

use of techniques that rely on the measurement of changes in the electrical properties occurring in plants, such as field effect transistors [8], [9], resistive [10], electrophysiological [11], and impedimetric [12] techniques. Such methods demonstrate the potential for cost-effective and continuous proximal plant monitoring, offering valuable insights into plant health conditions. Among these, bioimpedance is attracting an increasing interest in the field, especially for what concerns its application in measuring the electrical impedance of biological tissues when exposed to an alternating current (ac) stimulus, which can be in the form of either voltage or current [13]. The impedance of biological tissues is influenced by a variety of factors, such as their health status, structural attributes, and chemical composition. When an ac stimulus is applied, at lower frequencies, the current predominantly flows through the fluid surrounding the cells, whereas at higher frequencies, it tends to pass through the cells themselves [14]. The impedance properties of plant tissues are intimately linked to several passive electrical characteristics, including the ion content in the cells, the integrity of the cell membranes, and the viscosity of the tissue [15]. Although bioimpedance has been previously employed in plant monitoring [16], [17], [18], [19], to the best of our knowledge, the use of this technique for the continuous assessment of the effects of iron deficiency on plant health remains largely unexplored. This study focuses on the investigation of the impact of iron deficiency on 8 tomato plants by monitoring the impedance of their stems over a duration of 38 days. The experiment was carried out in a glasshouse, with controlled environmental conditions, reducing the effect of the environmental variability, thus increasing scientific rigor in comparison with alternative literature with a comparable, usually smaller population of plants [17], [18], [20], [21], [22]. The impedance data were collected using a semiportable impedance analyzer in the 100 Hz–10 MHz frequency range. As an initial assessment, the evolution of the bioimpedance magnitude at fixed frequency was analyzed over time showing a deviation between the control and iron-stressed plants. Subsequently, the entire bioimpedance data were fitted to the Cole model equivalent circuit [23]. Analysis of variance (ANOVA) proved the statistical difference between the equivalent circuit parameter extracted for control and stressed plant. Based on this, the equivalent circuit parameters were employed as features to successfully train a data classification model to discriminate between iron-deficient and control plants. The study emphasizes the potential of stem impedance measurements as indicators of plant response to iron deficiency, presenting a novel method for monitoring plant health and enhancing crop management.

II. MATERIALS AND METHODS

A. Plant Material

Eight tomato seedlings (*Solanum lycopersicum* cv. Pomodoro Tondo) were first transferred to soilless pots (diameter 21 cm/4 L) filled with an inert (i.e., free of nutrient components) perlite substrate (Karl Bachl Kunststoffverarbeitung GmbH & Co. KG) and subsequently grown for 7–8 weeks in a greenhouse with a controlled environment, as reported in Fig. S1 (day:

14 h, 24 °C, 70% relative humidity, 250 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$; night: 10 h, 19 °C, 70% relative humidity), maintaining these at approx. 60% water holding capacity, by watering them once a day with tap water. The necessary nutritional supply was provided by the addition of a Hoagland solution [24] composed of main macronutrients and micronutrients with the following composition: $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, K_2SO_4 , KCl , KH_2PO_4 , H_3BO_3 , $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, CuSO_4 , $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, and Fe-EDTA [25], allowing complete control over the distribution of nutritional constituents. The control group, consisting of four plants, remained exposed to a complete Hoagland nutrient solution [24] throughout the entire experiment. In contrast, the four iron-stressed plants underwent three distinct phases over the duration of the experiment, carried out within the optimal tomato growth window and lasting a total of 38 days. The first phase represented the optimal conditions, characterized by optimal nutrient availability, from 29th of July to 4th of August. This initial phase was designed to bring all the plants to comparable health conditions before the application of iron stress. The second phase, from 5th of August to 27th of August, represents the iron deficiency condition, wherein iron was deliberately removed from the nutrient solution. Finally, in the recovery phase, the iron was reintroduced into the nutrient solution until 5th September.

B. Electrical Impedance Spectroscopy

Bioimpedance data for the tomato plant were collected using a semiportable impedance analyzer (the Digilent Analog Discovery 2), which allows capacitance between 50 pF and 500 μF to be measured, with a 14-b resolution, for our purpose, it was equipped with the Impedance Analyzer add-on, setting a 10 k Ω feedback resistor, representative for an expected biological samples magnitude between 1 and 20 k Ω , within a frequency range of 100–10 MHz. This was done over 200 logarithmically spaced frequency points using a two-electrode configuration. Electrical contact with the plant stem was established using a pair of stainless steel subcutaneous needle electrodes (Technomed, Medical Accessories), each 13 mm long and 0.4 mm in diameter (27 g). These electrodes were vertically inserted into the plant stem 5 cm above the substrate, maintaining a consistent 0.5 cm distance between them. All plants were connected to the impedance analyzer at the same time, as in the representation in Fig. 1, and reducing the contribution of cables, by performing an open-/short-circuit compensation prior measurement. The switching between different plants was synchronized with data collection using a centralized Python script, which controlled the multiplexer channels via an Arduino setup. Measurements were continuously acquired for a period of 38 days with 1-h acquisition interval, resulting in a final dataset consisting in a total of 7557 impedance spectra, respectively, divided into 3881 spectra from the control and 3676 spectra from the iron-stressed plants.

C. Equivalent Circuit Component Analysis

The bioimpedance data were organized, postprocessed, and analyzed using MATLAB R2022b (The MathWorks Inc.,