

POTENTIAL OF THE PHYSIOLOGICAL RESPONSE OF PEA PLANTS (*Pisum sativum* L.) TO IRON DEFICIENCY (DIRECT OR LIME- INDUCED)

POTENCIAL DA RESPOSTA FISIOLÓGICA DAS PLANTAS DE ERVILHA (*Pisum sativum* L.) À DEFICIÊNCIA DE FERRO (DIRETA OU INDUZIDA PELO CALCÁRIO)

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ABSTRACT: Pea (*Pisum sativum* L.) is an important food crop in Tunisia, where calcareous soils represents the major limiting factor for agriculture production. In the present study a greenhouse experiment was conducted to assess the effects of direct and bicarbonate- induced iron deficiency on plant growth, chlorophyll fluorescence, photosynthesis, spad index and iron nutrition in two Tunisian pea genotypes (*Pisum sativum* L.). Plants were grown hydroponically and iron deficiency was induced for 3 weeks. Iron deficiency decreased all the above physiological parameters. The direct Fe deficiency is more drastic than bicarbonate- induced Fe deficiency. A close relationship between plant growth, photosynthesis and SPAD index was observed. Fe use efficiency for plant growth and Fe use efficiency for photosynthesis discriminates clearly the studied genotypes and seems to be the main reason of the tolerance of Kelvedon, as compared to Lincoln.

KEYWORDS: Chlorophyll fluorescence- Fe use efficiency- iron deficiency- photosynthesis- Spad index.

INTRODUCTION

In calcareous soils, which constitute the major part of cultivated land, the soil solution does not provide more than 10% of the plant requirements for Fe (MORTVEDT, 1991). The total Fe content in these soils is high but the available fraction for the plant is insufficient. This is caused by the very low solubility of iron oxides at the alkaline pH conditions that are buffered by the presence of bicarbonate in these soils. Ammari and Mengel (2006) indicated that Fe concentration found in calcareous soil was high enough to meet the plant's demand and prove that Fe chlorosis on such soils is not a question of the Fe availability in the soil.

Iron (Fe) is known to be essential for many physiological and biochemical processes such as: photosynthesis, respiration, DNA synthesis, nitrogen assimilation and symbiotic nitrogen fixation and participates in the electron transfer through reversible redox reactions, cycling between Fe²⁺ and Fe³⁺. Fe deficiency affects the structure, development and function of the entire photosynthetic apparatus (ABADIA et al., 1999). It has been shown that Fe deficiency decreases light-harvesting pigments, particularly chlorophylls (MORALES et al., 2000), and promotes antenna disconnection in PSII (MORALES et al., 2001). Furthermore, Fe-deficient leaves show lower PSII efficiency and a decrease in the proportion of open PSII reaction centers (qp) (LARBI et al., 2006).

Salahi et al. (2017) demonstrated that foliar iron sprays improve the performance of oriental plane tree in calcareous soil better than soil treatments.

Differences among species and genotypes in plant response to iron deficiency have been reported. Krouma et al. (2008) and Slatni et al. (2009) reported clear genotypic differences in the response of common bean to iron deficiency. Soybean seed yield decreased by 20% per unit of visual leaf chlorosis (FeDC) when grown on calcareous soil (FROEHLICH; FEHR, 1981). Various authors reported that Fe resupply to deficient plants restores many plant functions. For instance, it leads within a few days to increases in chlorophyll concentration and photosynthetic activity in several annual species, including sugar beet (LARBI et al., 2004) and tobacco (PUSHNIK; MILLER, 1989). Paula et al. (2016) observed in *Lotus japonicus* that interveinal chlorosis was associated with a reduced Fe²⁺ shoot content in all sensitive ecotypes and a decline in photosynthesis rate and PSII performance compared to the control. In some apple cultivars, bicarbonate treatment reduced active and total Fe and total chlorophyll concentrations, and FCR (leaf ferric chelate reductase) activity (SAHIN et al., 2017)

The objective of this study was to examine the physiological parameters associated with the tolerance of pea to iron deficiency in order to establish useful test for screening program, and to assess the relationships between plant growth,

SPAD index, photosynthesis, chlorophyll fluorescence and iron nutrition.

MATERIALS AND METHODS

Plant material and experimental conditions

Two pea genotypes were used, Kelvedon and Lincoln largely cultivated in the North and North West of Tunisia. Seeds were disinfected with 2% hypochlorite calcium solution then rinsed in deionized water. Germination was made in Petri dishes containing moistened filter paper for 6 days at 20 °C. Seven-day-old seedlings were then transferred to a half strength aerated nutrient solution for 7 days and then similar sized seedlings were selected and cultured as groups of 10 plants in 10 L of full strength aerated nutrient solution.

The composition of the nutrient solution was: 1.25mM Ca(NO₃)₂, 1.25mM KNO₃, 0.5mM MgSO₄, 0.25mM KH₂PO₄ and 10 µM H₃BO₃, 1 µM MnSO₄, 0.5 µM ZnSO₄, 0.05 µM (NH₄)₆Mo₇O₂₄ and 0.4 µM CuSO₄. Three treatments were established for 21 days as follows: control (solution added with 30 µM Fe: C), direct iron deficiency (no iron added) and bicarbonate-induced iron deficiency (solution added with 30 µM Fe + 0.5 g L⁻¹ CaCO₃ + 10 mM NaHCO₃). Iron was supplied in the form of Fe(III)-EDTA. NaHCO₃ and CaCO₃ were added to the nutrient solution to simulate the natural condition in calcareous soil. Aerated hydroponic cultures were maintained in a growth chamber with a day/ night regime of 16/8 h, 24/ 18 °C and a relative humidity of 70%. The solution was renewed every 2 days.

After 21 days of treatments, SPAD index, chlorophyll fluorescence and gas exchange parameters were measured, and then plants were separated into shoots and roots, dried at 60 °C for 72 hours, and then pulverized into a fine powder.

SPAD Index

The degree of chlorosis was estimated non-destructively in the youngest fully expanded apical leaves from five plants of each treatment using a portable SPAD-502 meter (Minolta, Osaka, Japan). Five SPAD readings were recorded for each leaf, homogeneously distributed from the apex to the base of the leaf, to obtain a representative degree of leaf chlorosis.

Active iron

Measurements of active iron (Fe²⁺) were performed according to Köseoglu and Acikgöz (1995) The extraction was made in 25 mg of leaves fine powder shacked in 10 ml of 1N HCl.

Gas exchange measurement

Gas exchange measurements were made with an LI-6400 (LI-COR, Inc.) portable gas exchange system. Measurements were made on the 3 youngest fully expanded leaves. Photosynthesis was induced with saturating light (1000 µmol m⁻² s⁻¹). This light was fitted to the standard 6-cm² 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.

Chlorophyll fluorescence measurements

Prior to the measurements, the attached leaves were dark adapted for 30min in leaf-clips. Values for maximum fluorescence (F_m) and initial fluorescence (F₀) from the fluorescence induction curve were measured with a portable chlorophyll fluorometer (OS1-FL). Photosynthetic photon flux density (PPFD) was lower than 0.4 µmol m⁻² s⁻¹ at the leaf surface. F_m was measured at 20 kHz with a 0.8 s pulse of 6000 µmol m⁻² s⁻¹ of white light (Morales et al., 1998).

Calculations

Fe use efficiency for plant growth (FeUEDW) was expressed as the ratio of biomass production to Fe accumulation in leaves [g dry weight. µmol⁻¹ Fe] and Fe use efficiency for net assimilation (FeUEAn) was expressed as the ratio of net assimilation to Fe accumulated in leaves [(µmol CO₂ m⁻² s⁻¹). µmol⁻¹ Fe].

Statistical analysis

Variance analysis of data (one-way ANOVA) was performed using the SPSS 10.0 program, and means were separated to Duncan's test at p ≤ 0.05. Data shown are means of five repetitions (photosynthetic parameters, SPAD index and chlorophyll fluorescence) or nine (Biomass and Fe content) replicates for each treatment.

RESULTS

Plant growth and SPAD index

All plants subjected to iron deficiency exhibited a clear decrease of biomass production. This effect was more important when plants are cultivated on Fe- free medium. Nevertheless, the negative effect of iron deficiency on plant growth is more pronounced in Lincoln than Kelvedon. The decrease of biomass production was estimated to 8% and 40% in Lincoln subjected to direct or bicarbonate- induced Fe deficiency, respectively; and 9% and 17% in Kelvedon subjected to direct or

bicarbonate- induced Fe deficiency, respectively (fig 1).

The SPAD index values (table 1) followed the same scheme of variation of plant growth parameters. Iron deficiency decreased SPAD index, the effect is more drastic in direct than indirect Fe deficiency. Kelvedon remain the less affected

genotype as compared to Lincoln. The values of SPAD index decreased with 11 % and 16 % in Kelvedon and with 24 % and 28 % in Lincoln, respectively subjected to direct or bicarbonate-induced iron deficiency.

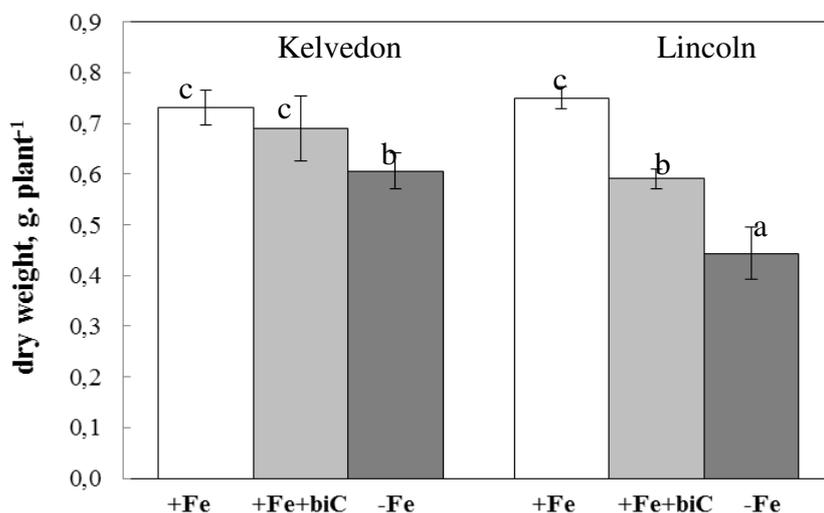


Figure 1. Biomass production in pea plants subject to bicarbonate- induced or direct iron deficiency. Vertical bars represent \pm standard errors of means of 9 replicates, $p \leq 0.05$.

Table 1. SPAD index in leaves of two pea genotypes subjected direct (- Fe) or bicarbonate- induced (+Fe +biC) Fe deficiency compared to control treatment (+Fe). Standard errors of means of 9 replicates, $p \leq 0.05$.

	Genotypes	+Fe	+Fe +biC	-Fe
SPAD Index	Kelvedon	38.56 \pm 0.85	34.33 \pm 0.95	32.4 \pm 1.63
	Lincoln	39.76 \pm 0.86	30.33 \pm 1.56	28.66 \pm 0.97

Photosynthesis and chlorophyll fluorescence

Iron deficiency decreased net photosynthesis in the two studied genotypes. Independently of iron deficiency origin, this effect was less pronounced in Kelvedon than Lincoln (fig 2). In the first genotype A_n decreased by 15% in plants subjected to induced Fe chlorosis (+Fe + biC) and by 32% in plants subjected to direct Fe chlorosis (- Fe). In the second genotype (Lincoln), this decrease was estimated to 34% in plants

suffering from induced Fe chlorosis and 49% in plants suffering from direct Fe deficiency.

Concerning the other photosynthetic parameters, the same trend of variation was observed. Direct or induced Fe chlorosis decreased transpiration (fig 3a), stomatal conductance (fig 3b). The lack of iron in the medium is more drastic than its deficiency induced by bicarbonate. Kelvedon remain the less affected genotype as compared to Lincoln.

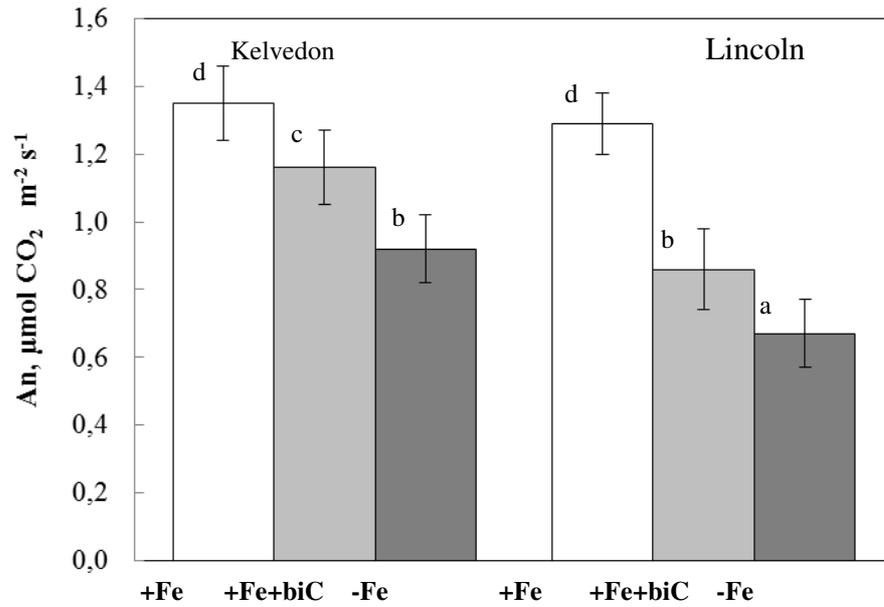


Figure 2. Net assimilation (An) in two pea genotypes subjected to direct (-Fe) or induced Fe chlorosis (+Fe +biC) as compared to control treatment (+Fe). Vertical bars represent \pm standard errors of means of 5 replicates, $p \leq 0.05$.

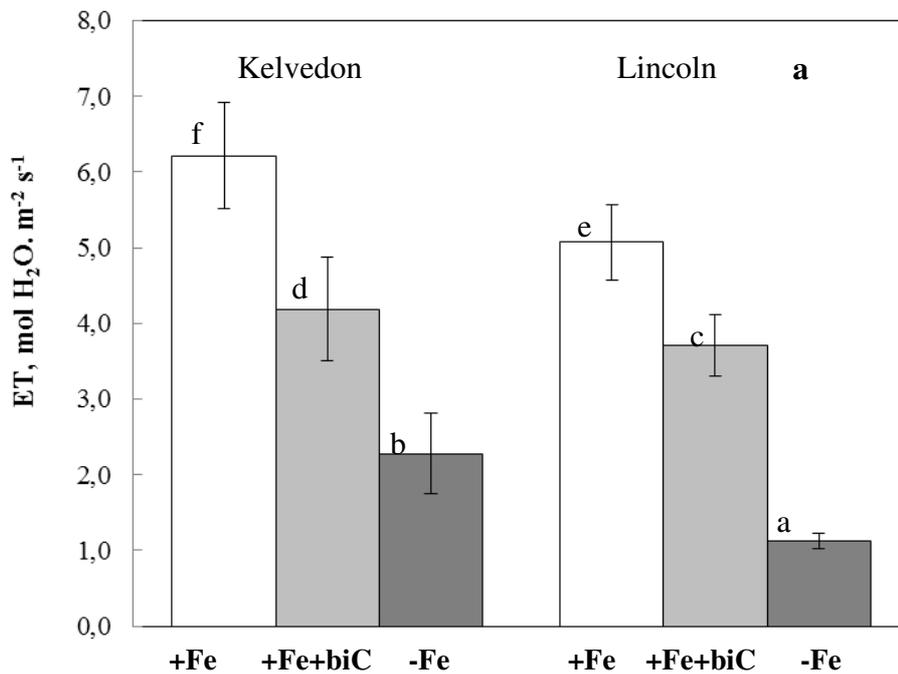


Figure 3a. Evapotranspiration (ET) in two pea genotypes subjected to direct (-Fe) or induced Fe chlorosis (+Fe +biC) as compared to control treatment (+Fe). Vertical bars represent \pm standard errors of means of 5 replicates, $p \leq 0.05$.

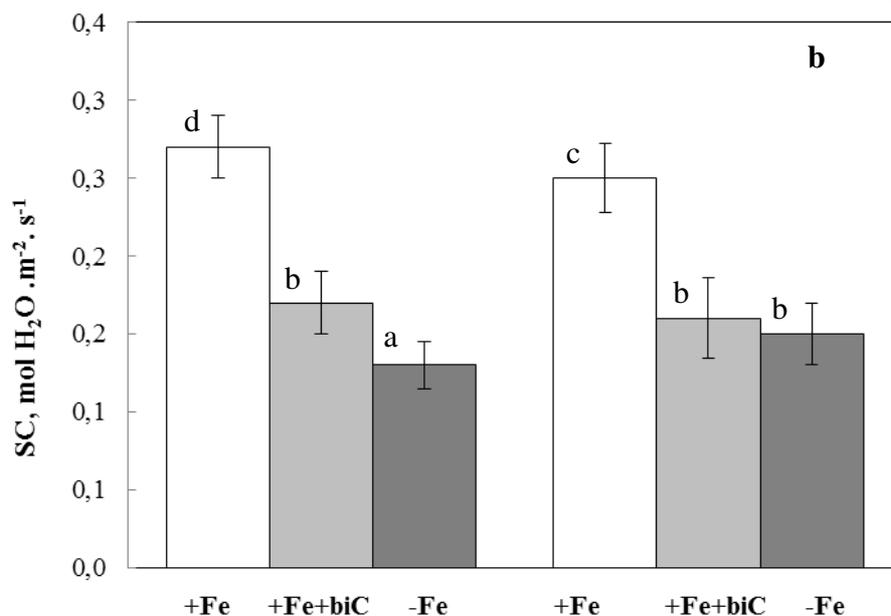


Figure 3b. Stomatal Conductance (SC) in two pea genotypes subjected to direct (-Fe) or induced Fe chlorosis (+Fe +biC) as compared to control treatment (+Fe). Vertical bars represent \pm standard errors of means of 5 replicates, $p \leq 0.05$.

For internal concentration of CO₂ (fig 4), a slight decrease of this element was observed under iron deficiency but without significant effect.

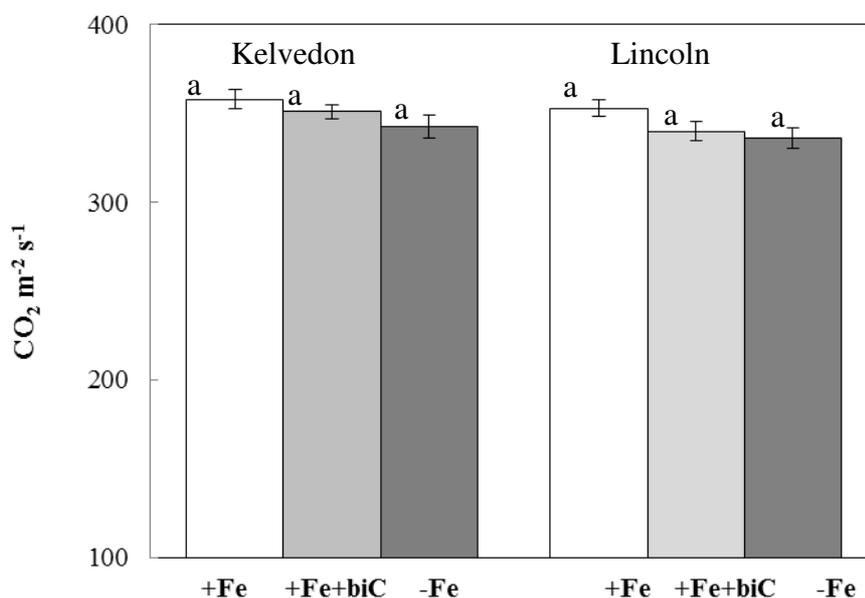


Figure 4. Internal CO₂ concentration in two pea genotypes subjected to direct (-Fe) or induced Fe chlorosis (+Fe +biC) as compared to control treatment (+Fe). Vertical bars represent \pm standard errors of means of 5 replicates, $p \leq 0.05$.

Measurements made on chlorophyll fluorescence demonstrate important genotypic differences in the maximum quantum yield of PSII in dark as in light test (table 2). In fact, no significant effect of direct and induced Fe chlorosis

observed in Kelvedon. However, in Lincoln genotype, iron deficiency induced a clear decrease of Fv/Fm (- 8% in bicarbonate- induced and - 12% in direct iron deficiency) and F'v/F'm (- 9% in bicarbonate- induced and - 10% in direct iron

deficiency). The calculation of non- photochemical quenching (NPQ) demonstrates an important effect of iron deficiency in Lincoln (- 10% in bicarbonate-induced and – 21% in direct iron deficiency) but not

in Kelvedon (– 6% only in direct iron deficiency) (table 2).

Table 2. Maximum quantum yield of PSII (Fv/Fm and F'v/F'm) and non - photochemical quenching (NPQ) in two pea genotypes subjected direct (- Fe) or bicarbonate- induced (+Fe +biC) Fe deficiency compared to control treatment (+Fe). Standard errors of means of 5 replicates, $p \leq 0.05$.

	Genotypes	+ Fe	+ Fe + biC	- Fe
Fv/Fm	Kelvedon	0,76 ± 0.03	0,76 ± 0.02	0,74 ± 0.01
	Lincoln	0,79 ± 0.02	0,73 ± 0.01	0,69 ± 0.02
F'v/F'm	Kelvedon	0,60 ± 0.03	0,58 ± 0.02	0,59 ± 0.01
	Lincoln	0,60 ± 0.02	0,54 ± 0.01	0,53 ± 0.02
NPQ	Kelvedon	2.51± 0.02	2.57± 0.03	2.35± 0.03
	Lincoln	2.71± 0.03	2.44± 0.03	2.13± 0.02

Iron nutrition

The analysis of the extractable active fraction of Fe in leaves (fig 5) demonstrated that iron deficiency induced a significant decrease of this micronutrient. When subjected to induced Fe chlorosis, this decrease was estimated to 35% and

33%, respectively in Kelvedon and Lincoln, while reaching 54% and 52%, respectively in Kelvedon and Lincoln, subjected to direct iron deficiency. This result indicated a clear problem of iron allocation to leaves.

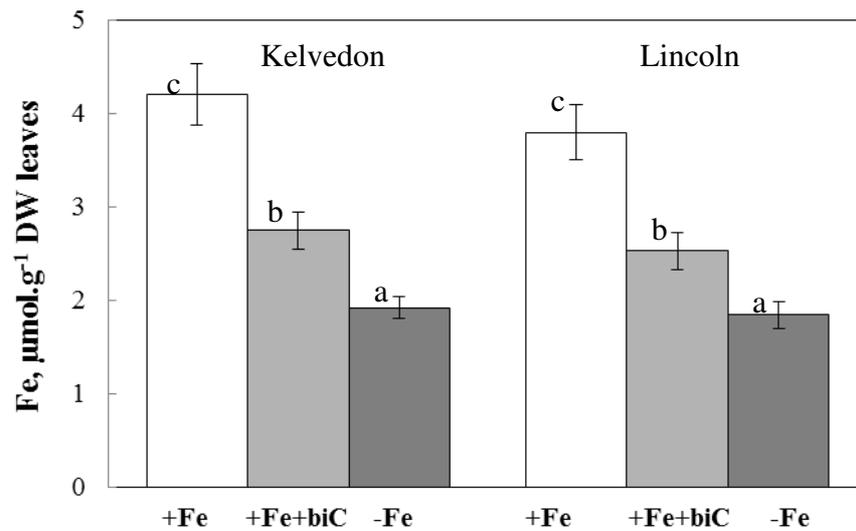


Figure 5. Active iron (FeII) concentrations in leaves of pea genotypes subjected to direct (-Fe) or induced Fe chlorosis (+Fe +biC) as compared to control treatment (+Fe). Vertical bars represent \pm standard errors of means of 9 replicates, $p \leq 0.05$. DW: dry weight

The calculation of the Fe use efficiency for plant growth and photosynthetic activity demonstrated that this parameter increased with iron deficiency in Kelvedon and remain without

significant changes in Lincoln (table 3). The first genotype expressed higher efficiency of Fe use in Fe depletion conditions as compared to Lincoln.

Table 3. Fe use efficiency for plant growth (FeUEDW, expressed as the ratio of biomass production to Fe accumulation in leaves, g DW. μmol^{-1} Fe) and Fe use efficiency for net assimilation (FeUEAn, calculated as the ratio of net assimilation to Fe accumulated in leaves), in two pea genotypes subjected to direct or induced Fe deficiency.

	Genotypes	+Fe	+Fe+biC	-Fe
FeUE DW	Kelvedon	0.40 \pm 0.04	0.78 \pm 0.05	1.12 \pm 0.09
	Lincoln	0.32 \pm 0.03	0.66 \pm 0.07	0.82 \pm 0.07
FeUE An	Kelvedon	0.73 \pm 0.06	1.15 \pm 0.08	1.71 \pm 0.09
	Lincoln	0.55 \pm 0.04	0.96 \pm 0.09	1.23 \pm 0.11

DISCUSSION

Typically, Fe and P are the two main nutrients that limit plant growth on calcareous soils (MARSCHNER, 1995). Our results show that iron deficiency decreased biomass production and SPAD

index in the two studied genotypes and significantly inhibit active iron (FeII) accumulation. Direct Fe deficiency is more drastic than bicarbonate- induced Fe deficiency and Lincoln is usually more affected than Kelvedon. As documented by several authors (ZOCCHI et al., 2007; KROUMA et al., 2008), Fe

deficiency adversely affected plant growth and shoot length in several plant species. In fact, Fe is known to be essential for many physiological and biochemical processes such as: photosynthesis, respiration, DNA synthesis, nitrogen assimilation and symbiotic fixation. Iron is shown as an indispensable element for chlorophyll and carotenoids biosynthesis (THOIRON et al., 1997), the photosynthesis (JELLELI et al., 2011) as well as the metabolism of plastidial proteins (SPENCE et al., 1991) and therefore, the induced iron chlorosis and the decrease of SPAD index observed in this study can be explained by a drastic decrease of iron availability for these organs. İncesu et al. (2015) observed significant differences in SPAD and iron chlorosis scale reading between rootstocks and Fe treatments. In fact, fig 6 which correlates the leaves Fe content with their SPAD index demonstrated a close relationship between these two parameters ($R^2 = 0.99$). The genotypic variability observed in this study was found in common bean subjected to iron deficiency (KROUMA et al., 2008). It has been shown that Fe deficiency decreased light-harvesting pigments, particularly chlorophylls (MORALES et al., 2000); and promotes antenna disconnection in PSII (MORALES et al., 2001; MOSELEY et al.,

2002). Furthermore, many micronutrients (e.g. Fe, Mn, Cu and Zn) that are freely available in acid soils are only sparingly available in calcareous soils, due to their poor solubility at high pH (BRADY and WEIL, 1999). Experiments have shown calcifuges plants (those which cannot establish well on calcareous soils e.g. pea and common bean) to be primarily excluded from growth in calcareous soils due to poor P and Fe use efficiency (KERLEY et al., 2001). The calculation of the Fe use efficiency for plant growth (FeUEDW, table 3) expressed as the ratio of biomass production to Fe accumulation in leaves ($\text{g DW} \cdot \mu\text{mol}^{-1} \text{Fe}$) screened clearly the studied genotypes. The values of FeUEDW were 2 times more important in bicarbonate-induced Fe deficiency plants as compared to control ones and 3 times in direct Fe deficiency. The most attractive result at this level is that Kelvedon developed more important efficiency of iron use than Lincoln with the same quantities of iron accumulated in leaves when subjected to iron deficiency. The FeUEDW is 1.2 and 1.4 times higher in Kelvedon than Lincoln when subjected to induced and direct Fe deficiencies, respectively. This efficiency seems to be the origin of Kelvedon tolerance.

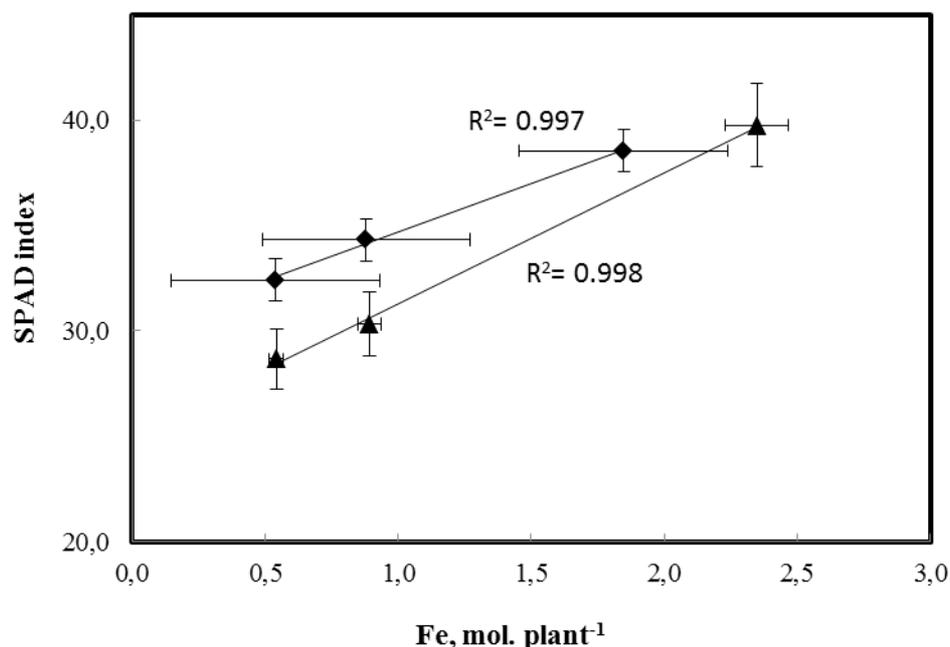


Figure 6. Relationship between active iron (FeII) concentrations in leaves and SPAD index in two pea genotypes subjected to induced or direct iron deficiency.

The measurements made on gas exchange parameters and chlorophyll fluorescence show that independently of its origin, iron deficiency decreased net assimilation and other photosynthetic

parameters. The maximum quantum yield of PSII is significantly affected by iron deficiency in Lincoln but not in Kelvedon. Genotypic differences previously observed were maintained and Kelvedon

develops a better preservation of its photosynthetic apparatus. Previous works demonstrated that the maximum quantum yield of photosystem II decreased in Fe deficient leaves of citrus (PESTANA et al., 2005), pear (DONNINI et al., 2009) and peach rootstocks (MOLASSIOTIS et al., 2006). Similar results were found by Donnini et al. (2009) showing different reorganization of the photosynthetic apparatus between tolerant and sensitive genotypes of pear and quince cultivated in the presence of bicarbonate. In addition, Sharma (2007) investigated the adaptation of photosynthesis under Fe deficiency in maize plants and suggested an involvement of nuclear- chloroplast signaling in mediating adaptive changes in the photosynthetic machinery triggered by redox status and possibly, accumulation of chlorophyll biosynthesis intermediates. Kara (2016) concluded that low temperature, high net photosynthesis rate, high internal CO₂ concentration/ambient CO₂ ratio and low transpiration rate might be used as reliable selection criteria in further triticale breeding programs. In the present study, the calculation of Fe

use efficiency for photosynthesis (FeUEAn, calculated as the ratio of net assimilation to Fe accumulated in leaves, $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1} \cdot \mu\text{mol}^{-1} \text{ Fe}$) (table 3), demonstrated a clear increase of this parameter in plants subjected to iron deficiency. Kelvedon maintain its performance, as compared to Lincoln, in the two Fe deficiency origin with values of FeUEAn 1.4 times more important than lincoln.

It appears clearly that FeUEDW and FeUEAn discriminates the two studied pea genotypes. The better efficiency of Kelvedon genotype, compared to Lincoln one, gives us a new explanation of its performance in a limiting iron availability condition like calcareous soil. In fact, our results suggested that the tolerance of Kelvedon is probably linked to two parameters: firstly a better ability to allocate more iron to shoots to maintain photosynthetic activity and plant growth; and secondly, to its efficiency of iron use. We suggest that Fe use efficiency for plant growth and Fe use efficiency for photosynthesis might be used as reliable selection criteria in further plant breeding programs.

RESUMO: A ervilha (*Pisum sativum* L.) é uma cultura alimentar importante na Tunísia, onde os solos calcários representam o principal fator limitante para a produção agrícola. No presente estudo, foi conduzido um experimento em estufa para avaliar os efeitos da deficiência de ferro direta e induzida por bicarbonato sobre o crescimento de plantas, a fluorescência da clorofila, a fotossíntese, o índice SPAD e a nutrição de ferro em dois genótipos de ervilha da Tunísia (*Pisum sativum* L.). As plantas foram cultivadas hidroponicamente e a deficiência de ferro foi induzida durante 3 semanas. A deficiência de ferro diminuiu todos os parâmetros fisiológicos acima. A deficiência de Fe direta é mais drástica do que a deficiência de Fe induzida por bicarbonato. Observou-se uma estreita relação entre o crescimento das plantas, a fotossíntese e o índice SPAD. A eficiência de uso de Fe para o crescimento de plantas e a eficiência de uso de Fe para a fotossíntese discriminam claramente os genótipos estudados e parecem ser a razão principal da tolerância de Kelvedon, em comparação com a de Lincoln.

PALAVRAS-CHAVE: Fluorescência de clorofila. Eficiência de uso de Fe. Deficiência de ferro. Fotossíntese. Índice Spad.

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