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RESEARCH ARTICLE

# Do calcareous soils affect morphological and physiological characteristics of maize hybrids and their iron efficiency?

**Chitdeshwari Thyagarajan[1*], Ravikesavan Rajasekaran[2], Sivamurugan A P[3*] & Senthil Alagarswamy[4]**

[1]Department of Sericulture, Forest College and Research Institute, Tamil Nadu Agricultural University, Mettupalayam, Coimbatore 638 109, Tamil Nadu, India

[2]Directorate of Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

[3]Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

[4]Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

*Email: chitdeshwari@tnau.ac.in / chithuragul@gmail.com

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## Abstract

Maize (*Zea mays* L.) is the third most cultivated cereal crop in the world after wheat and rice. Soil calcareousness is the key growth limiting factor causing a substantial decline in plant growth and yield in many arid and semiarid regions globally. This study aimed to understand the variability in morphological and physiological traits among maize hybrids as well as their iron (Fe) efficiency on calcareous soils. Five ruling maize hybrids [COH (M) 6, COH (M) 7, COH (M) 8, COH (M) 9 and COH (M) 10] and their six inbreds [UMI 1200, UMI 1201, UMI 1205, UMI 1210, UMI 1220 and UMI 1230] were evaluated on three calcareous soils having various free $CaCO_3$ content (6.23 to 17.3 %) with and without Fe supply. The experiment was carried out in the greenhouse of the Department of Soil Science, Tamil Nadu Agricultural University, Coimbatore, in 2019. Soil calcareousness reduced the morphological and photosynthetic parameters of all the maize hybrids and the reduction was more in highly calcareous soils (17.3 %). Significant variation in Fe efficiency among maize hybrids was observed and shows good association with physiological and photosynthetic characteristics. The maize hybrids COH (M) 6, COH (M) 8 and COH (M) 7 record higher growth attributes, photosynthetic parameters and active Fe content with better Fe efficiency. Lower Fe efficiency was noted with inbreds UMI 1230 and UMI 1220. The study showed that maize hybrids and their inbreds were sensitive to higher soil calcareousness, but the magnitude of differences varied with genotypes.

## Keywords

active Fe content; calcareous soils; Fe efficiency; maize hybrids; morphological and photosynthetic parameters

## Introduction

Calcareous soils are widespread in regions having arid and semi-arid climates and occur as inclusions in humid regions, affecting 800 million ha of land worldwide. These soils were identified by the higher presence of calcium carbonate as an accumulation of lime and the extent of calcareous soil in India is about 230 million ha (70 % of the total area), while in Tamil Nadu it accounts for 4.0 million ha (29.1 % of the total area) (1). Soil calcareousness is a primary growth-hindering factor for plant productivity due to lower soil organic matter and availability of all essential plant nutrients coupled with higher pH (2). Iron, despite its high abundance in the earth's crust, is extremely insoluble in calcareous soils due to its high free $CaCO_3$ content and gets converted into insoluble $Fe^{3+}$ oxy-hydroxides (3). Poor availability of Fe in soil, insufficient uptake and inactivation within the plants are the main factors that cause growth and yield loss in crops grown on calcareous soils (4, 5). Further, Fe deficiency in calcareous soils led to reduced crop productivity due to its involvement in plant photosynthetic activity, chlorophyll synthesis, nitrogen fixation and many enzyme activities (1, 6). Furthermore, Fe is most important for boosting antioxidant activities (catalase and peroxidase) and is involved in cell detoxification.

Iron deficiency significantly reduced the growth and yield of maize hybrids grown on calcareous soils due to lesser chlorophyll and active Fe content (2, 5). Nutrient fixation, adsorption and precipitation are the common constraints in calcareous soils due to reduced nutrient use efficiency of applied fertilizer inputs even after adopting best management practices for crop cultivation (7). Traditionally, the application of iron-containing inorganic fertilizers and chelates *via* foliar and soil has been advocated as a remedy for alleviating iron deficiency (8, 9). Use of Fe-containing fertilizers are the cheaper sources used for Fe nutrition of crops. Apart from this, commercial chelate products such as Fe-EDDHA and Fe-EDDHMA also opted for correcting Fe deficiency. However, their higher solubility and cost make them unsustainable. Foliar spraying of 1 % ferrous sulphate along with 0.10% citric acid was also advocated to improve the yield of crops in calcareous soils, but multiple applications are often necessary, which limits the economic feasibility of this practice (5, 10). Under Fe deficiency, the physiological and biochemical changes that occur to promote Fe uptake differ with genotypes; hence, identifying crops with higher Fe efficiency and better morpho-physiological parameters is a good approach for getting more crop yield on calcareous soils (1, 7).

Plants have developed several methods to improve Fe availability, mobility and absorption from Fe-deficient soils. Iron uptake and utilization by plants depends on various physiological and morphological parameters of the crop, which differ with genotypes. Research reports suggested that the Fe absorption efficiency of maize hybrids grown on calcareous soils ranged from 5 to 40% and chlorotic yellowing of the crop is influenced by chlorophyll content, photosynthetic parameters and active Fe concentration in plants. The secretion of mugineic acid compounds is a key metabolic process in maize plants for Fe absorption due to their role in solubilizing and complexing inorganic Fe and being made available to plants (6, 3, 9). The quantity of phytosiderophores synthesized in the root and released in the rhizosphere varies with genotype and its root architecture (5, 11, 12). Genetic variability to soil calcareousness and Fe efficiency has been reported in different crops (1, 13); however, it needs to be exploited continuously for cultivar development. Hence, understanding the morphological and physiological variability that exists among the maize hybrids for tolerating soil calcareousness and Fe efficiency is necessary for yield maximization in crops grown on calcareous soils. We hypothesized that our study delivers few insights on the differences in morphological and physiological parameters, Fe absorption and utilization efficiency of maize hybrids and inbreds on calcareous soils.

## Materials and Methods

### *Pot experiment*

A pot experiment with five maize hybrids, *viz*., COH (M) 6, COH (M) 7, COH (M) 8, COH (M) 9 and COH (M) 10, along with their six inbreds, *viz*., UMI 1200, UMI 1201, UMI 1205, UMI 1210, UMI 1220 and UMI 1230, was conducted on three calcareous soils having the free $CaCO_3$ content of 6.23 ($S_1$), 10.7 ($S_2$) and 17.3 % ($S_3$) in the greenhouse of the Department of Soil Science and Agricultural Chemistry, Tamil Nadu Agricultural University, Coimbatore during 2019. The seeds of maize hybrids were obtained from the Department of Millets, Tamil Nadu Agricultural University, Coimbatore. A preliminary survey was conducted for collecting calcareous soils and based on the soil calcareousness, three different soils were chosen for the study and bulk samples were collected from farmers' fields and TNAU farms. The soils were processed, sieved through a 2 mm sieve and analyzed for initial soil properties (Table 1). About 10 kg of the processed soil was filled in the earthen pots. Healthy, uniform-sized seeds of all the maize hybrids and their inbreds (2 seeds per pot) were sown in pots and a total of 198 pots were maintained. Mineral fertilizers such as urea, single super phosphate and muriate of potash at 125:37.5:37.5 mg NPK $kg^{-1}$ soil were applied basally along with and without Fe as a control and 25 mg $FeSO_4$ $kg^{-1}$ soil. The first irrigation was done soon after sowing, followed by irrigations at an interval of 4-6 days. The experimental design followed was a factorial completely randomized design with three replications. The maize hybrids were grown for 40 days and harvested.

### *Morphological and physiological parameters*

Plant height was recorded by measuring the plant length from the collar region of the shoot, while the root length was recorded by accounting for the length from the base of the stem to the tip of the longest root and both parameters were expressed in centimeters. The dry matter production was measured using a single plant weight of all hybrids. The plant was uprooted, washed with water and dried in a hot air oven at 70°C until it was completely dried to reach constant weight. To measure the relative chlorophyll concentration in the youngest fully expanded leaf, a SPAD meter (SPAD 502) was used to record the SPAD readings and five readings were taken around the midpoint of each leaf blade in a plant(14).

**Table 1.** Selected physico-chemical properties of the experimental soils

| Parameters | Slightly calcareous soil | Moderately calcareous soil | Highly calcareous soil |
|---|---|---|---|
| pH | 8.21 | 8.72 | 8.93 |
| EC (dS $m^{-1}$) | 0.43 | 0.98 | 0.59 |
| Organic carbon (g $kg^{-1}$) | 2.30 | 4.20 | 5.30 |
| Free $CaCO_3$ (%) | 6.23 | 10.7 | 17.3 |
| Available nutrients (kg $ha^{-1}$) | | | |
| Nitrogen | 178 | 292 | 245 |
| Phosphorus | 10.6 | 12.9 | 6.25 |
| Potassium | 172 | 142 | 210 |
| DTPA micronutrients (mg $kg^{-1}$) | | | |
| Zinc | 0.62 | 0.55 | 0.48 |
| Iron | 3.30 | 2.60 | 2.00 |
| Manganese | 2.20 | 1.90 | 1.42 |
| Copper | 1.22 | 1.01 | 0.88 |
| Texture | Sandy loam | Sandy clay loam | Sandy clay |

The average of the five readings was taken and expressed as the SPAD index. The photosynthetic parameters were measured in triplicate at morning hours (8-10 am) on the uppermost fully expanded leaf after 40 days and performed in the morning to avoid a high vapour pressure deficit. A portable photosynthesis system equipped with an infrared gas analyzer (IRGA, LI 6800, wavelength of 940 nm) was used to determine the photosynthetic rate and gas exchange parameters of the maize hybrids.

### *Active Fe*

The active Fe content was estimated from the fresh leaf samples collected from plants by washing once with water followed by 0.1 N HCl and then rinsed with double-distilled water and chopped with a stainless steel knife (9, 15). The chopped leaf sample (2 g) was immediately weighed and transferred to 100 mL glass bottles and added with 20 mL of o -phenanthroline solution. The stoppered bottles were allowed to stand for 16 hr at room temperature. The contents were filtered and the active iron content was estimated using an atomic absorption spectrophotometer (Model: GBC, PM Avanta).

### *Iron (Fe) efficiency indices*

Based on the dry matter production (DMP) and active Fe content, the maize genotypes were grouped into tolerant and susceptible to Fe deficiency using the following formula.

Fe efficiency (%) = [DMP from no Fe control/DMP from Fe applied pots] ×100 (Eqn. 1)

Fe absorption efficiency (%) = [Fe absorption at no Fe control/ Fe absorption at Fe applied condition] ×100 (Eqn. 2) (16)

### *Statistical analysis*

Data generated from the experiment are statistically analyzed using a randomized block design with three replications using SPSS 16.0 software (17). Based on the significant differences between treatments, the mean comparisons were made using Fisher's least significant difference (LSD) test at $p=0.05$ (18). The critical difference (CD) was calculated and the non-significant comparisons were indicated as NS.

## Results

### *Morphological characteristics*

Soil calcareousness reduced the plant height, root length and lateral root length of all the maize hybrids and the extent of reduction was lesser in maize hybrids than in their inbreds (Tables 2-4). Greater reduction in the growth parameters of all the maize hybrids was noted in moderately calcareous (18.5 to 20 %) and highly calcareous soils (16.9 to 39.9 %) than in slightly calcareous soil. The plant height varied from 89.8 to 142 cm, 60.7 to 123 cm and 45.8 to 90.9 cm with the root length of 17.2 to 25.6cm, 14.2 to 19.1 cm and 13.6 to 23.4 cm, respectively, slightly in, moderately and highly calcareous soils. The addition of Fe to calcareous soils improved the growth attributes of all the maize genotypes, irrespective of soil calcareousness. However, the response to added Fe was higher in slightly calcareous soil, followed by moderately and highly calcareous soils. As expected, the Fe-efficient maize hybrids were less affected by Fe deficiency in all the calcareous soils than the Fe-inefficient maize hybrids. Higher values of all the morphological parameters were observed with Fe-efficient COH (M) 6 genotype (86.8 to 142 cm, 19.1 to 25.6 cm and 14.4 to 18.7 cm, respectively, for plant height, root length and lateral root length), followed by COH (M) 8 and COH (M) 10 in all three calcareous soils. The Fe-inefficient maize hybrid COH (M) 9 recorded lesser plant height (72.4 to 123 cm), root length and lateral root length (15.8 to 21.3 cm and 13.3 to 16.4 cm, respectively). All the maize inbreds registered poor growth parameters and UMI 1230 (45.8 to 89.8 cm, 13.6 to 17.2 cm and 8.50 to 13.8 cm, respectively for plant height, root length and lateral root length) was found highly susceptible to increasing soil calcareousness and Fe deficiency, followed by UMI 1220, which was evident from their poor growth parameters. The reduction in maize hybrid growth was marked and linear with increasing soil calcareousness and about 35 to 50 % of growth reduction was observed in highly calcareous soils.

The quantification of the shoot, root and total dry weight of maize hybrids showed significant reduction with increasing soil calcareousness and the mean shoot, root and total DMP of maize hybrids varied from 4.84 to 21.5 g, 0.92 to 3.05 g and 5.80 to 24.5 g pot$^{-1}$, respectively Fig.1. The decline in dry weight of maize hybrids was 30 to 50% in calcareous soils, which differs widely with soil calcareousness. The maize hybrids COH (M) 6 (37.8 and 22.1 g pot$^{-1}$) and COH (M) 8 (33.9 and 20 g pot$^{-1}$) were less affected by soil calcareousness. The inbreds were highly sensitive to soil calcareousness and showed severe Fe deficiency symptoms in the plants. The inbreds UMI 1230 and UMI 1220 registered very poor dry matter production in all three calcareous soils (0.96 to 6.52 g pot$^{-1}$) and exhibited their greater sensitivity to soil calcareousness.

### *Photosynthetic parameters*

The SPAD index, photosynthetic rate, stomatal conductance and transpiration rate of all the maize hybrids and inbreds were measured after 40 days and furnished in Table 5 and Fig. 2-4. The Fe fertilization increased all the photosynthetic characteristics of maize hybrids, whereas increasing soil calcareousness reduced the photosynthetic activity. The SPAD index, which is the indicator of greenness in plants, decreased with increasing soil calcareousness (16.5 to 31.9) and the reduction was greater in highly calcareous soil than in moderately and slightly calcareous soils. The highest SPAD index was recorded in COH (M) 6 (31.9), followed by COH (M) 10 (29.1), COH (M) 8 (28.7) and COH (M) 7 (27.7). Generally, maize inbreds show very low SPAD values; however, UMI 1200 and UMI 1201 show higher SPAD indexes (26.0 and 25.8) and the least is observed with UMI 1230 (16.5).

Significant differences in the photosynthetic parameters were observed between cultivars and the inclusion of Fe showed a 10-15 % improvement in all the photosynthetic parameters. Although increasing soil calcareousness reduced the photosynthetic parameters, few maize hybrids such as COH (M) 6, COH (M) 10 and COH (M) 8 had better attributes that were closely associated with their higher Fe efficiency (66.7 to 73.2 %). The photosynthetic rate, stomatal conductance and transpiration rate of maize hybrids varied from 11.5 to 28.6 μmol m$^{-2}$ s$^{-1}$, 0.17 to 1.02 μmol m$^{-2}$s$^{-1}$

**Table 2.** Plant height (cm) of maize hybrids and inbreds on calcareous soils

| Maize hybrids | Slightly calcareous soil | | Mean | Moderately calcareous soil | | Mean | Highly calcareous soil | | Mean | Grand mean |
|---|---|---|---|---|---|---|---|---|---|---|
| | (-) Fe | (+Fe) | | (-) Fe | (+Fe) | | (-) Fe | (+Fe) | | |
| CO H (M) 6 | 131$^{ab}$ | 152$^{a}$ | 142 | 119$^{ab}$ | 126$^{ab}$ | 123 | 84.7$^{abc}$ | 89.0$^{ac}$ | 86.8 | 131 |
| CO H (M) 7 | 115$^{ab}$ | 128$^{ab}$ | 122 | 97.5$^{b}$ | 102$^{ab}$ | 99.9 | 89.2$^{bc}$ | 92.7$^{abc}$ | 90.9 | 115 |
| CO H (M) 8 | 126$^{ab}$ | 147$^{a}$ | 137 | 112$^{ab}$ | 121$^{ab}$ | 116 | 75.7$^{abc}$ | 87.3$^{ac}$ | 81.5 | 126 |
| CO H (M) 9 | 121$^{ab}$ | 126$^{ab}$ | 123 | 96.5$^{b}$ | 104$^{ab}$ | 100 | 67.2$^{bc}$ | 77.6$^{abc}$ | 72.4 | 121 |
| CO H (M) 10 | 123$^{ab}$ | 140$^{a}$ | 132 | 110$^{ab}$ | 116$^{ab}$ | 113 | 82.3$^{abc}$ | 92.5$^{ac}$ | 87.4 | 123 |
| UMI 1200 | 108$^{abc}$ | 114$^{ac}$ | 111 | 87.6$^{bc}$ | 95.6$^{abc}$ | 91.6 | 51.6$^{bc}$ | 63.8$^{ac}$ | 57.7 | 108 |
| UMI 1201 | 95.3$^{abcd}$ | 113$^{acd}$ | 104 | 80.7$^{bcd}$ | 87.7$^{abcd}$ | 84.2 | 56.6$^{bcd}$ | 61.9$^{acd}$ | 59.3 | 95.3 |
| UMI 1205 | 97.3$^{abde}$ | 110$^{ade}$ | 104 | 72.7$^{bde}$ | 82.8$^{abde}$ | 77.8 | 52.6$^{acde}$ | 61.3$^{acde}$ | 57.0 | 97.3 |
| UMI 1210 | 90.7$^{abd}$ | 104$^{ad}$ | 97.2 | 77.0$^{bd}$ | 86.3$^{abd}$ | 81.7 | 58.6$^{bcd}$ | 64.7$^{adc}$ | 61.7 | 90.7 |
| UMI 1220 | 85.7$^{abe}$ | 98.3$^{ae}$ | 92.0 | 68.5$^{be}$ | 80.6$^{abe}$ | 74.6 | 50.5$^{bce}$ | 55.5$^{ace}$ | 53.0 | 85.7 |
| UMI 1230 | 84.7$^{abe}$ | 95.0$^{ae}$ | 89.8 | 56.4$^{be}$ | 65.0$^{abe}$ | 60.7 | 43.2$^{bce}$ | 48.4$^{ace}$ | 45.8 | 84.7 |
| Mean | 107 | 121 | 114 | 88.9 | 97.0 | 92.9 | 64.8 | 72.2 | 68.5 | 107 |
| | S | F | G | S×F | F×G | S×G | S×F×G | | | |
| SEd | 2.03 | 1.66 | 3.89 | 2.87 | 5.51 | 6.75 | 9.54 | | | |
| CD (p=0.05) | 4.02 | 3.28 | 7.70 | 5.69 | 10.9 | 13.3 | 18.8 | | | |

*The data were analyzed in a three-factor completely randomized block design (FCRD) in SPSS 16.0 for the following: SEd-standard error of difference, CD-critical difference and p- p value (level of probability) for G- genotypes, F- iron level, S- calcareous soils and their interactions G×F, S×F, S×G.

*The numerical values in a column followed by dissimilar letters in the superscript are significantly different at (ANOVA, LSD test, p≤0.05).

**Table 3.** Root length (cm) of maize hybrids and inbreds on calcareous soils

| Maize hybrids | Slightly calcareous soil | | Mean | Moderately calcareous soil | | Mean | Highly calcareous soil | | Mean | Grand mean |
|---|---|---|---|---|---|---|---|---|---|---|
| | (-) Fe | (+Fe) | | (-) Fe | (+Fe) | | (-) Fe | (+Fe) | | |
| CO H (M) 6 | 24.2$^{ab}$ | 27.0$^{ab}$ | 25.6 | 17.5$^{ab}$ | 20.6$^{ab}$ | 19.1 | 20.2$^{ab}$ | 21.3$^{ab}$ | 20.8 | 24.2 |
| CO H (M) 7 | 19.6$^{abc}$ | 21.4$^{abc}$ | 20.5 | 16.0$^{bc}$ | 18.0$^{abc}$ | 17.0 | 20.8$^{bc}$ | 21.2$^{abc}$ | 21.0 | 19.6 |
| CO H (M) 8 | 23.5$^{ab}$ | 28.3$^{a}$ | 25.9 | 15.5$^{ab}$ | 22.0$^{ab}$ | 18.8 | 23.3$^{ab}$ | 23.4$^{ab}$ | 23.4 | 23.5 |
| CO H (M) 9 | 19.3$^{abc}$ | 23.2$^{ac}$ | 21.3 | 15.2$^{bc}$ | 16.4$^{abc}$ | 15.8 | 16.1$^{bc}$ | 19.0$^{abc}$ | 17.6 | 19.3 |
| CO H (M) 10 | 26.9$^{ab}$ | 28.1$^{a}$ | 27.5 | 21.8$^{ab}$ | 23.5$^{ab}$ | 22.7 | 22.2$^{ab}$ | 22.0$^{ab}$ | 22.1 | 26.9 |
| UMI 1200 | 18.6$^{abcd}$ | 23.7$^{acd}$ | 21.2 | 14.5$^{bcd}$ | 17.7$^{abcd}$ | 16.1 | 13.1$^{bcd}$ | 15.9$^{abcd}$ | 14.5 | 18.6 |
| UMI 1201 | 14.6$^{abe}$ | 16.9$^{ae}$ | 15.7 | 12.7$^{be}$ | 15.6$^{abe}$ | 14.1 | 11.7$^{bc}$ | 13.6$^{abe}$ | 12.6 | 14.6 |
| UMI 1205 | 19.1$^{abcd}$ | 20.0$^{acd}$ | 19.5 | 16.1$^{bcd}$ | 15.4$^{abcd}$ | 15.8 | 15.5$^{bcd}$ | 16.6$^{abcd}$ | 16.1 | 19.1 |
| UMI 1210 | 19.7$^{abcd}$ | 19.3$^{acd}$ | 19.5 | 12.8$^{bcd}$ | 15.7$^{abcd}$ | 14.3 | 17.5$^{bcd}$ | 18.5$^{abcd}$ | 18.0 | 19.7 |
| UMI 1220 | 21.2$^{abcd}$ | 17.7$^{acd}$ | 19.5 | 14.3$^{bcd}$ | 18.3$^{abcd}$ | 16.3 | 17.2$^{bcd}$ | 15.4$^{abcd}$ | 16.3 | 21.2 |
| UMI 1230 | 16.9$^{abde}$ | 17.5$^{ade}$ | 17.2 | 16.9$^{bde}$ | 11.5$^{abde}$ | 14.2 | 12.2$^{bde}$ | 15.0$^{abde}$ | 13.6 | 16.9 |
| Mean | 20.3 | 22.1 | 21.2 | 15.8 | 17.7 | 16.7 | 17.2 | 18.4 | 17.8 | 20.3 |
| | S | F | G | S×F | F×G | S×G | S×F×G | | | |
| SEd | 0.73 | 0.59 | 1.40 | 1.03 | 1.98 | 2.42 | 3.43 | | | |
| CD (p=0.05) | 1.44 | 1.18 | 2.77 | 2.04 | 3.91 | 4.79 | 7.00 | | | |

*The data were analyzed in a three-factor completely randomized block design (FCRD) in SPSS 16.0 for the following: SEd-standard error of difference, CD-critical difference and p- p value (level of probability) for G- genotypes, F- iron level, S- calcareous soils and their interactions G×F, S×F, S×G.

*The numerical values in a column followed by dissimilar letters in the superscript are significantly different at (ANOVA, LSD test, p≤0.05).

**Table 4.** Lateral root length (cm) of maize hybrids and inbreds on calcareous soils

| Maize hybrids | Slightly calcareous soil | | Mean | Moderately calcareous soil | | Mean | Highly calcareous soil | | Mean | Grand mean |
|---|---|---|---|---|---|---|---|---|---|---|
| | (-) Fe | (+Fe) | | (-) Fe | (+Fe) | | (-) Fe | (+Fe) | | |
| CO H (M) 6 | 16.7$^{ab}$ | 20.6$^{a}$ | 18.7 | 15.8$^{b}$ | 17.5$^{ab}$ | 16.7 | 13.3$^{b}$ | 15.5$^{ab}$ | 14.4 | 16.7 |
| CO H (M) 7 | 12.0$^{ab}$ | 20.0$^{a}$ | 16.0 | 18.2$^{b}$ | 22.6$^{ab}$ | 20.4 | 14.3$^{b}$ | 17.3$^{ab}$ | 15.8 | 12.0 |
| CO H (M) 8 | 15.6$^{ab}$ | 21.6$^{a}$ | 18.6 | 12.1$^{b}$ | 16.1$^{ab}$ | 14.1 | 15.1$^{b}$ | 17.8$^{ab}$ | 16.5 | 15.6 |
| CO H (M) 9 | 14.6$^{ab}$ | 18.1$^{a}$ | 16.4 | 16.0$^{b}$ | 15.9$^{ab}$ | 16.0 | 12.8$^{b}$ | 13.8$^{ab}$ | 13.3 | 14.6 |
| CO H (M) 10 | 17.0$^{ab}$ | 21.6$^{a}$ | 19.3 | 15.7$^{b}$ | 21.2$^{ab}$ | 18.4 | 17.6$^{b}$ | 16.1$^{ab}$ | 16.9 | 17.0 |
| UMI 1200 | 14.2$^{ab}$ | 17.5$^{a}$ | 15.9 | 15.9$^{b}$ | 11.9$^{ab}$ | 13.9 | 11.4$^{b}$ | 14.4$^{ab}$ | 12.9 | 14.2 |
| UMI 1201 | 14.1$^{ab}$ | 15.3$^{a}$ | 14.7 | 12.9$^{b}$ | 18.5$^{ab}$ | 15.7 | 11.5$^{b}$ | 11.5$^{ab}$ | 11.5 | 14.1 |
| UMI 1205 | 14.9$^{ab}$ | 17.1$^{a}$ | 16.0 | 12.3$^{b}$ | 12.4$^{ab}$ | 12.3 | 11.5$^{b}$ | 12.8$^{ab}$ | 12.2 | 14.9 |
| UMI 1210 | 14.1$^{ab}$ | 18.9$^{a}$ | 16.5 | 12.8$^{b}$ | 13.3$^{ab}$ | 13.0 | 12.3$^{b}$ | 12.2$^{ab}$ | 12.3 | 14.1 |
| UMI 1220 | 13.9$^{ab}$ | 16.7$^{a}$ | 15.3 | 10.8$^{b}$ | 14.1$^{ab}$ | 12.5 | 9.20$^{b}$ | 10.4$^{ab}$ | 9.80 | 13.9 |
| UMI 1230 | 12.9$^{ab}$ | 14.6$^{a}$ | 13.8 | 10.3$^{b}$ | 11.6$^{ab}$ | 11.0 | 8.23$^{b}$ | 8.70$^{ab}$ | 8.50 | 12.9 |
| Mean | 14.6 | 18.4 | 16.5 | 13.9 | 15.9 | 14.9 | 12.5 | 13.7 | 13.1 | 14.6 |
| | S | F | G | S×F | F×G | S×G | S×F×G | | | |
| SEd | 0.75 | 0.61 | 1.44 | 1.06 | 2.04 | 2.50 | 3.54 | | | |
| CD (p=0.05) | 1.49 | 1.21 | 2.85 | 2.11 | 4.04 | 4.95 | 7.00 | | | |

*The data were analyzed in a three-factor completely randomized block design (FCRD) in SPSS 16.0 for the following: SEd-standard error of difference, CD-critical difference and p- p value (level of probability) for G- genotypes, F- iron level, S- calcareous soils and their interactions G×F, S×F, S×G.

*The numerical values in a column followed by dissimilar letters in the superscript are significantly different at (ANOVA, LSD test, p≤0.05).

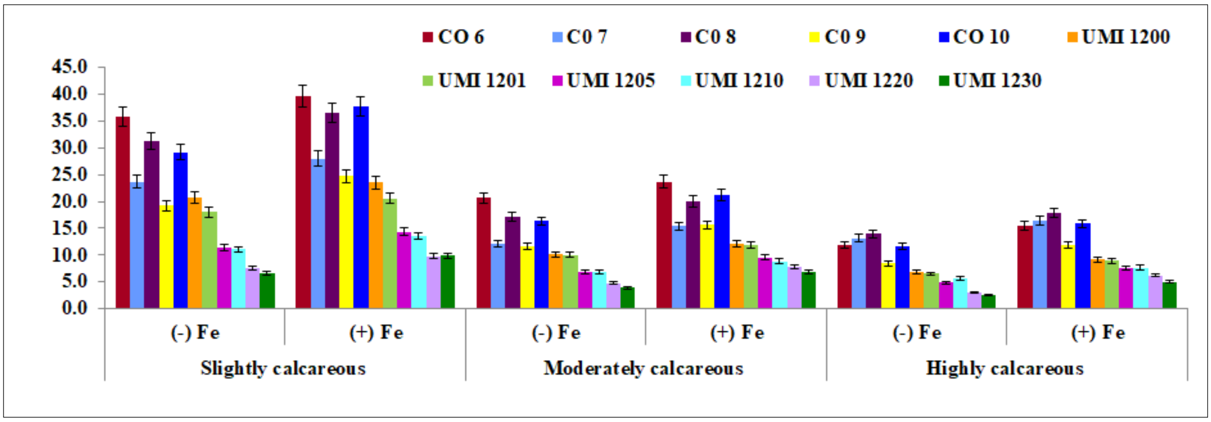


**Fig. 1.** Dry matter production of various maize hybrids and their inbreds on calcareous soils.

**Table 5.** SPAD index of maize hybrids and inbreds on calcareous soils

| Maize hybrids | Slightly calcareous soil | | Mean | Moderately calcareous soil | | Mean | Highly calcareous soil | | Mean | Grand mean |
|---|---|---|---|---|---|---|---|---|---|---|
| | (-) Fe | (+Fe) | | (-) Fe | (+Fe) | | (-) Fe | (+Fe) | | |
| CO H (M) 6 | 31.9$^{ab}$ | 36.0$^{a}$ | 34.0 | 27.2$^{ab}$ | 32.2$^{ab}$ | 29.7 | 16.7$^{abc}$ | 20.5$^{ac}$ | 18.6 | 31.9 |
| CO H (M) 7 | 27.7$^{ab}$ | 31.0$^{ab}$ | 29.4 | 22.2$^{b}$ | 26.9$^{ab}$ | 24.6 | 20.1$^{bc}$ | 22.2$^{abc}$ | 21.2 | 27.7 |
| CO H (M) 8 | 28.7$^{ab}$ | 32.5$^{ab}$ | 30.6 | 25.3$^{ab}$ | 27.9$^{ab}$ | 26.6 | 19.5$^{abc}$ | 22.5$^{abc}$ | 21.0 | 28.7 |
| CO H (M) 9 | 26.9$^{abc}$ | 30.8$^{ac}$ | 28.9 | 20.2$^{cd}$ | 23.3$^{abc}$ | 21.8 | 14.1$^{bc}$ | 17.5$^{ac}$ | 15.8 | 26.9 |
| CO H (M) 10 | 29.1$^{ab}$ | 33.4$^{ab}$ | 31.3 | 23.9$^{b}$ | 26.8$^{ab}$ | 25.4 | 15.5$^{bc}$ | 20.8$^{abc}$ | 18.2 | 29.1 |
| UMI 1200 | 26.0$^{abc}$ | 29.4$^{ac}$ | 27.7 | 18.7$^{bc}$ | 23.3$^{abc}$ | 21.0 | 17.4$^{bc}$ | 22.1$^{ac}$ | 19.8 | 26.0 |
| UMI 1201 | 25.6$^{abcd}$ | 30.4$^{acd}$ | 28.0 | 17.7$^{bcd}$ | 21.8$^{abcd}$ | 19.8 | 14.5$^{bcd}$ | 19.3$^{acd}$ | 16.9 | 25.6 |
| UMI 1205 | 21.6$^{abe}$ | 26.1$^{ae}$ | 23.8 | 16.0$^{be}$ | 19.7$^{abe}$ | 17.9 | 10.6$^{bce}$ | 14.1$^{ace}$ | 12.4 | 21.6 |
| UMI 1210 | 25.8$^{abd}$ | 29.3$^{ad}$ | 27.6 | 18.0$^{bd}$ | 23.2$^{abd}$ | 20.6 | 11.3$^{bcd}$ | 14.3$^{acd}$ | 12.8 | 25.8 |
| UMI 1220 | 19.7$^{abe}$ | 24.6$^{ae}$ | 22.2 | 13.4$^{be}$ | 17.1$^{abe}$ | 15.3 | 10.4$^{bce}$ | 15.7$^{ace}$ | 13.0 | 19.7 |
| UMI 1230 | 16.5$^{abf}$ | 21.6$^{af}$ | 19.1 | 11.2$^{bf}$ | 15.2$^{abf}$ | 13.2 | 9.5$^{bcf}$ | 14.3$^{acf}$ | 11.9 | 16.5 |
| Mean | 25.4 | 29.6 | 27.5 | 19.5 | 23.4 | 21.4 | 14.5 | 18.5 | 16.5 | 25.4 |
| | S | F | G | S×F | F×G | S×G | S×F×G | | | |
| SEd | 0.47 | 0.38 | 0.91 | 0.67 | 1.29 | 1.58 | 2.23 | | | |
| CD (p=0.05) | 0.94 | 0.77 | 1.80 | NS | 2.55 | 3.12 | 4.42 | | | |

*The data were analyzed in a three-factor completely randomized block design (FCRD) in SPSS 16.0 for the following: SEd-standard error of difference, CD-critical difference and p- p value (level of probability) for G- genotypes, F- iron level, S- calcareous soils and their interactions G×F, S×F, S×G.

*The numerical values in a column followed by dissimilar letters in the superscript are significantly different at (ANOVA, LSD test, p≤0.05).

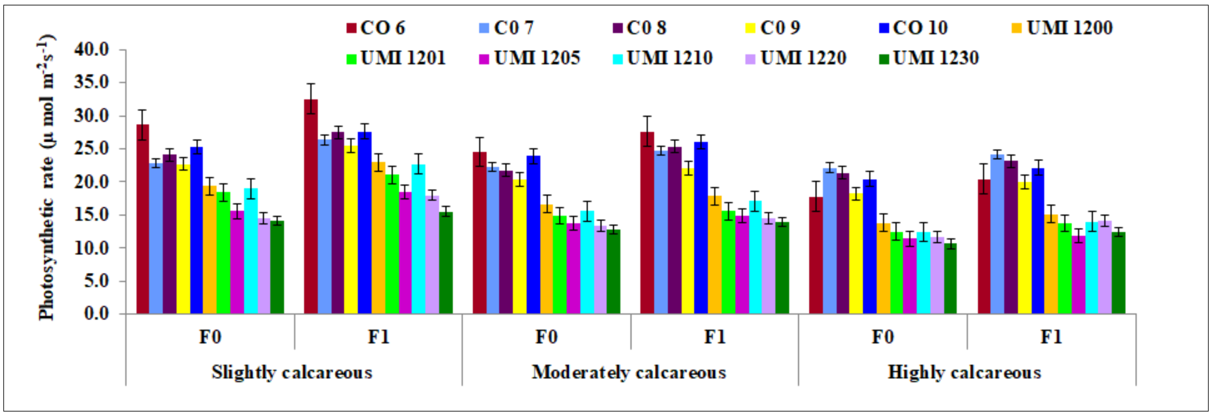


**Fig. 2.** Photosynthetic rate (µ mol $m^{-2}s^{-1}$) of maize hybrids and inbreds on calcareous soils.

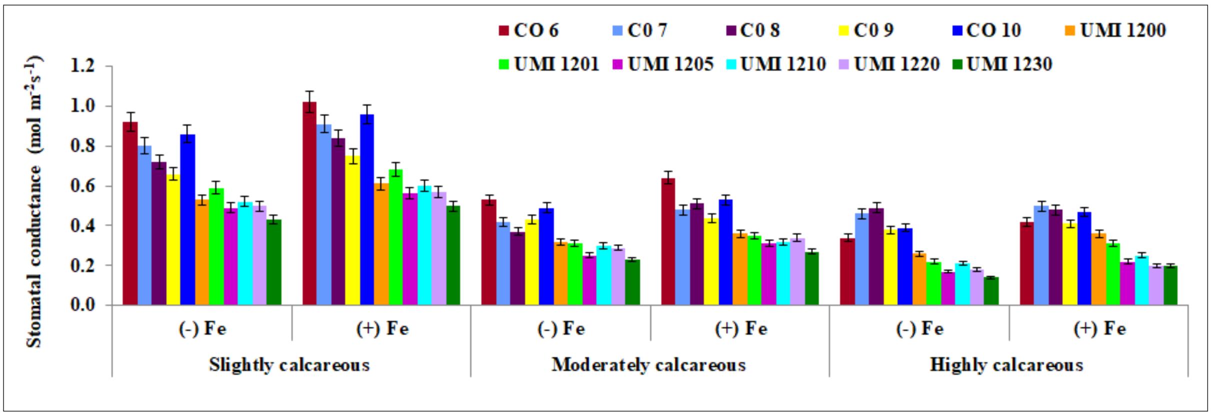


**Fig. 3.** Stomatal conductance (mol $m^{-2}s^{-1}$) of maize hybrids and inbreds on calcareous soils.

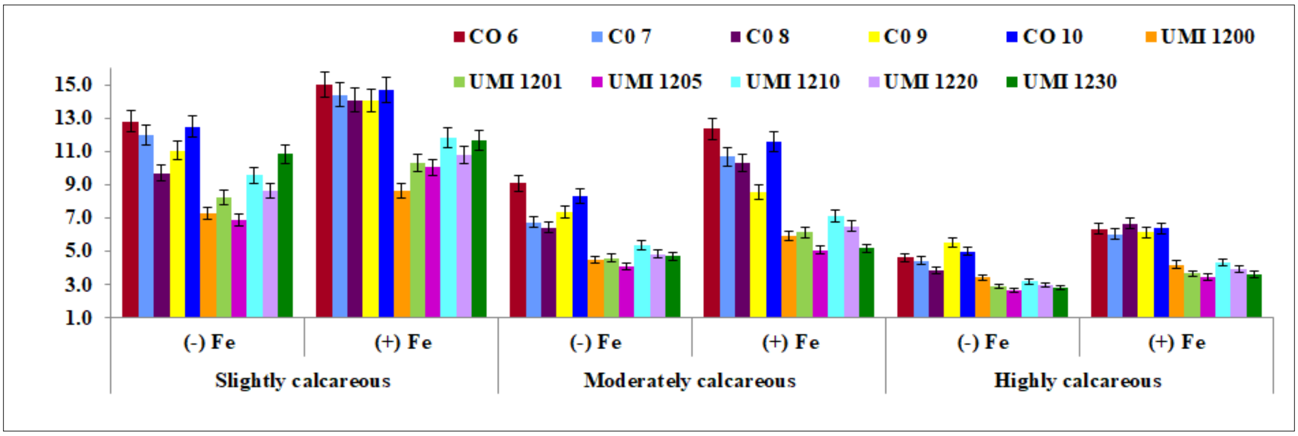


**Fig. 4.** Transpiration rate (mol m$^{-2}$s$^{-1}$) of maize hybrids and inbreds on calcareous soils.

and 3.23 to 15.0 mmol m$^{-2}$ s$^{-1}$, respectively. The maize inbreds UMI 1220 and UMI 1230 showed very poor photosynthetic parameters, indicating their sensitivity to soil calcareousness. Similarly, the transpiration rate and stomatal conductance of maize hybrids were also decreased with increasing soil calcareousness and greater reduction was recorded with the inefficient UMI 1230 and UMI 1210 maize inbreds (10-30 %). The efficiency of maize hybrids based on dry weight and Fe uptake also confirmed the present results, suggesting wider genotypic variability exists among maize hybrids, which could be exploited for growing maize hybrids on calcareous soils.

### *Active Fe content*

The estimation of active Fe content in plants demonstrated a drastic reduction in Fe uptake and utilization by maize hybrids and inbreds when grown on calcareous soils (Fig. 5). The active Fe content in the plants declined with increasing soil calcareousness even in the presence of Fe addition and differs with maize hybrids. The maize hybrid COH (M) 6 had more active Fe content in plants grown on slightly (20.8 mg kg$^{-1}$) and moderately calcareous soils (17.5 mg kg$^{-1}$) while in highly calcareous soil, the hybrids COH (M)7 and COH (M)8 had higher active Fe content. This result shows the nature of the higher tolerance of these genotypes, which might be due to increased Fe absorption and translocation. The inbreds UMI 1230 and UMI 1220 had lesser active Fe content (6.98 to 11.4 mg kg$^{-1}$) and indicating their poor ability to accumulate Fe in plants due to their sensitiveness to soil calcareousness. The results were well correlated with the SPAD index, photosynthetic rate, stomatal conductance and transpiration rate.

## Discussion

From the present study, we observed a wide variation in morphological and photosynthetic parameters among maize hybrids and their inbreds in response to varying intensities of soil calcareousness. The significant variation in plant growth, dry weight and net photosynthetic parameters among maize hybrids and their inbreds implies that considerable improvement could be made for improved crop yield, Fe absorption and utilization on calcareous soils. The higher pH and bicarbonate concentrations in calcareous soils were primarily responsible for such growth hindrance and the differential response of Fe-efficient and inefficient genotypes to pH and soil bicarbonate content was wider. The higher severity of Fe deficiency symptoms in maize inbreds indicates their sensitivity to soil calcareousness and Fe deficiency, which was also attributed to the contribution of less than 10 % of plants Fe needs from calcareous soils (1). The increase in bicarbonate concentration in calcareous soils decreased the active Fe content in plants, thus reducing the photosynthetic activity and resulting in yellowing of leaves, poor growth and biomass. Further, the low soil Fe availability led to its immobilization and precipitation in plant cells, thus resulting in chlorotic leaves, senescence and tissue death (19, 20). In agreement with our results, several studies showed limited plant growth and crop yield after 30 to 40 days when grown on calcareous soils and attributed to a lack of chlorophyll, reduced photosynthetic parameters, Fe availability and absorption by plants (2, 21 , 22).

Soil application of 25 mg kg$^{-1}$ $FeSO_4$ along with FYM showed positive results in improving plant growth and

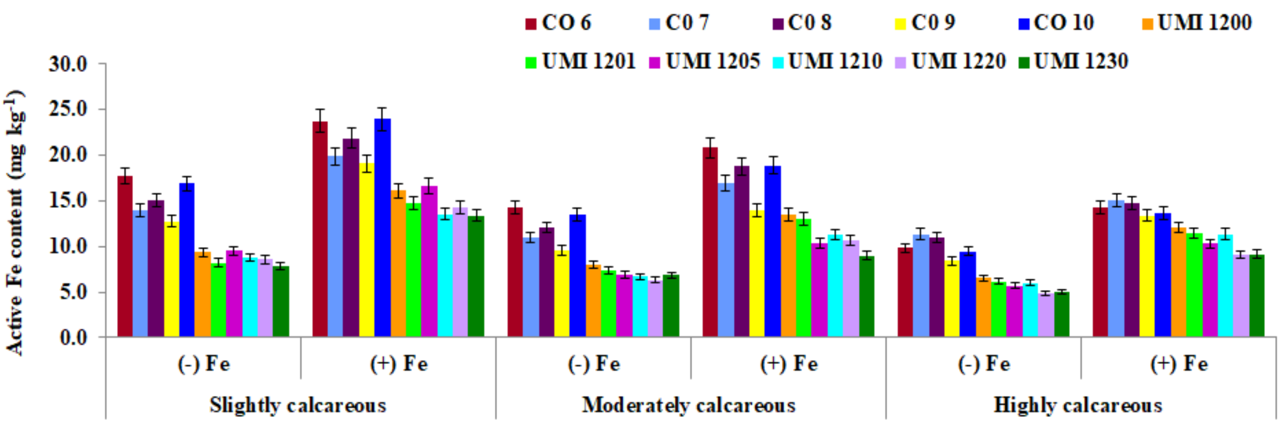


**Fig. 5.** Active Fe content in the plant tissues of maize hybrids and inbreds on calcareous soils.

photosynthetic parameters. The significant role played by Fe in chlorophyll synthesis might have activated the cell division and enhanced the plant growth attributes (23, 24). The maize hybrids COH (M) 6, COH (M) 8 and COH (M) 10 were able to record higher morphological and photosynthetic parameters even in highly calcareous soils and our results were in agreement with the results reported in sorghum genotypes exhibiting better crop growth and greater photosynthetic activity under alkaline soils (25, 26). Under limited Fe availability in calcareous soils, two times increased photosynthetic activity by tolerant wheat (1), barley (27) and sorghum genotypes were reported (28, 29) by researchers. Maize being a $C_4$ plant generally transpires less water per molecule of carbon dioxide fixed during photosynthesis and the effectiveness of reducing water loss varies with the efficiency of genotypes. Higher transpiration rates registered by the Fe-efficient genotypes in the present study were also ascribed to higher photosynthetic rates and stomatal conductance (30). However, the hybrid COH (M) 9 was highly sensitive to Fe deficiency, even slight soil calcareousness reduced the growth and biomass production, which could be ascribed to a lesser photosynthetic rate and SPAD index by poor light interception and nutrient acquisition by the genotype. Researchers reported a similar differential response of crops to Fe deficiency on calcareous soils, stating that Fe-efficient genotypes absorb more Fe and produce higher growth and dry weight (2, 5, 20). As compared to maize hybrids, the inbreds were severely affected by Fe deficiency and soil calcareousness and recorded very fdry weight, morphological and photosynthetic parameters. The inbreds, UMI 1230 and UMI 1220 were highly sensitive to Fe deficiency in calcareous soils with decreased photosynthetic rates and efficiency, leading to reduced $CO_2$ assimilation and ultimately resulting in poor growth and Fe efficiency. Further lesser photosynthetic parameters and chlorophyll content intercept less light and directly decrease the photosynthetic rate as well as Fe acquisition and utilization efficiency by maize hybrids (1, 13).

Significant differences in active Fe content of maize hybrids and inbreds were observed and it was substantially improved by the addition of Fe. Higher active Fe content was registered by Fe-efficient COH (M) 6, followed by COH (M) 7 and COH (M) 10 in all three calcareous soils. An average of 50 to 150 mg Fe $kg^{-1}$ in plants was reported, which varies with the carbon fixing pathway of crop species and to utilize the Fe effectively, plants exude more organic acids to acidify the root zone and reduce the insoluble Fe to soluble ferrous ions for better transport in plants (30-32). Higher biomass produced by these genotypes in calcareous soils had a significant relationship with higher active Fe content and photosynthetic parameters. Lesser active Fe content was noted with the inbreds UMI 1230 and UMI 1220, indicating their inefficiency to absorb and translocate more Fe within the plant system (1, 8, 33). The indices worked out using dry weight and active Fe content were well correlated with each other for their Fe efficiency and inefficiency (Fig. 6). The maize hybrids COH (M) 6 > COH (M) 8 > COH (M)7 > COH (M)10 were found to be Fe-efficient in tolerating soil calcareousness, while CO H (M) 9 was Fe-inefficient and sensitive to Fe deficiency in calcareous soils. The efficiency based on biomass and Fe uptake varied from 49.2 to 90.5 % and 32.2 to 81.2 %, respectively. The inbreds UMI 1230 and UMI 1220 were found Fe-inefficient, which indicated their differential genetic efficiency to Fe stress (1, 13, 22). Further, Fe efficiency differs widely with genotypes due to differential active iron content and plant photosynthetic parameters (9, 25 -27, 33). In addition to morphological and physiological mechanisms of maize genotypes for their adaptation to low Fe stress on calcareous soils studied, several other mechanisms, such as plant differences in root architecture, secretion of organic acids, phyto-sideropores, soil-related constraints due to varied carbonate and bicarbonate ion concentrations, pH and relationship with environmental factors *viz.,* temperature and $CO_2$ underpinning the Fe efficiency of maize genotypes when grown on calcareous soils, need future detailed studies (34).

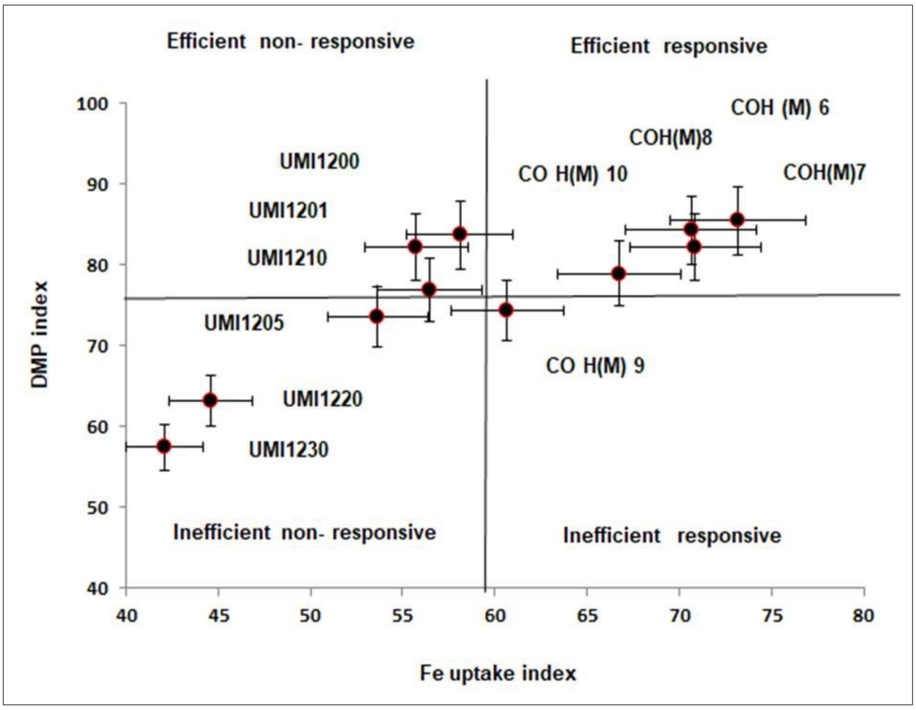


**Fig. 6.** Grouping of maize genotypes for their Fe efficiency and inefficiency.

## Conclusion

The present study focused on the genotypic variability of plant morphological, photosynthetic parameters and Fe efficiency by maize hybrids cultivated on slightly calcareous to highly calcareous soils. The results suggested that all the morphological parameters, SPAD index, active Fe content and efficiency in utilizing Fe depend on each other and decrease with increasing soil calcareousness. However, the maize hybrids COH (M) 6, COH (M) 8 and COH (M) 10 recorded better morphological and photosynthetic parameters and were highly Fe-efficient thus indicating their tolerance to soil calcareousness and Fe deficiency. However, the inbreds, *viz.*, UMI 1230 and UMI 1220, were highly susceptible to soil calcareousness with greater inefficiency in utilizing the native and applied Fe by registering lesser morphological and photosynthetic parameters. Hence the growth traits, photosynthetic parameters and active Fe content could be important in identifying maize hybrids for calcareous soils to reduce yield loss and farm income.

## Authors' contributions

CT conceptualized and carried out the investigation and wrote the original draft. RR associated with the collection of maize genotypes and facilitated in conducting the experiment. SAP and SA involved in coordinating the study. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflicts of interest**: Authors do not have any conflict of interests to declare.

**Ethical issues**: None

## References


1. Salhi K, Hajlaoui H, Krouma A. Genotypic differences in response of durum wheat (*Triticum durum* Desf.) to lime-induced iron chlorosis. Plant Direct. 2022;6(1):e377. https://doi.org/10.1002/pld3.377
2. Brindhavani PM, Chitdeshwari T. Calcareousness on the seed germination and seedling growth of hybrid maize genotypes-an *in vitro* study. J Soil Sci Plant Nutr. 2022;22(1):87-98. https://doi.org/10.1007/s42729-021-00634-6
3. Niu L, Wei J, Tang HL. Responses of the roots of different maize genotypes of nitrogen efficiency to iron deficiency. Anhui Agricultural Sciences. 2019;47(3):128-32. https://doi.org/10.1371/journal.pone.0239075
4. Zhao K, Wu Y. Effects of Zn deficiency and bicarbonate on the growth and photosynthetic characteristics of four plant species. Plos One. 2017;12(1):e0169812. https://doi.org/10.1371/journal.pone.0169812
5. Long W, Li Q, Wan N, Feng D, Kong F, Zhou Y, et al. Root morphological and physiological characteristics in maize seedlings adapted to low iron stress. PLoS One. 2020;15(9):e0239075. https://doi.org/10.1371/journal.pone.0239075
6. Ferhi J, Gharsalli M, Abdelly C, Krouma A. Potential of the physiological response of pea plants (*Pisum sativum* L.) to iron deficiency (direct or lime-induced). J Biosci. 2017;33(5):1208-18. 10.14393/BJ-v33n5a2017-36988
7. Rukmani N, Thiyagarajan C. Coated multi-nutrient fertilizers: effect on kernel yield, biochemical constituents and antioxidant enzymes in groundnut grown on a calcareous soil. Agrochimica. 2022;66 (2):199-213. https://doi.org/10.12871/000218572022237
8. Mann A, Singh AL, Shubhangi Oza SO, Nisha Goswami NG, Deepti Mehta DM, Vidya Chaudhari VC. Effect of iron source on iron deficiency induced chlorosis in groundnut. Legume Research-An International Journal. 2017;40(2):241-49. http://10.0.73.117/lr.v0iOF.6849
9. Chen XL, Xu JQ, Yu FT. Study on the iron efficiency of different maize inbred lines at seedling stage. Journal of South China Agricultural University. 2017;22(11):49-57.
10. Xu J, Chen X, Yu F. Effects of BPDS-Fe(II) on the difference in tolerance to iron deficiency of maize seedlings under different ammonium/nitrate ratios. Scientia Agricultura Sinica. 2017;50 (7):1223-33. https://doi.org/10.3864/j.issn.0578-1752. 2017.07.005
11. Masuda H, Shimochi E, Hamada T, Senoura T, Kobayashi T, Aung MS, et al. A new transgenic rice line exhibiting enhanced ferric iron reduction and phytosiderophore production confers tolerance to low iron availability in calcareous soil. PLoS One. 2017;12 (3):e0173441. https://doi.org/10.1371/journal.pone.0173441
12. Singh SP, Keller B, Gruissem W, Bhullar NK. Rice nicotianamine synthase 2 expression improves dietary iron and zinc levels in wheat. Theor Appl Genet. 2017;130:283-92. https://doi.org/10.1007/s00122-016-2808-x
13. Su Y, Zhang Z, Su G, Liu J, Liu C, Shi G. Genotypic differences in spectral and photosynthetic response of peanut to iron deficiency. J Plant Nutr. 2015;38(1):145-60. https://doi.org/10.1080/01904167.2014.920392
14. Peng S, Garcia FV, Laza RC, Cassman KG. Adjustment for specific leaf weight improves chlorophyll meter's estimate of rice leaf nitrogen concentration. Agronomy Journal. 1993;85(5):987-90. https://doi.org/10.2134/agronj1993.00021962008500050005x
15. Katyal JC, Sharma BD. A new technique of plant analysis to resolve iron chlorosis. Plant and Soil. 1980;55:105-19. https://doi.org/10.1007/BF02149714
16. Erdem H, Torun MB. Assessment of sulphur efficiency of durum and bread wheat genotypes. Fresenius Environmental Bulletin. 2017;26 (10):5891-99.
17. Snedecor GW, Cochran WG. Statistical methods 6th edition. The Iowa State University. USA;1967.
18. Gomez KA, Gomez AA. Statistical procedures for agricultural research. John Wiley andSons; 1984.
19. Akhtar S, Bangash N, Iqbal MS, Shahzad A, Arshad M, UlHassan F. Comparison of foliar and soil applications for of iron deficiency in peanut (*Arachis hypogaea* L.). Pakistan J Bot. 2019;51:1121-27. https://doi.org/10.30848/PJB2019-3(13) -19
20. Chao ZF, Chao DY. Similarities and differences in iron homeostasis strategies between graminaceous and non-graminaceous plants. New Phytol. 2022;236:1655-60. https://doi.org/10.1111/nph.18482-20
21. Celik H, Katkat AV. Effects of bicarbonate and iron-deprivation on growth of different maize varieties. American-Eurasian Journal of Agricultural and Environmental Science. 2008;3(2):169-78.
22. Celletti S, Mimmo T, Pii Y, Cesco S, Ciaffi M, Astolfi S. The effect of excess sulfate supply on iron accumulation in three graminaceous plants at the early vegetative phase. Environ Exp Botany. 2016;128:31-38. https://doi.org/10.1016/j.envexpbot.2016.04.004
23. Kong J, Dong Y, Xu L, Liu S, Bai X. Effects of exogenous salicylic acid on alleviating chlorosis induced by iron deficiency in peanut seedlings (*Arachis hypogaea* L.). J Plant Growth Regul. 2014;33:715-29. https://doi.org/10.1007/s00344-014-9418-0
24. Krouma A. Differential response of pea (*Pisum sativum* L.) genotypes to iron deficiency in relation to the growth, rhizosphere acidification and ferric chelate reductase activities. Aust J Crop Sci. 2021;15(6):925-31. https://doi.org/10.21475/ajcs.21.15.06.p3171
25. Luna DF, Aguirre A, Pittaro G, Bustos D, Ciacci B, Taleisnik E. Nutrient deficiency and hypoxia as constraints to Panicum coloratum growth in alkaline soils. Grass Forage Sci. 2017;72(4):640-53. https://doi.org/10.1111/gfs.12263

26. Luna DF, Pons AB, Bustos D, Taleisnik E. Early responses to Fe-deficiency distinguish *Sorghum bicolor* genotypes with contrasting alkalinity tolerance. Environmental and Experimental Botany. 2018;155:165-76. https://doi.org/10.1016/j.envexpbot.2018.06.030

27. Motesharezadeh B, Hesam-Arefi A, Savaghebi GR. The effect of bicarbonate on iron (Fe) and zinc (Zn) uptakes by soybean varieties. Desert. 2017;22(2):145-55.

28. Zhao Y, Sun M, Liang Z, Li H, Yu F, Liu S. Analysis of contrast iron chlorosis tolerance in the pear cv.'Huangguan' grafted onto Pyrus betulifolia and quince A grown in calcareous soils. Sci Hortic. 2020; 271:109488. https://doi.org/10.1016/j.scienta. 2020.109488

29. Saito A, Shinjo S, Ito D, Doi Y, Sato A, et al. Enhancement of photosynthetic iron-use efficiency is an important trait of *Hordeum vulgare* for adaptation of photosystems to iron deficiency. Plants. 2021;10(2):234. https://doi.org/10.3390/plants10020234

30. Martín-Barranco A, Thomine S, Vert G, Zelazny E. A quick journey into the diversity of iron uptake strategies in photosynthetic organisms. Plant Signal Behav. 2021;16:1975088. https://doi.org/10.1080/15592324.2021.1975088

31. Merry R, Dobbels AA, Sadok W, Naeve S, Stupar RM, Lorenz AJ. Iron deficiency in soybean. Crop Sci. 2022;62:36-52. https://doi.org/10.1002/csc2.20661

32. Malhotra H, Pandey R, Sharma S, Bindraban PS. Foliar fertilisation: Possible routes of iron transport from leaf surface to cell organelles. Arch Agron Soil Sci. 2020;66:279-300. https://doi.org/10.1080/03650340 .2019.1616288

33. LópezP, José L, Fabián PL. Iron nutrition management in calcisol soils as a tool to mitigate chlorosis and promote crop quality- An overview. J appl biol. 2024;12:17-29.

34. Hasanuzzaman M, Zhou M, Shabala S. How does stomatal density and residual transpiration contribute to osmotic stress tolerance? Plants. 2023;12:494-501. https://doi.org/10.3390/plants12030494