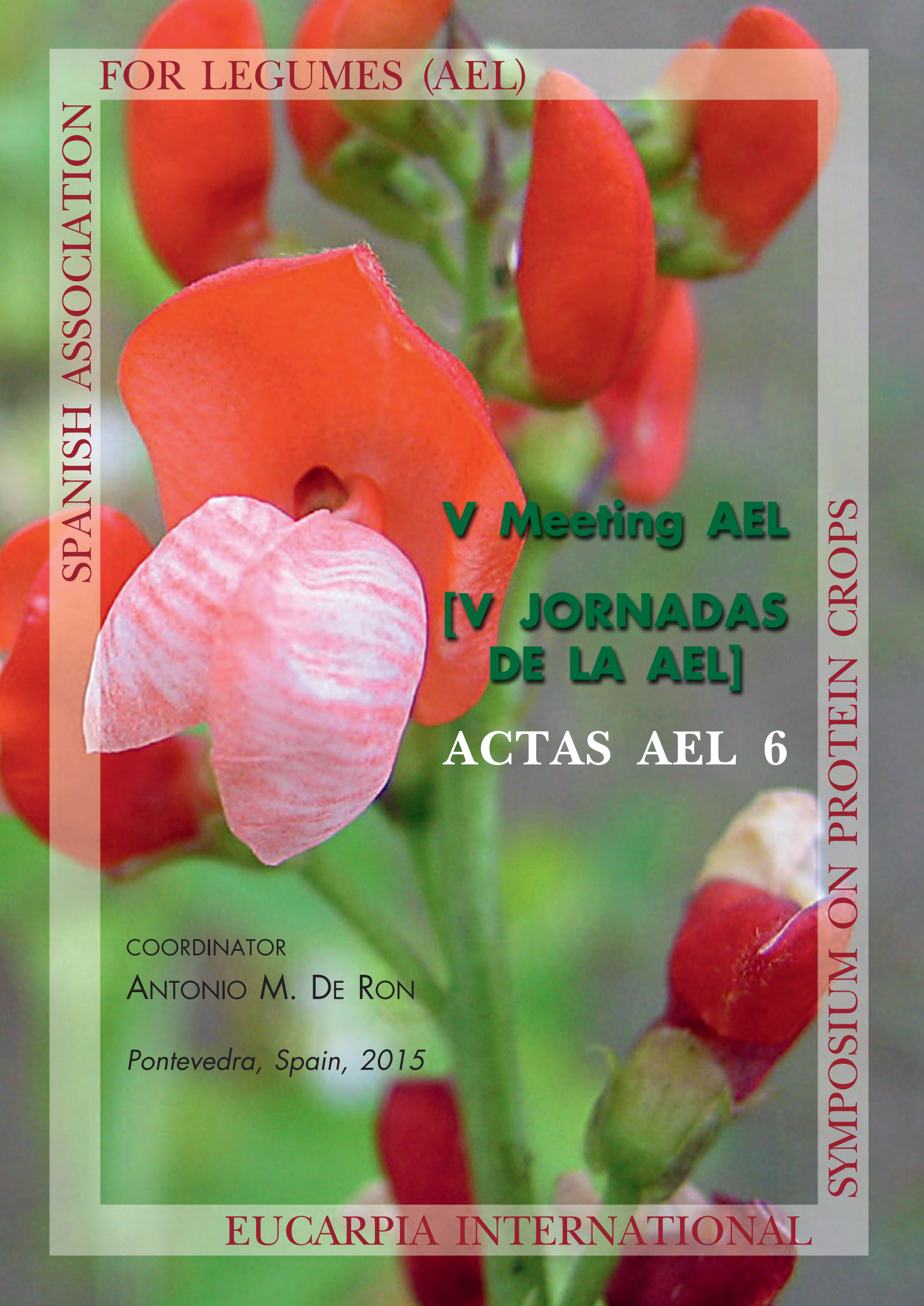

SPANISH ASSOCIATION FOR LEGUMES (AEL)

V Meeting AEL

[V JORNADAS DE LA AEL]

ACTAS AEL 6

SYMPOSIUM ON PROTEIN CROPS

COORDINATOR

ANTONIO M. DE RON

Pontevedra, Spain, 2015

EUCARPIA INTERNATIONAL

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THE IMPACT OF IRON DEFICIENCY ON THE TETRAPYRROLE AND ANTIOXIDATIVE SYSTEMS IN SOYBEAN PLANTS (*Glycine max* L.)

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Introduction

Iron deficiency chlorosis (IDC) is a serious physiological disorder (OU agronomical problem), prevalent on calcareous soil, contributing to decreased crop growth and yield. Its main visible symptom is leaf yellowing due to decreased chlorophyll concentrations. Understanding the mechanisms underlying iron uptake, trafficking and homeostasis is essential in order to prevent IDC and enhance plant productivity. The tetrapyrrole cycle is involved in the biosynthesis of chlorophyll and heme, and it starts with the formation of 5-aminolevulinic acid (ALA). As iron is necessary for hemoproteins (heme containing proteins, such as Fe reductases, catalases and peroxidases) and chlorophyll synthesis, its absence leads to decreased photosynthetic capacity and damaged antioxidative defence. In this study photosynthetic pigments, Fe accumulation, root reductase activity, ALA and hemin (the oxidized version of heme) concentration and antioxidant enzymes activity were evaluated to better understand the impact of Fe deficiency on the tetrapyrrole cyle.

Material and Methods

An efficient (EF - PI437929 / VIR 316) *G. max* accession for Fe deficiency (Vasconcelos and Grusak, 2014) was selected from the USDA (United States Department of Agriculture) germplasm collection via GRIN (Germplasm Resources Information Network) (http://www.ars-grin.gov/). Germinated seedlings were transferred to hydroponic conditions and maintained for 14 days under Fe sufficiency (+Fe; n=5) and Fe deficiency (-Fe; n=5). At the end of the assay, the photosynthetic pigments concentration was measured. The activity of heme containing enzymes root iron reductase, catalase and ascorbate peroxidase was determined in roots and trifoliate leaves. Protocols for dALA quantification and hemin extraction were optimized based on Mauzerall and Granick (1956) and Weinstein and Beale (1983), respectively. Hemin quantification was performed using the Hemin Assay Kit (Sigma-Aldrich). Quantifications were assessed on roots and trifoliate leaves. Samples were digested and the iron concentration calculated by inductively coupled plasma optical emission spectrometry (ICP-OES), as described by Roriz et al. (2014).

Results and Discussion

In plants, chlorophylls are the most abundant tetrapyrroles, and one of the most common symptoms of Fe deprived plants is lower chlorophyll levels, leading to leaf yellowing (Tanaka et al., 2011). Here, plants under Fe deficiency presented a 37% decrease of anthocyanins,

30% of total chlorophylls (Fig. 1A), and 30% decrease of carotenoids ($P < 0.05$). Total Fe concentration results corroborate this chlorophyll decrease, as plants under Fe deficiency presented 80% lower Fe concentration in the trifoliate leaves (data not shown). Moreover, root iron reductase activity increased about 4-fold under Fe deprivation (Fig. 1B). The increased activity of this enzyme is one of the main biochemical reactions to Fe-stress, in order to enhance the amount of readily available Fe(II) for uptake (García et al., 2013).

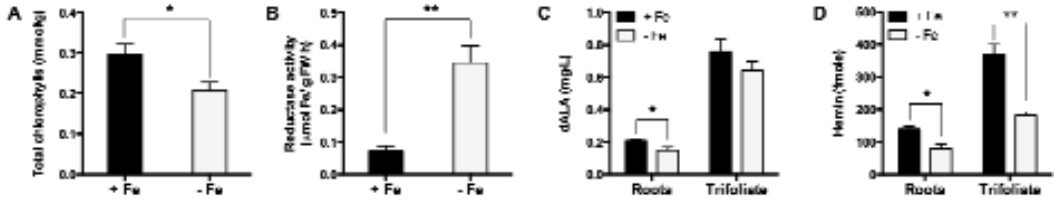

Figure 1. Leaf total chlorophylls (A), root ferric reductase activity (B) and dALA (C) and hemin (D) concentrations of roots and trifoliate leaves of *G. max* plants grown under Fe-sufficiency (+Fe) and Fe-deficiency (-Fe). Significant differences are indicated $*P < 0.05$, $**P < 0.001$.

The biosynthesis of 5-aminoleculinic acid (ALA), the precursor for heme (Briat et al., 2015), was significantly lower in the roots under Fe deficiency (Fig. 1C). This lower ALA levels could justify the lower root hemin (the oxidized form of heme) concentrations (Fig. 1D) and, consequently, the significantly lower activity of heme containing proteins catalase and ascorbate peroxidase (data not shown). Interestingly, despite the lower heme levels in Fe deficient roots, an increase in the heme containing protein root ferric reductase was observed (Fig. 1B). Iron reductase is a membrane bound protein and here we measured free heme, which could explain the observed differences. This study reveals the impact of Fe deficiency on crucial components of the tetrapyrrole and antioxidative systems, and further elucidates the role of heme in soybean biochemical responses to Fe deprivation.

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