration in the medium was inversely correlated with the excretion of PS.

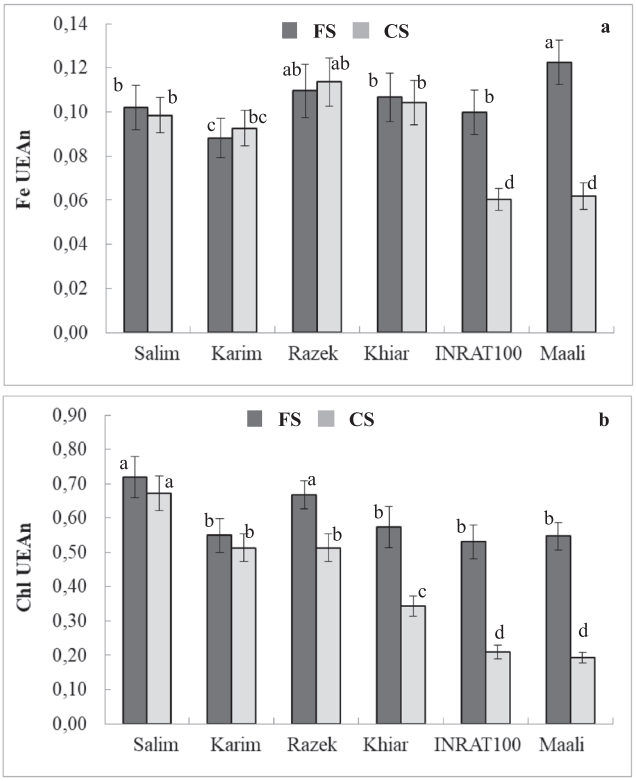

FIGURE 7 Fe use efficiency for photosynthesis (FeUEAn, a) expressed as the ratio of net photosynthesis (*An*, μmol CO_2 m^{-2} s^{-1}) to shoot Fe concentration (μg g^{-1} DW) and chlorophyll use efficiency for photosynthesis (ChlUEAn, b) expressed as the ratio of net photosynthesis (*An*, μmol CO_2 m^{-2} s^{-1}) to SPAD index. Within columns, means with the same letter are not significantly different at $\alpha = .05$ according to Fisher's least significant difference. Bars on the columns represent the standard error of the mean ($n = 10$)

Along with these effects on plant growth and chlorophyll biosynthesis, our results showed a significant reduction of net photosynthetic assimilation (*An*). The salim genotype maintained the highest capacity of photosynthesis on calcareous soil, whereas maali revealed to be the most sensitive genotype. Under these conditions of induced Fe limitation, salim expressed photosynthetic activity 2 times higher than that of khiar, 3.5 times higher than that of inrat100, and 4 times higher than that of maali. In agreement with these results, Luna et al. (2016) demonstrated in sorghum grown in an alkaline pH solution that the tolerant genotype displayed higher plant growth with a greater photosynthetic activity.

Fe is an essential micronutrient in the plant. As a critical factor, it controls plant growth, photosynthesis, and chlorophyll biosynthesis. In our study, we obtained a close positive relationship between biomass production and shoot Fe content (Figure 5) and between photosynthesis and shoot Fe content (Figure 6). Wheat tolerance increased with increasing Fe uptake and allocation to shoots leading to the above ranking of genotypes. The calculation of FeT to shoots (Table 2) supports this explanation. Indeed, the most tolerant genotype, salim, showed the lowest decrease of Fe allocation to shoots on calcareous soil (−8%), whereas the most sensitive one, maali, showed the most decrease (−72%). Under these conditions of low availability of Fe on calcareous soil, the tolerant genotypes salim expressed FeT capacity 1.5 to 3.3 times greater than that expressed by the other genotypes (Table 2). Thus, a close relationship between wheat tolerance to Fe deficiency and its capacity to uptake and allocate this micronutrient to the shoots becomes increasingly clear and confirmatory. In accordance with our investigations, Chaves et al. (2009) demonstrated that photosynthesis was among the most affected parameters by Fe deficiency, leading to a reduced growth. Shahsavandi et al. (2020) indicated that bicarbonate and Fe deficiency significantly decreased leaf chlorophyll index and photosynthetic rate. Typically, about 80% of Fe is found in photosynthetic cells where it is essential for the biosynthesis of cytochromes and other heme molecules, including chlorophyll, the electron transport chain, and the biosynthesis of the complex Fe–S (Briat et al., 2007; Hansch & Mendel, 2009). In the photosynthetic apparatus, two or three Fe atoms are found in molecules directly related to photosystem II (PSII), 12 atoms in photosystem I (PSI), five in the cytochrome complex, and two in the ferredoxin molecule (Varotto et al., 2002). Such distributions show that Fe is directly involved in plants' photosynthetic activity and, consequently, its productivity (Briat et al., 2007). Otherwise, Fe is an important component of electron transfer enzymes (cytochromes and iron–sulfur [Fe–S] proteins involved in redox reactions). Thus, any disruption of Fe nutrition would have a direct consequence on photosynthetic activity. Considering all these data, which are closely in accordance with ours, we observed in this study a clear genotypic variability in the response of wheat to lime-induced Fe chlorosis on calcareous soil. The tolerant genotype salim showed higher photosynthetic activity with better SPAD index and plant growth. These parameters decreased with increasing sensitivity. Nevertheless, the calculation of Fe use efficiency for photosynthesis (FeUEAn, Figure 7a) and chlorophyll use efficiency for photosynthesis (ChlUEAn) (Figure 7b) discriminated clearly the studied genotypes and confirmed the relative tolerance of salim, which was able to maintain high FeUEAn and ChlUEAn as compared with the others. On calcareous soil, salim expressed FeUEAn 1.6 times greater than that of inrat100 and maali and ChlUE An 2.0 times higher than that of khiar, 3.2 times higher than that of inrat100, and 3.5 times higher than that of maali. In accordance with these results that confirm the importance of Fe use efficiency in shoots, Zhao et al. (2020) suggested that the iron efficiency is attributed to an iron activation mechanism in leaves. Saito et al. (2021) demonstrated that varieties of barley originating from alkaline soils increased their photosynthetic iron use efficiency in response to Fe-deficiency. They suggest that the ability of Fe-deficiency tolerant varieties of barley to increase their iron use efficiency is related to optimizing the electron flow downstream of PSII, including cytochrome b6f and photosystem I. Hantzis et al. (2018) and Kroh and Pilon (2020) suggested that cyt b6f and PSI are the most Fe-requiring photosynthetic apparatus and the primary sufferer of Fe-deficiency in plants. Saito et al. (2021) postulate that the key mechanism for increased photosynthesis iron use efficiency under Fe-deficiency to exist downstream of PSII seems reasonable.

Nevertheless, Durum wheat is known by its capacity of roots release exudates into the rhizosphere (strategy II plant releasing PS). These organic compounds actively shape the root microbiome (Rolfe et al., 2019). Yu et al. (2021) demonstrated that plants abundantly form beneficial associations with diverse members of their root microbiome. Such mutualistic interactions provide important services to the plant, including improved nutrient uptake, optimized root architecture, plant growth promotion, or enhanced protection against pathogens and pests.

5 | CONCLUSION

The present study focused on the physiological behavior of six Tunisian genotypes of wheat (*T. durum* Desf.) cultivated on calcareous soil, as compared with fertile soil. The results suggested that plant growth, photosynthesis, chlorophyll biosynthesis, and Fe content are interdependent. Regarding all these parameters, the wheat genotypes involved in this study can be classified, from the least to the most tolerant to lime-induced Fe chlorosis as follows: Maali- inrat100- khiar- razek- karim- salim. The relative tolerance of some wheat genotypes to lime-induced iron chlorosis is linked to (1) their capacity of Fe remobilization in the rhizosphere, (2) their high FeT to shoots to support photosynthesis and plant growth, and (3) their high efficiency of Fe use for photosynthesis (FeUEAn) and their high efficiency of chlorophyll use for photosynthesis (ChlUEAn). The FeT, FeUEAn, and ChlUEAn can be used as a plant tolerance marker to Fe deficiency in screening programs.

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AUTHOR CONTRIBUTIONS

Krouma Abdelmajid set up the experimental protocol, followed its realization, and formatted the last version of the article. Hajlaoui Hichem has placed at our disposition certain equipment for plant analyses. Salhi Khaled conducted the experiments, carried out analyzes, and wrote the first version of the paper.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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