Research Square

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Functional analysis of the genotypic differences in response to calcareous-induced iron deficiency in pea plants (*Pisum sativum* L.)

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Research Article

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Abstract

Background

Lime-induced iron deficiency in Pea plants is a major nutritional disorder causing severe plant growth and yield reduction in calcareous soils of Tunisia. Other the chemical fertilization for iron chlorosis correction, the exploration of the genotypic differences in response to this constraint remains the most efficient approach due to its coast, environmental benefits, and sustainability. This approach allows as to screen tolerant genotypes and identify useful traits of tolerance.

Results

calcareous-induced iron deficiency reduced SPAD index, plant growth, net photosynthesis, and tissues Fe concentration against a significant stimulation of the oxidative stress indicators, H2O2 and Malondialdehyde (MDA). In the same time, we have reported significant induction of SOD activity in shoots and CAT activity in roots of the genotype Alexandra (ne clear behavior observed in the other genotypes). Fe use efficiency increased on calcareous soil and clearly discriminates the studied genotypes.

Conclusion

Genotypic differences were observed, and Alex was found to be the most tolerant. This genotype protect its tissues against oxidative stress by stimulating SOD activity in shoots and CAT içn roots, and expressed significant efficiency of Fe uptake and use on calcareous soil. The Fe use efficiency for photosynthesis and for SOD and CAT activities clearly discriminates the studied genotypes and can be used as a useful trait for further screening programs.

Background

Iron chlorosis is a yellowing of plant leaves caused by iron deficiency that affects many agricultural crops in the calcareous soils of Tunisia [1, 2]. The primary symptom of iron deficiency is interveinal chlorosis, the development of a yellow leaf with a network of dark green veins. In these soils having bicarbonate, high pH, and ferric form of iron, Fe deficiency is a major nutritional disorder for plants. These physicochemical conditions of soil affects the bioavailability of Fe and hamper plant growth and yield through the inhibition of Fe-dependent functions such as chlorophyll biosynthesis, photosynthesis, respiration, and protein formation [3]. Previous studies revealed the inhibitory effect of calcareous-induced iron chlorosis on several crops [1, 2, 4] and attributed the observed Fe deficiency not to the total Fe concentration in these soils but to the available fraction for the plant. Lindsay and Schwab [5] demonstrated that the very low solubility of iron oxides in calcareous soils is the result of the alkaline pH, buffered by the presence of bicarbonate ions. Ammari and Mengel [6] indicated that measured Fe

concentrations in calcareous soils exceeds from a way the plant's demand and mention that 50–90% of this Fe was complexed by organic molecules.

The physiological role of iron (Fe) in plants is well documented. Its main functions concern respiration, the synthesis of chlorophyll and photosynthesis. The first consequence of a limited iron availability is the lack of chlorophyll, then young leaves yellowing [7]. The function of various cytochromes containing heme Fe in the electron transport of photosynthesis is well established, as well as the role of ferredoxin. Because its close relationship with some antioxidant enzymes (some of them contain Fe, either in heme, POD and CAT, or non-heme, Fe-SOD, 8) iron catalyzes the free radical generation through the Fenton reaction. Krouma et al. [9] demonstrated that a significant stimulation of POD activity was observed in the tolerant genotype of common bean subjected to iron deficiency. SOD and CAT activities were maintained at high level. In another context, the high respiratory activity of plant cells can cause problem of oxygen conversion to reactive forms such as hydrogen peroxide (H2O2), superoxide anion (O2.) and hydroxyl radical (HO.) which are highly destructive to lipid membranes. Iron deficiency induces oxidative stress and generates reactive oxygen species (ROS) in plants causing cell membranes peroxidation [9, 10 11]. In order to defend themselves against oxidants, plants have developed protective mechanisms including antioxidant enzymes such as GPX, CAT, SOD, and non-enzymatic antioxidant substances [12, 13, 14]. It has been suggested that activities of these enzymes could be used as biochemical indicators of Fe availability in plants [15, 16]. Prity et al. [10] demonstrated that antioxidant defense either through metabolites or antioxidant enzymes is not efficient in counteracting oxidative damage in Fe-deprived sorghum.

Despite all these nutritional problems observed in calcareous soils, differences among species, varieties and genotypes in response to Fe deficiency have been reported [1, 2, 4, 17, 18, 19]. In a previous study conducted on the same pea genotypes, Krouma [7] demonstrated that Alex showed high tolerance to Fe deprivation as compared to other genotypes. Important H-ATPase and Fe- chelate reductase activities are the main reasons of this tolerance. In order to continue in this strategy of exploration of the genotypic differences in response to Fe deficiency and identify the useful traits of tolerance, we planned the present work on the same Pea genotypes cultivated on fertile or calcareous soil in order to simulate the natural Fe deficiency induced in calcareous soils. Primordial interest was granted to photosynthesis, ROS production and antioxidant activity io order to establish a logical reasoning between the physiological behaviour and some biochemical responses of pea to iron deficiency, that can explain the tolerance of some genotypes. For this purpose, three pea (*pisum sativum* L.) genotypes sourced from the Ministry of Agriculture, Water resources and Fishing are used, in a greenhouse experiment. A deep dissection of some physiological and biochemical parameters was made in order to express the genotypic differences and highlight useful traits for further screening programs.

Results

Plant growth, chlorophyll and gas exchange

All plants cultivated on calcareous soil (CS) exhibited a clear decrease of biomass production as compared to those cultivated on fertile soil (FS). Nevertheless, the negative effect of calcareous-induced iron deficiency is more pronounced in MK than Alex and DP. The decrease of biomass production was estimated to 30% in MK subjected to calcareous soil, 25% in DP and 10 % in Alex (fig. 1). Even if significantly decreased on calcareous soil, plant growth in Alex remains 30 % more important than that of DP and 40 % than that of MK.

The chlorophyll accumulation in shoots estimated by SPAD index demonstrated that cultivation of plants on calcareous soil induced a significant decrease of this pigment. As compared to fertile soil, SPAD index decreased by 32 % in Alex, 60 % in DP and 47 % in MK cultivated on calcareous soil, fig. 2). On this problematic soil, Alex accumulates 70 % more chlorophyll than DP and 30 % more chlorophyll than MK.

As compared to fertile soil, the cultivation of pea plants on calcareous soil decreased significantly net photosynthesis assimilation (An) in all genotypes. This decrease was estimated to - 33 % in Alex, - 55 % in MK and - 67% in DP (fig. 3a). Even if grown on calcareous soil, An was 2.2 higher in Alex than DP and 1.4 higher in Alex than MK.

The measurements made on evapotranspiration (ET, fig. 3b) and stomatal conductance (SC, fig. 3c) showed the same scheme of variation with a less pronounced effect than An. Alex remain the less affected genotype on calcareous soil.

Iron nutrition

The analysis of extractible active fraction of iron in plant organs demonstrated that roots represent the main organ of Fe accumulation, as compared to leaves particularly in fertile soil (fig. 4). On calcareous soil, the inhibitory effect of Fe uptake was more significant in roots (Fe concentration decreased by 43 % in Alex, 57 % in DP and 64 % in MK, fig. 4b) than shoots (Fe concentration decreased by 37 % in Alex, 57 % in DP and 68 % in MK, fig. 4a).

Enzyme assays

All genotypes exhibited a significant increase of H_2O_2 accumulation in plant organs when cultivated on calcareous soil with some genotypic differences (fig. 5). In Alex, H_2O_2 Increased by 10 % in shoots (fig. 5a) and by 23 % in roots (fig. 5b) of plants cultivated on calcareous soil, as compared to fertile soil. In DP, H_2O_2 was 2.50 times in shoots (fig. 5a) and 1.56 times in roots (fig. 5b) more important in calcareous soil as compared to fertile soil. In MK H_2O_2 was 6.80 and 2.16 times more important in calcareous soil as compared to fertile soil, respectively in shoots (fig. 5a) and roots (fig. 5b). This phenomenon reflects the existence of an oxidative stress in these organs and Alex is the less affected genotype. When comparing plant organs, we noticed that shoots are more affected than roots, values of H_2O_2

concentration are more important in shoots than roots. Thereby, we analyzed the MDA concentrations in the same organs (fig. 6). This substance reflected the lipid peroxidation of the cell membranes. Obtained results demonstrated a substantial increase of MDA in shoots and roots of DP and MK cultivated on calcareous soil (+ 50 % in shoots and + 36 % in roots of DP and + 70 % in shoots and + 96 % in roots of MK, fig 6a, b). In Alex we noticed an inverse behavior, MDA decreased by 10 % in shoots and 20 % in roots (fig. 6a, b).

The analysis of superoxide dismutase activity demonstrated no clear modifications in shoots of DP and MK cultivated on calcareous soil, as compared to fertile soil against a substantial increase in Alex (+ 60 %) (fig. 7a). In roots, all genotypes exhibited a clear decrease on calcareous soil (fig. 7b). The above discriminated genotype (Alex) expressed SOD activity about two times more important in shoots than roots on calcareous soil. This behavior lacked in the other genotypes (values of SOD are comparable).

For catalase, we measured a negligible value in all plant organs of DP and MK independently of treatments and organs (fig. 8). In Alex, we observed an inverse behavior than SOD, clear decrease of CAT activity in shoots (fig 8a) and a significant increase (2 times) in roots (fig. 8b).

In order to express other traits of pea response to iron deficiency and explore the genotypic variability, we calculated the Fe use efficiency for photosynthesis (FeUE-An), Fe use efficiency for SOD activity in shoots (FeUE-SOD-Sh) and SOD activity in roots (FeUE-SOD-R) and Fe use efficiency for CAT activity shoots (FeUE-CAT-Sh) and CAT activity in roots (FeUE-CAT-R) (table 2). For photosynthesis, Alex maintained high efficiency of Fe use for photosynthesis on calcareous soil (- 7 %), as compared to DP (- 54 %) and MK (- 47 %). FeUE-SOD-Sh increased significantly in plants cultivated on calcareous soil, as compared to fertile soil (+ 20 % in DP, + 40 % in MK and + 120 % in Alex) (table 2). In roots, this parameter decreased in Alex and DP, and increased slightly in MK. For catalase, we noticed a very low values in all plant organs of DP and MK with negligible variations. Nevertheless, a very high expression was measured in Alex with significant stimulation in roots of plants cultivated on calcareous soil (3 times higher in calcareous soil than fertile soil, table 2).

Discussion

In this study, we investigated the influence of bicarbonate induced Fe-deficiency stress on some physiological parameters and antioxidant enzymes activity in three pea genotypes. Under these experimental conditions, plant growth, chlorophyll accumulation, net photosynthesis and iron concentrations were significantly decreased, while these reductions are less pronounced in Alex. In fact, it is well established that chloroplasts benefit from the most important amount of leaf iron, thus any degree of Fe deficiency affect structure, function and chlorophyll content of the chloroplasts (Fu et al. 2016; 27]. The influence of Fe on leaf chlorophyll content has been reported in several studies. For example, the presence of bicarbonate in the nutrient solution decreased leaf chlorophyll concentration in arabidopsis [28] and in apple [29]. Krouma et al. [1] demonstrated that common bean cultivation on calcareous soil induced young leaves chlorosis, decreased significantly chlorophyll concentration and plant growth and

reduced Fe concentration. The relationship between Fe and chlorophyll can be explained by its implication in the biosynthesis of the chlorophyll precursors δ-aminolevulinic acid and protochlorophyllide [30]. The lower leaf sapd index in the calcareous-induced Fe-deficiency may be also explained by the prevented or retarded thylakoid formation [31]. Ristic et al. [32] observed a negative linear correlation between chlorophyll content and damage to thylakoid membranes in wheat. Since Fe is directly involved in the biosynthesis of chlorophyll, any decrease of its availability leads to the disruption of the biosynthesis of this pigment [33, 34]. Liu et al. [35] indicated that growth inhibition and photosynthesis depression under iron deficiency are mediated by systemic auxin signaling. Chaves et al. [36] demonstrated that photosynthesis is among the most affected parameters by iron deficiency, leading to reduced growth. About 80% of iron is found in photosynthetic and serves for the biosynthesis of cytochromes and other heme molecules, including chlorophyll, the electron transport chain and the biosynthesis of the complex Fe-S [37, 38]. In the photosynthetic apparatus, two or three iron 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 [39]. Such distributions confirms the direct involvement of iron in photosynthesis and, consequently, plant yield [37].

In the present study, the genotypic differences observed consists in the relative tolerance of Alex that maintains higher plant growth and photosynthetic activity on calcareous soil as compared to DP and MK. This genotype expressed better capacity of iron uptake and allocation to shoots (Fe concentration was 1.7 and 2.1 times higher in shoots of Alex as compared to DP and MK, respectively and 1.4 and 2.2 times higher in roots of Alex as compared to DP and MK, respectively). Thus, the relative tolerance of Alex can be explained by its capacity of iron remobilization, uptake, and distribution in the plant to support chlorophyll accumulation, photosynthesis and plant growth. Previously, Briat et al. [40] demonstrated that the main nutrients that limit plant growth on calcareous soils is iron.

It is well established that iron deficiency induced oxidative stress in plant tissues due to increased accumulation of ROS such as superoxide radicals and H2O2 [41, 42]. MDA is an indicator of this oxidative radicals that damage lipid membranes [9]. In the present study, H2O2 accumulation increased significantly in all plant organs grown on calcareous soil, except shoot of Alex. MDA increased also in DP and MK organs, except shoots and roots of Alex. These results let us think that the relative tolerance of Alex to calcareous-induced Fe-deficiency spotted on some physiological levels can be also explained by a particular ability of this genotype to protect its photosynthetic organs against their injury by ROS. However, analyses made on antioxidant enzymes activity demonstrated a significant increase of SOD in shoots and CAT in roots of Alex. In accordance with our result, several studies demonstrated SOD activity increase in quince rootstocks subjected to direct and bicarbonate induced Fe-deficiency [11]. An increase of the SOD activity in response to Fe-deficiency was observed also in other plants [29, 42, 43, 44]. Page et al. [45] suggested that the increase of SOD activity in chloroplast under Fe-deficiency is due to de novo biosynthesis of Fe-SOD over other plastid proteins and, the induction of a chloroplastic Mn-SOD. In literature, SOD is usually considered the first line of defense against oxidative stress [3]. By controlling the steady-state superoxide levels, SOD plays an important protective role against cellular oxidative damage, because superoxide acts as a precursor of more cytotoxic or highly reactive oxygen derivatives, such as

peroxynitrite or hydroxyl radical [46]. CAT, APX and GPX are also H2O2 scavenger that are able to convert H2O2 to H2O [14, 47, 48]. In our study, SOD activity increased in shoots while CAT increased in roots of Alex cultivated on calcareous soil (no clear behavior in the other genotypes). It becomes clear that other than its ability to mobilize and uptake more iron, Alex expressed more efficient antioxidant mechanism that protect its tissues against ROS. SOD plays a key role in shoots while CAT take over this role in roots.

By progressing in the elucidation of the mechanisms of Pea tolerance to calcareous-induced Fe-deficiency, we noticed an important increase of Fe use efficiency for photosynthesis and SOD activity in shoots and for CAT activity in roots mainly in Alex. This result adds to our investigation another tool of explanation of Alex tolerance to induced Fe-deficiency. This genotype uses efficiently the low available amount of iron to express the maximum potentialities of photosynthesis, plant growth and antioxidant activity.

Conclusions

Taken together, our results demonstrated that calcareous- induced iron deficiency induced young leaves chlorosis, decreased chlorophyll and plant growth and disrupts photosynthesis and Fe nutrition in the studied Pea genotypes. Some genotypic differences were observed, and Alex was found to be relatively tolerant. This genotype is characterized by (1) high capacity of iron uptake and distribution in the plant organs on calcareous soil, (2) important ability to stimulate SOD and CAT activities protecting tissues against their injury by ROS and, (3) better Fe use efficiency.

FeUE-An, FeUE-SOD and FeUE-CAT can be used as a useful trait for further screening programs.

Methods

Plant Materials and experimental conditions

Three pea genotypes were used, Alexandra, Douce de Provence and Merveille de Kelvedon largely cultivated in Tunisia. Seeds were disinfected with 2% hypochlorite calcium solution and sown individually in 1kg pots filled with a fine, mixed fertile soil (FS) sampled in the region of Gatrana (Sidi Bouzid: Long= 9.64301650° Lat=35.14827290° WGS84 (GPS): Long= 9.64301650 ° Lat= 35.14827290 °) or calcareous soil (CS) sampled in the region of Faiedh (sidi Bouzid: Long= 9.67560230° Lat= 35.07737120° WGS84 (GPS): Long= 9.67560230° Lat= 35.07737120 °). Experiments were conducted in the greenhouse under natural light. The main soils characteristics are given in Table 1. Soil moisture was maintained near field capacity using tap water. At the beginning of flowering, 45 days after germination, non-destructive measurements (SPAD index and gas exchange) were made then plants were harvested and separated into shoots and roots. The last organs are soaked in 0.01 M CaCl2 solution and washed thoroughly and successively in 3 baths of ultra-pure water in order to avoid the contamination of roots with iron and elements from the soil. Organs intended for biochemical analysis (MDA, proteins, enzymes) were harvested in liquid nitrogen then stored at -80°C until use.

SPAD Index

The degree of chlorosis was estimated non-destructively on the third fully expanded apical leaves from ten plants of each treatment and genotype using a portable SPAD-502 meter (Minolta, Osaka, Japan).

Gas exchange

Gas exchange measurements were made with an LI-6400 (LI-COR, Inc.) portable gas exchange system. Measurements were made on the same third fully expanded apical leaves from ten plants of 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 pCO2, flow rate, and temperature were kept constant at 362 mbar, 500 μmol s^{-1}, and 25 °C, respectively.

Active iron

Measurements of active iron (Fe^{2+}) were performed according to Köseoglu and Acikgöz [20]. The extraction was made in 25 mg of plant organ's fine powder shacked in 10 ml of 1N HCl.

Protein extraction

Aliquots of frozen plant material (100 mg FW) were ground to a fine powder with liquid nitrogen and extracted at 4°C in 300 μl of 100 mM Tris HCl buffer (pH 8.0) containing 10 mM EDTA, 50 mM KCl, 20 mM $MgCl_2$, 0.5 mM PMSF, 1 mM DTT, 0.1% (V/V) Triton X-100, and 10 % (W/W) PVP. The homogenate was centrifuged at 14000g for 30 min at 4°C, and the supernatant was used for determination of protein content and activities of antioxidative enzymes. Three replicates per treatment and genotype were used and measurements were repeated twice. Protein concentration was determined according to Bradford [21], using bovine serum albumin as a standard.

Enzyme assays

Total superoxide dismutase (SOD) activity was assayed according to Scebba et al. [22]. Increasing volumes (5, 10, 20, and 40 μl) of tissue crude extract were added to the reaction mixture at a final volume of 3 ml. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.8), 0.1 mM EDTA, 13 mM L-methionine, 2 μM riboflavin and 75 lM MTT (3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium

bromide). The reaction was started by exposing the mixture to cool white, fluorescent light at a photosynthetic photon flux of 50 μmol m^{-2} s^{-1} for 15 min. The developed blue colour in the reaction mixture was measured spectrophotometrically at 560 nm. The volume of sample causing 50% inhibition in colour development was taken as one unit of SOD activity [23] and the activity was expressed as units per mg protein in each plant organ.

Catalase (CAT) activity was measured spectrophotometrically according to the method of Aebi [24], by monitoring the decline in the absorbance at 240 nm, as H2O2 was consumed. The final volume (3 ml) of the reaction mixture contained 50 mM sodium phosphate buffer (pH 7.0), to which 30 mM H2O2 (1 ml) was added (OD = 0.52–0.55 at 240 nm). The reaction was activated by adding 100 μl of the tissue extract to this solution. CAT activity was expressed as units (mmol of H2O2 decomposed per min) per mg protein in each plant organ.

Malondialdehyde concentration

Iron deficiency induced oxidative damage (membrane lipid peroxidation) was assessed by measuring the amount of malonyldialdehyde (MDA) in tissue. Fresh tissues samples were homogenized in 0.1% (w/v) TCA solution. The homogenate was centrifuged at 15,000·g for 10 min. An aliquot of the supernatant was added to 0.5% TBA in 20% TCA. The mixture was heated at 90°C for 30 min in a shaking water bath, and then cooled in an ice bath. The samples were centrifuged at 10000·g for 5 min, and the absorbance of the supernatant was read at 532 and 600 nm (Esterbauer 1993). The MDA concentration was calculated as the difference of absorbance at 600 nm and 532 nm.

Hydrogen peroxide (H_2O_2) concentration

Hydrogen peroxide concentration of the plant tissue was determined using the method of Alexieva et al. [25]. A fresh tissues sample (0.1 g) was homogenized with 1 mL 0.1% (w/v) TCAA on ice. After centrifugation at 12,000 g for 15 min, 0.5 mL of the supernatant transferred to 0.5 mL of K-phosphate buffer (100 mM, pH 7.0) and 2.0 mL of 1 M potassium iodide (KI). The reaction was kept for 1 h in darkness and absorbance of incubation mixture measured at 390 nm. The amount of H_2O_2 was calculated based on a standard curve prepared with known concentrations of H_2O_2.

Statistical analysis

Data and statistical analyses were performed using the software StatPlus Pro. All data are presented as mean ± standard error. Analysis of variance (ANOVA) was performed to determine whether effects of treatments (FS and CS) on the respective factor were significant. The significance of differences among

treatment means was determined by Fisher's least significant difference test (LSD) 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.

Abbreviations

Alex: Alexandra; An: net photosynthesis; CS: calcareous soil; CAT: catalase; DP: douce de provence; ET: evapotranspiration; FS: fertile soil; MDA: malondialdehyde; MK: merveille de kelvedon; ROS: reactive oxygen species; SC: stomatal conductance; SOD: superoxide dismutase.

Declarations

Ethics approval and consent to participate

Not applicable.

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The authors declare that they have no competing interests.

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Authors' contributions

SM, carried out plant experiments and physiological and biochemical analysis. AK planned, designed the research and analyzed critically the results. HS, helped for enzyme analysis.

All authors read and approved the final manuscript.

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Tables

Table 1. Main characteristics of used soils. **FS**: Fertile soil sampled in the region of Gatrana (Sidi Bouzid (35°9'52.366" N 9° 40' 23.689" E), **CS**: calcareous soil sampled in the region of Faiedh (sidi Bouzid, 35°4'38.536" N 9°40' 32.167" E).

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

Table 2. Fe use efficiency for photosynthesis (FeUE-An), Fe use efficiency for SOD activity in shoots (FeUE-SOD-Sh) and in roots (FeUE-SOD-R), and Fe use efficiency for CAT activity in shoots (FeUE-CAT-Sh) and in roots (FeUE-CAT-R) of pea plants (*Pisum sativum* L.) cultivated on fertile soil (FS) or calcareous soil (CS). Within columns, means with the same letter are not significantly different at $\alpha = 0.05$ according to Fisher's Least Significant Difference. Standard errors of means of 10 replicates.

		Alex	DP	MK
FeUE-An	FS	0.103 ± 0.011^{b}	0.103 ± 0.017^{b}	0.132 ± 0.018^{a}
	CS	0.096 ± 0.007^{bc}	0.047 ± 0.001^{b}	0.084 ± 0.007^{c}
FeUE-SOD-Sh	FS	1.53 ± 0.11^{d}	2.17 ± 0.17^{c}	2.13 ± 0.18^{c}
	CS	3.35 ± 0.22^{a}	2.70 ± 0.24^{b}	2.95 ± 0.20^{ab}
FeUE-SOD-R	FS	2.26 ± 0.21^{c}	3.15 ± 0.22^{b}	3.27 ± 0.25^{b}
	CS	1.60 ± 0.13^{d}	2.90 ± 0.21^{bc}	3.68 ± 0.22^{a}
FeUE-CAT-Sh	FS	6.74 ± 0.48^{a}	0.14 ± 0.01^{e}	0.25 ± 0.018^{d}
	CS	5.15 ± 0.41^{b}	0.27 ± 0.02^{d}	0.47 ± 0.031^{c}
FeUE-CAT-R	FS	2.23 ± 0.18^{b}	0.13 ± 0.011^{d}	0.15 ± 0.011^{d}
	CS	6.06 ± 0.43^{a}	0.15 ± 0.01^{d}	0.52 ± 0.044^{c}

Figures

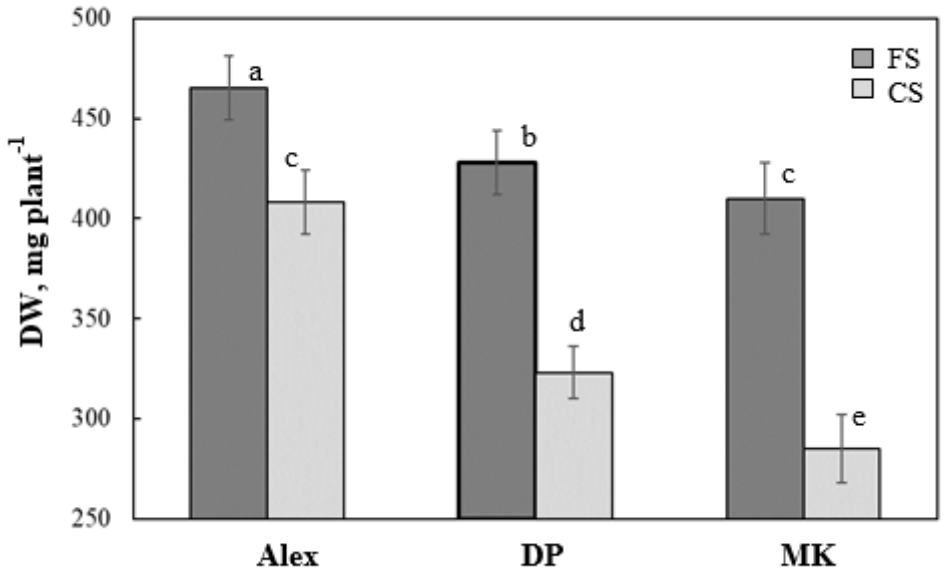

Figure 1

Biomass production (mg plant-1) in three Pea (Pisum sativum L.) cultivated on fertile soil (FS) or calcareous soil (CS). Within columns, means with the same letter are not significantly different at α = 0.05 according to Fisher's Least Significant Difference. Bars on the columns represent the standard error of the mean (n= 10).

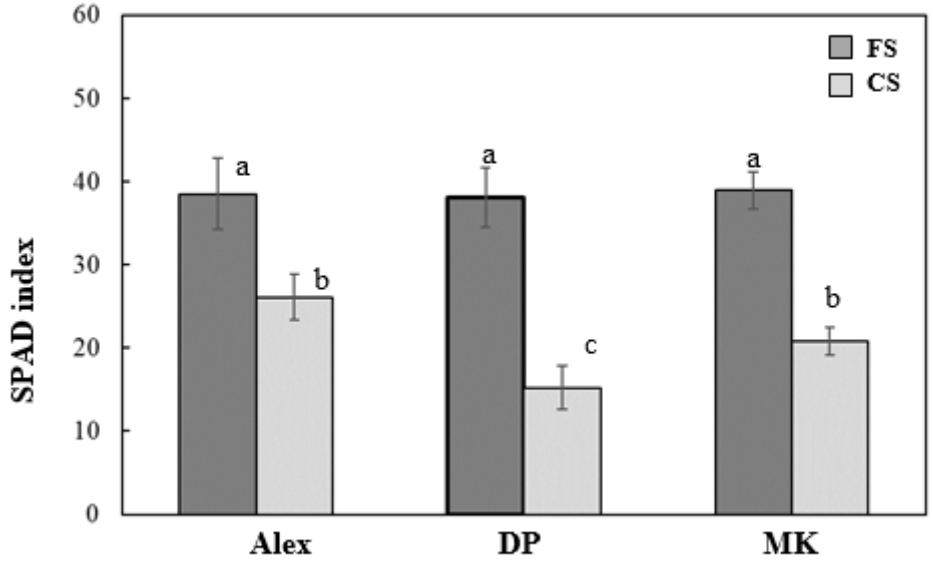

Figure 2

SPAD index in Pea plants (Pisum sativum L.) cultivated on on fertile soil (FS) or calcareous soil (CS). Within columns, means with the same letter are not significantly different at α = 0.05 according to Fisher's Least Significant Difference. Bars on the columns represent the standard error of the mean (n= 10).

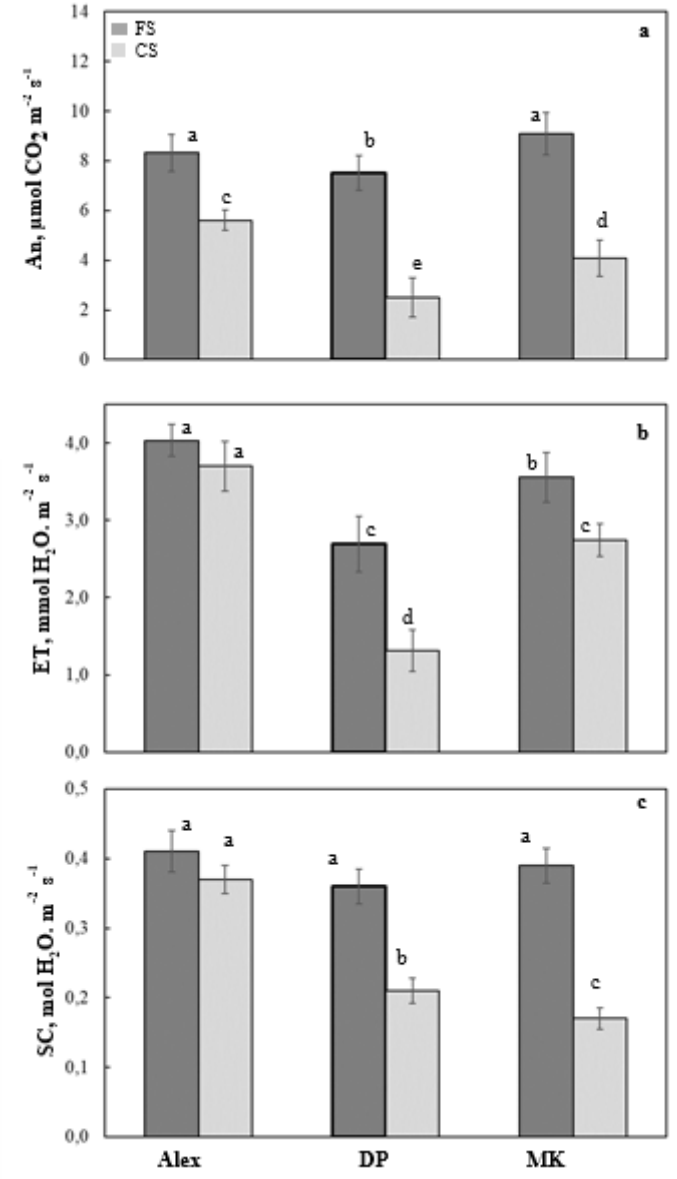

Figure 3

Net photosynthesis activity (An, µmol CO2 m-2 s-1, a), evapotranspiration (ET, mol H2O m-2 s-1, b) and stomatal conductance (SC, mol H2O m-2 s-1, c) in pea genotypes cultivated on fertile soil (FS) or calcareous soil (CS). Within columns, means with the same letter are not significantly different at α = 0.05 according to Fisher's Least Significant Difference. Bars on the columns represent the standard error of the mean (n= 10)

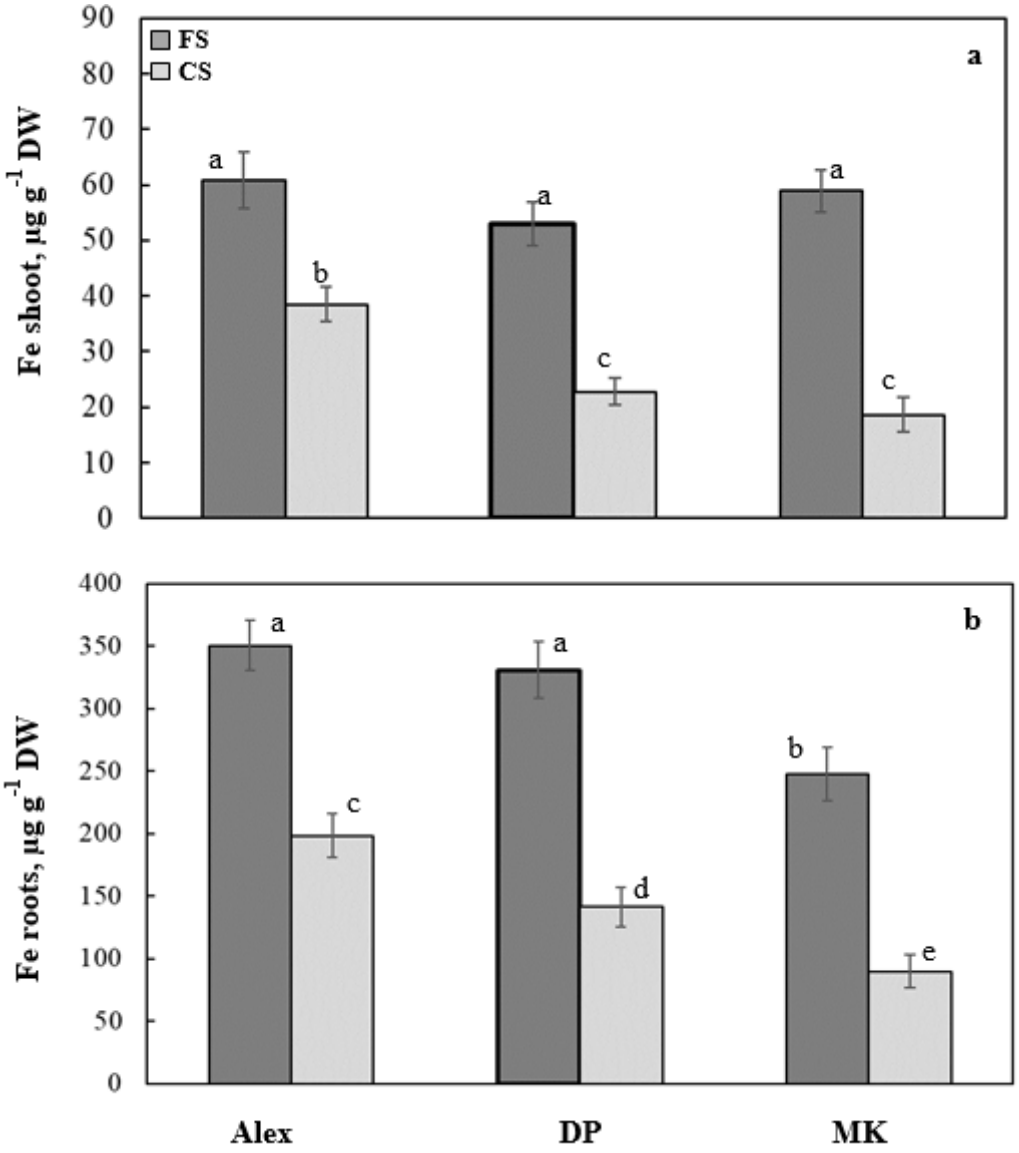

Figure 4

Active Fe concentration (µg g-1DW) in shoots (a) and roots (b) of Pea plants (Pisum sativum L.) cultivated on on fertile soil (FS) or calcareous soil (CS). Within columns, means with the same letter are not significantly different at α = 0.05 according to Fisher's Least Significant Difference. Bars on the columns represent the standard error of the mean (n= 10).

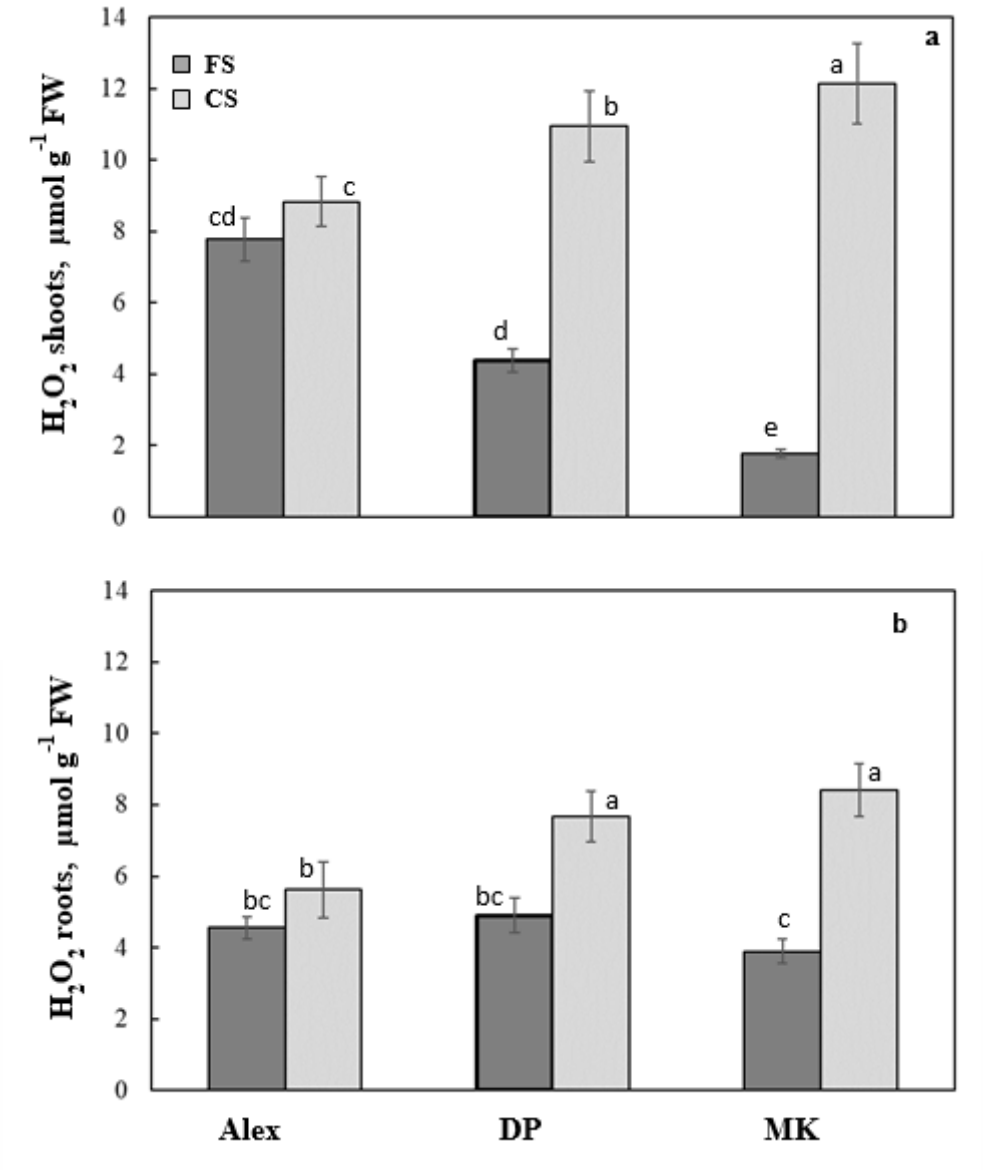

Figure 5

H2O2 concentration (µmol g-1 FW) in shoots (a) and roots (b) of Pea plants (Pisum sativum L.) cultivated on on fertile soil (FS) or calcareous soil (CS). Within columns, means with the same letter are not significantly different at α = 0.05 according to Fisher's Least Significant Difference. Bars on the columns represent the standard error of the mean (n= 6, three replicates repeated twice).

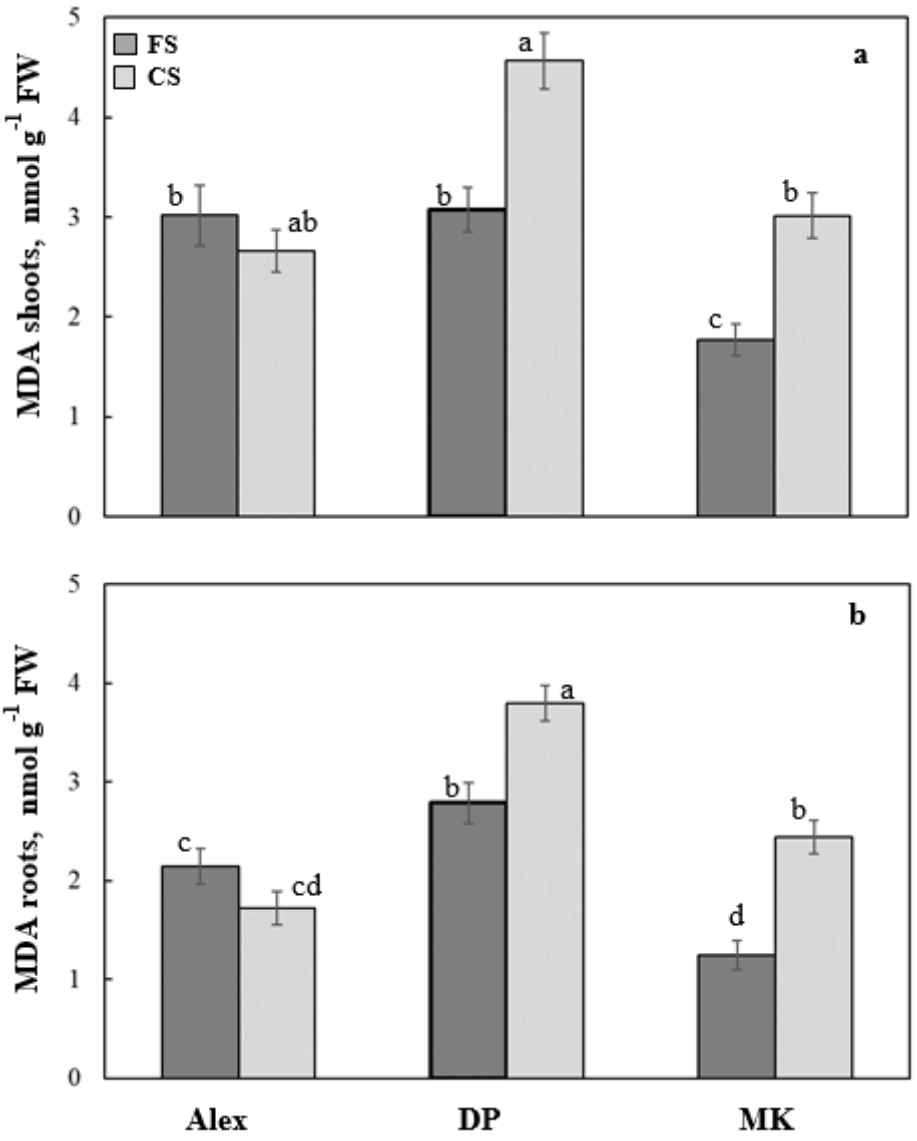

Figure 6

MDA concentration (µmol g-1 FW) in shoots (a) and roots (b) of Pea plants (Pisum sativum L.) cultivated on on fertile soil (FS) or calcareous soil (CS). Within columns, means with the same letter are not significantly different at α = 0.05 according to Fisher's Least Significant Difference. Bars on the columns represent the standard error of the mean (n= 6, three replicates repeated twice).

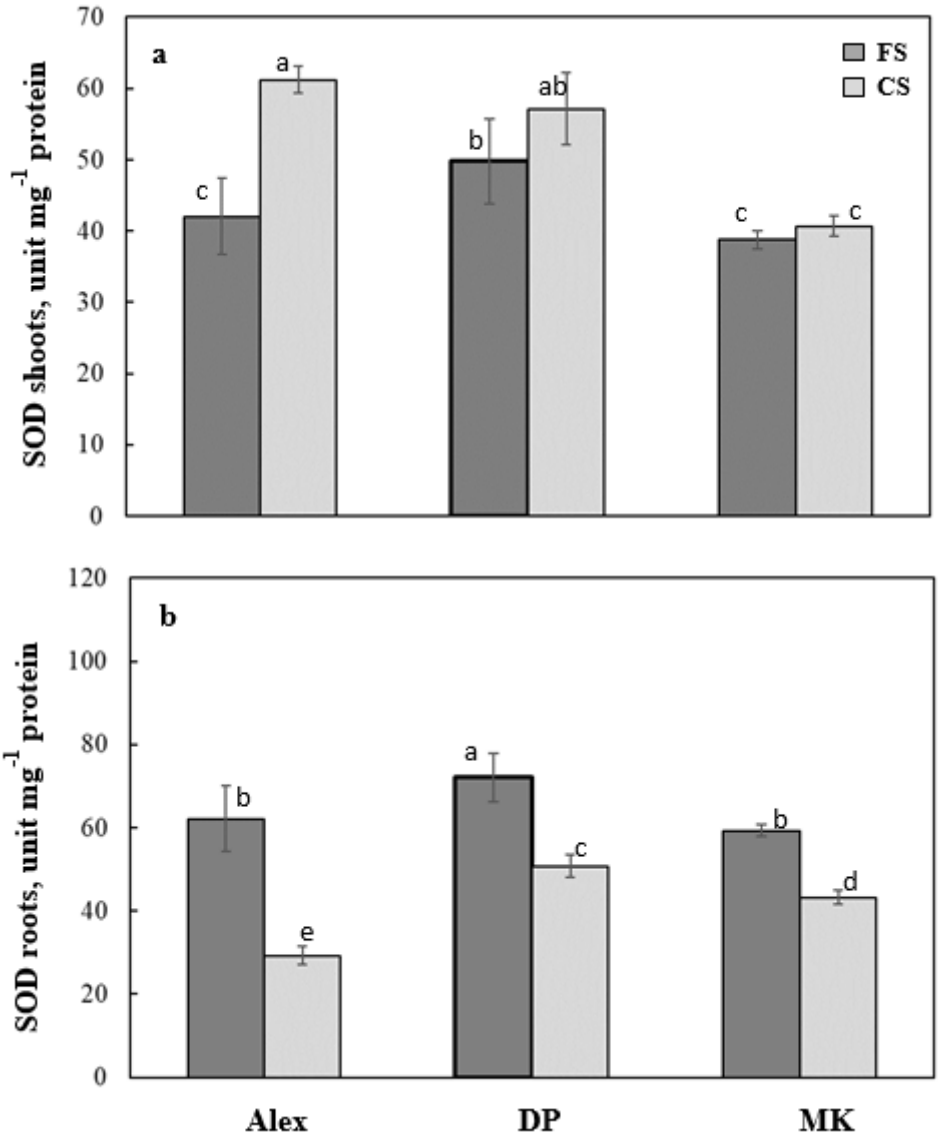

Figure 7

SOD activity (unit mg-1 protein) in shoots (a) and roots (b) of Pea plants (Pisum sativum L.) cultivated on on fertile soil (FS) or calcareous soil (CS). Within columns, means with the same letter are not significantly different at α = 0.05 according to Fisher's Least Significant Difference. Bars on the columns represent the standard error of the mean (n= 6, three replicates repeated twice).

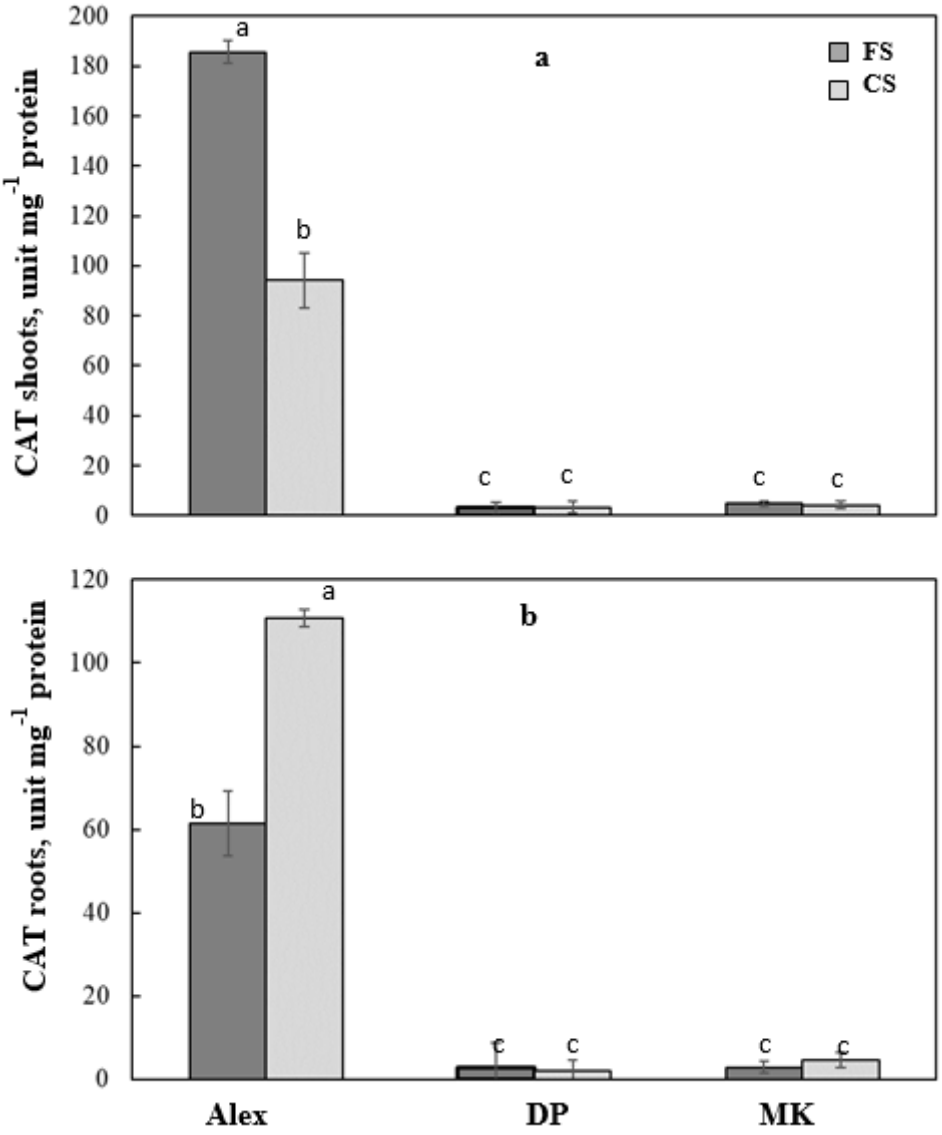

Figure 8

CAT activity (unit mg-1 protein) in shoots (a) and roots (b) of Pea plants (Pisum sativum L.) cultivated on on fertile soil (FS) or calcareous soil (CS). Within columns, means with the same letter are not significantly different at α = 0.05 according to Fisher's Least Significant Difference. Bars on the columns represent the standard error of the mean (n= 6, three replicates repeated twice).