

DISCRIMINATING FE DEFICIENT SOYBEAN PLANTS BY FOURIER TRANSFORM INFRARED SPECTROSCOPY

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INTRODUCTION

Iron deficiency chlorosis (IDC) is a serious environmental problem that affects several crops in the world. Under alkaline soils, iron (Fe) bioavailability is limited and plants suffer from leaf yellowing, stunted growth and yield losses. Finding early, non-invasive, detection methods for IDC is of great agronomic importance, as these may help farmers improve fertilization timings and avoid economic losses. Fourier transform infrared (FTIR) spectroscopy have been explored as an easy use, cheap, and environmentally friendly technique for several proposes as plant species and/or cultivars discrimination. The main goal of this work was to test the ability of FTIR spectroscopy to discriminate two groups of soybean (*Glycine max*) plants treated under different Fe stress conditions.

METHODS

Thirty *Glycine max* seeds were germinated for seven days in the dark at 25 °C. Germinated seedlings were transferred to 5 L vessels containing hydroponic solution with 20 mM FeEDDHA (Santos et al., 2019). Plants were maintained under these conditions for two weeks, after which, they were divided in two groups - one supplied with 20 mM FeEDDHA (Fe+; n=15) and other without Fe supplementation (Fe-; n=15). For the five following weeks, three plants of each treatment were collected / week and analysed. Chlorophyll concentration was measured according to Sims and Gamon (2002). The antioxidant activity was evaluated by the ABTS and DPPH radicals scavenging assays, as described by Vilas-Boas et al. (2020). Infrared spectra acquisition was performed according to the methods described in Páscoa et al. (2019).

Data were analyzed with GraphPad Prism version 6. Differences among treatments were examined using 2-way ANOVA with Fisher's LSD Test. Infrared spectra were pre-processed with standard normal variate (SNV) and Savitzky-Golay filter to remove baseline drifts and further mean centered. Spectra were subsequently modelled by partial least square discriminant analysis (PLSDA) to evaluate the potential of FTIR to discriminate between Fe- and Fe+ plant samples.

RESULTS and discussion

To evaluate the stress level of plants grown under the different Fe treatments, total chlorophyll concentration and antioxidant activity was measured in the trifoliolate leaves of Fe+ and Fe- plants (Table 1).

Table 1. Total chlorophylls (mmol/g FW), DPPH (mg/g) and ABTS (mg/g) concentrations in shoots of *Glycine max* plants grown under optimum conditions for two weeks and then treated either with (Fe+) or without (Fe-) ironj supplementation for the five following weeks[1]

		Week 1	Week 2	Week 3	Week 4	Week 5
Total chlorophylls	Fe +	0.021 ± 0.005 ^a	0.020 ± 0.001	0.019 ± 0.002 ^a	0.020 ± 0.001	0.024 ± 0.001 ^a
	Fe -	0.010 ± 0.001 ^b	0.015 ± 0.001	0.014 ± 0.001 ^b	0.017 ± 0.001	0.017 ± 0.002 ^b
DPPH	Fe +	0.860 ± 0.033	0.858 ± 0.026	0.808 ± 0.056 ^a	0.745 ± 0.022 ^a	0.734 ± 0.038 ^a
	Fe -	0.867 ± 0.027	0.846 ± 0.075	0.519 ± 0.005 ^b	0.491 ± 0.017 ^b	0.434 ± 0.034 ^b

ABTS	Fe +	0.541 ± 0.044	0.505 ± 0.079	0.595 ± 0.035 ^a	0.528 ± 0.020	0.411 ± 0.092
	Fe -	0.453 ± 0.099	0.463 ± 0.054	0.339 ± 0.051 ^b	0.378 ± 0.004	0.331 ± 0.046

As expected, total chlorophyll concentration was always higher in Fe+ treated plants than Fe- (Table 1). Since Fe plays a role in the biosynthesis of photosynthetic pigments, IDC has been associated with chlorophyll synthesis inhibition (Santos et al., 2019). Iron is also a main co-factor of several antioxidant enzymes. DPPH and ABTS show the radical scavenging ability of antioxidants. The results depicted in Table 1 show that, after two weeks under Fe deficiency, the antioxidant capacity of plants decreased significantly when compared to plants that were maintained under optimum conditions throughout the entire assay.

Having the physiological results confirming that the two groups of plants were under distinct stress conditions, the infrared spectra were analysed. The optimum spectral region for the discrimination between Fe+ and Fe- plants was dominated by carbohydrates functional groups vibrations enabling postulating that this class of compounds was highly affected by the Fe stress.

The PLSDA model (Figure 1A) exhibited two clusters corresponding to infrared spectra of Fe + (green) and Fe- (red) plants. The exception was noticed for spectra from week 1 for which Fe+ and Fe- infrared spectra were quite overlap (Figure 1B- model presented in figure 1A but colored by week). This was a somehow expected results once in week 1 plants were probably yet barely affected by the presence/deficiency.

[1] Values represent means ± SEM; different letters between Fe treatments of each parameter and of each week represent statistically significant differences.

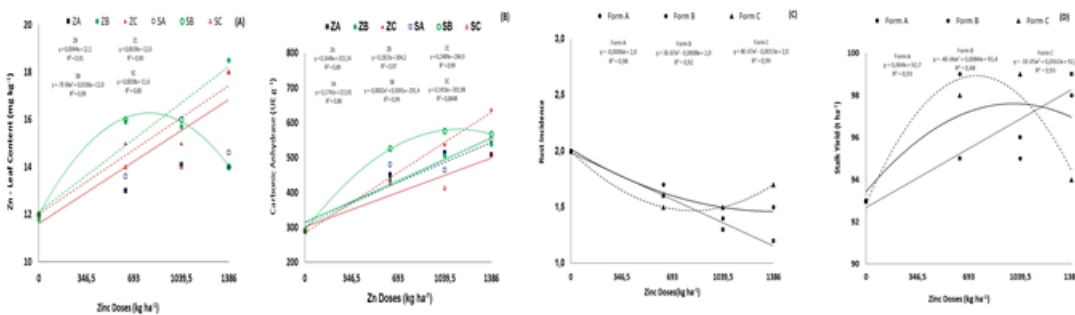


Fig. 1. Scores plot of the PLSDA regression model obtained for discrimination between Fe+ (?) and Fe- (?) plants. B: Scores plot of the PLSDA regression model obtained for discrimination between Fe+ and Fe- plants colored by week: 1st week (?); 2nd week (?); 3rd week (?); 4th week (?); 5th week (?).

CONCLUSIONS

FTIR analysis can potentially provide a rapid, easy, and non-invasive way to detect IDC. Further predictive models can help farmers deciding key factors, contributing for the implementation of sustainable agricultural practices.

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