Scientia Horticulturae 165 (2014) 148–155

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Chlorophyll fluorescence imaging as a tool to understand the impact of iron deficiency and resupply on photosynthetic performance of strawberry plants

Júlio Osório[a,*], Maria Leonor Osório[b], Pedro José Correia[a], Amarilis de Varennes[c], Maribela Pestana[a]

[a] Institute of Mediterranean Agricultural and Environmental Sciences – (ICAAM), Faculty of Sciences and Technology, University of Algarve, Ed. 8, Campus de Gambelas, 8005-139 Faro, Portugal
[b] Institute for Biotechnology and Bioengineering, Centre of Genomics and Biotechnology (IBB-CGB), Faculty of Sciences and Technology, University of Algarve, Ed. 8, Campus de Gambelas, 8005-139 Faro, Portugal
[c] Biosystems Engineering Center, Instituto Superior de Agronomia, University of Lisbon, Tapada da Ajuda, 1349-017 Lisbon, Portugal

ARTICLE INFO

Article history:
Received 8 February 2013
Received in revised form 31 July 2013
Accepted 29 October 2013

Keywords:
Chlorophyll fluorescence imaging
Energy partitioning
Fragaria × ananassa
Iron deficiency
Photosynthetic efficiency

ABSTRACT

Bare-root transplants of strawberry (Fragaria × ananassa Duch. cv 'Diamond') were grown in a Hoagland's nutrient solution with or without Fe. Forty two days after Fe deprivation, recovery was induced by addition of 10 μM of Fe (Fe-EDDHA) to the Fe(−) nutrient solution. Total leaf chlorophyll concentration in young leaves decreased progressively with time in Fe-deprived plants, and before Fe resupply it was only 6% of that of Fe(+) control plants. Spatio-temporal changes on photosynthetic efficiency were monitored by imaging chlorophyll a fluorescence in four areas of interest (AOIs) located on the midrib and on interveinal mesophyll areas of leaf blades. Chlorophyll fluorescence images (F_v/F_m, ϕ_{II}, NPQ, q_p) showed a large spatial variation, particularly at day 42, with greater values in midrib areas. Temporal changes were also observed in all measured parameters along the experimental period, but the onset and intensity of impact was clearly different between parameters. Maximal efficiency of PSII (F_v/F_m) was the last parameter to be affected, being the effects visible only in plants that had lost over 90% of their chlorophyll (day 42). In contrast, actual efficiency of PSII (ϕ_{II}) and photochemical quenching (q_p) were affected earlier on, showing noticeable changes by day 20, when chlorophyll concentration had declined by 38%. Decreases in ϕ_{II} were balanced by increases in quantum yield of non-regulated energy dissipation (ϕ_{NO}). Fluorescence parameters, with the exception of ϕ_{II} and Fe content, recovered within eight days following Fe resupply to values found in Fe(+) plants. The results of this study indicate that: (i) Chl fluorescence imaging is a useful technique to evaluate Fe deficiency (ii) Fe stress generates spatio-temporal heterogeneity in fluorescence response (iii) ϕ_{II} measured in interveinal mesophyll areas could be used as an early and fast indicator of Fe deficiency and could be applied for fertilization management.

1. Introduction

Iron (Fe) deficiency is a well-documented problem affecting crop production worldwide, in particular in calcareous soils of Mediterranean basin countries, including Portugal (Pestana et al., 2004). Fe-deficient plants are characterized by the development of a pronounced chlorosis occurring first on the youngest leaves and causing various morphological and physiological changes in plants. Among other effects, Fe deficiency affects the structure, development and function of the entire photosynthetic apparatus (Terry and Abadía, 1986; Abadía et al., 1999). It has been shown that Fe deficiency leads to decreases in light-harvesting pigments, particularly chlorophylls (Morales et al., 2000), and promotes antenna disconnection in PSII (Morales et al., 2001, Moseley et al., 2002). Furthermore, Fe-deficient leaves show lower actual PSII efficiency (ϕ_{II}) and a decrease in the proportion of open PSII reaction centers (q_p) (Larbi et al., 2006). However, many abiotic stresses, including Fe deficiency, do not result in uniform symptoms, but rather on patches of visible injuries on leaf surfaces, more drastic in mesophyll leaf areas than in midrib and veins in the case of this deficiency. It was also shown that Fe-deficient leaves accumulated more Fe in the midrib and veins, with lower Fe concentration in mesophyll leaf areas (Jiménez et al., 2009; Tomasi et al., 2009). Thus, this heterogeneous distribution of photosynthetic pigments and Fe in leaves could equally affect the photosynthetic efficiency.

* Corresponding author. Tel.: +351 289 800900; fax: +351 289 818419.
E-mail address: josorio@ualg.pt (J. Osório).

0304-4238/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.scienta.2013.10.042

In recent years, chlorophyll (Chl) fluorescence analysis has been applied as a rapid non-destructive tool to obtain information about the state of photosynthetic apparatus and especially of photosystemII (PSII). One of the major disadvantages of conventional Chl fluorescence measurements is that it provides information only on a single leaf spot, which is not representative of the physiological status of the whole leaf (Lichthentaler and Miehé, 1997). The development of instruments capable of imaging Chl fluorescence provided a powerful tool to identify spatial heterogeneity of leaf photosynthetic performance, and offered new possibilities to understand the operation and regulation of photosynthesis (Baker, 2008; Gorbe and Calatayud, 2012). This rapid non-destructive method has been used to assess the effects of other abiotic stresses on photosynthetic efficiency (Calatayud et al., 2006; Osório et al., 2011, 2012, 2013; Martins et al., 2013). The use of Chl fluorescence imaging to quantify the degree of photosynthetic leaf heterogeneity related with Fe nutrition seems obvious, but to our knowledge this is the first report of such a study. As stressors can modify the partitioning of absorbed light energy in leaves, analysis of the contribution of different routes for excitation energy utilisation/dissipation in PSII complexes is of huge importance to study the regulatory mechanisms involved in responses of the photosynthetic apparatus (Kornyeyev and Hendrickson, 2007). Furthermore, this approach also allows a deeper insight into the plant's capacity to cope with excess excitation energy (Klughammer and Schreiber, 2008).

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 (Nishio et al., 1985; Larbi et al., 2004), soybean (Hecht-Buchholz and Ortmann, 1986) and tobacco (Pushnik and Miller, 1989). It was also recorded that tolerance to Fe deficiency may vary between different varieties of the same species, and the capacity to recover from this stress was related with sensitivity to the deficiency (Mahmoudi et al., 2007). However, knowledge on the responses of chlorotic plants to Fe resupply is still scarce, although it may provide crucial information to optimize Fe-fertilization strategies (Abadía et al., 2011; Pestana et al., 2012).

Strawberries are a major crop in southern Portugal where calcareous soils with high levels of bicarbonate ions tend to decrease Fe availability, representing a major constraint to their production. A deeper understanding of the development of Fe stress and plant recovery after resupply is essential to develop suitable and sustainable Fe-fertilization programs for crop production on calcareous soils.

In the present study, we evaluated the potential of Chl fluorescence imaging to investigate the effects of Fe deficiency and Fe resupply on spatial and temporal efficiency of PSII in *Fragaria × ananassa* Duch. cv 'Diamond' grown in hydroponics. The specific aims of this work were: (i) to map leaf chlorosis across leaf blades of recently-expanded leaves; (ii) to assess the effects of Fe deficiency and Fe resupply on Chl and Fe concentrations throughout time; (iii) to check whether high Chl and Fe concentrations in vascular tissues of Fe-deficient leaves would also result in high photosynthetic efficiency; (iv) to identify an early and fast chlorophyll fluorescence indicator for Fe deficiency and Fe recovery.

2. Material and methods

2.1. Plant material and experimental conditions

Experiments were carried out with a strawberry cultivar (*Fragaria × ananassa* Duch. cv 'Diamond') commonly cultivated in the Algarve, southern Portugal. Forty eight plants were cultivated in six

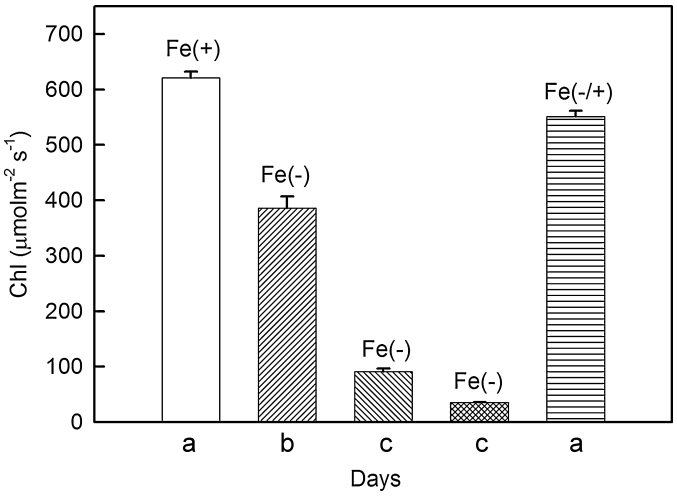

Fig. 1. Chlorophyll concentrations (Chl) as estimated by SPAD-502 meter during Fe treatments: moderately Fe-deficient (Fe(−)/Day 20), severely Fe-deficient (Fe(−)/Day 27), extremely Fe-deficient (Fe(−)/Day 42 and Fe-recovered plants (Fe(−/+)/Day 50). Fe(+) and Fe(−) indicate solutions with 10 μM of Fe (Fe-EDDHA) or 0 μM of Fe, respectively. Values shown are means ± SE of six measurements taken in five leaves per treatment. Different letters indicate significant differences between treatments ($P < 0.05$) according SNK test.

20-L poly-ethylene vessels filled with Hoagland's nutrient solution, which contained: 5 mM $Ca(NO_3)_2$, 5 mM KNO_3, 1.0 mM KH_2PO_4, 2.0 mM $MgSO_4$, 46.0 μM H_3BO_3, 0.8 μM $ZnSO_4$, 0.4 μM $CuSO_4$, 9.0 μM $MnCl_2$, and 0.02 μM MoO_3. Half of the plants were grown in the presence of 10 μM Fe added as Fe(III)-EDDHA, Fe(+), and half in the absence of Fe, Fe(−). The initial pH of the nutrient solution was 6.0 ± 0.2 and the electrical conductivity (EC) was 2.2 ± 0.1 dS m^{-1}. The aerated nutrient solution was replaced when the EC dropped to 1.7 dS m^{-1}. The experiments were performed in a glasshouse under natural photoperiod conditions: a photosynthetic photon flux density (PPFD) of 150 − 450 μmol m^{-2} s^{-1} at the top of the plants and a temperature ≤25 °C. After 42 days, Fe(+) plants remained green but Fe(−) plants were severely chlorotic (Fig. 1). At this point, in order to study recovery from Fe deficiency, half of the chlorotic plants were transferred to nutrient solution that contained Fe, and further grown for eight days. The treatments are described as follows: Fe-sufficient = Fe(+)/Day 0, moderately Fe-deficient = Fe(−)/Day 20, severely Fe-deficient = Fe(−)/Day 27, extremely Fe-deficient = Fe(−)/Day 42 and Fe-recovered plants = Fe(−/+)/Day 50. Measurements were taken on the youngest fully expanded leaves of five plants of each treatment. At the end of the experiment (a total of 50 days), the number of leaves and their fresh weights were registered.

2.2. SPAD readings, chlorophyll concentrations and Fe contents

The first new leaves appeared approximately 15 days after the beginning of the experiment and from then on 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). Six 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. SPAD readings were converted to total Chl using the calibration curve:

$$y = 0.45x^2 - 1.11x + 32.56 (R^2 = 0.98; n = 31; P < 0.001)$$

where y is the Chl concentration (μmol m^{-2}) measured spectrophotometrically and x is the SPAD readings measured in strawberry leaves (Pestana et al., 2011a).