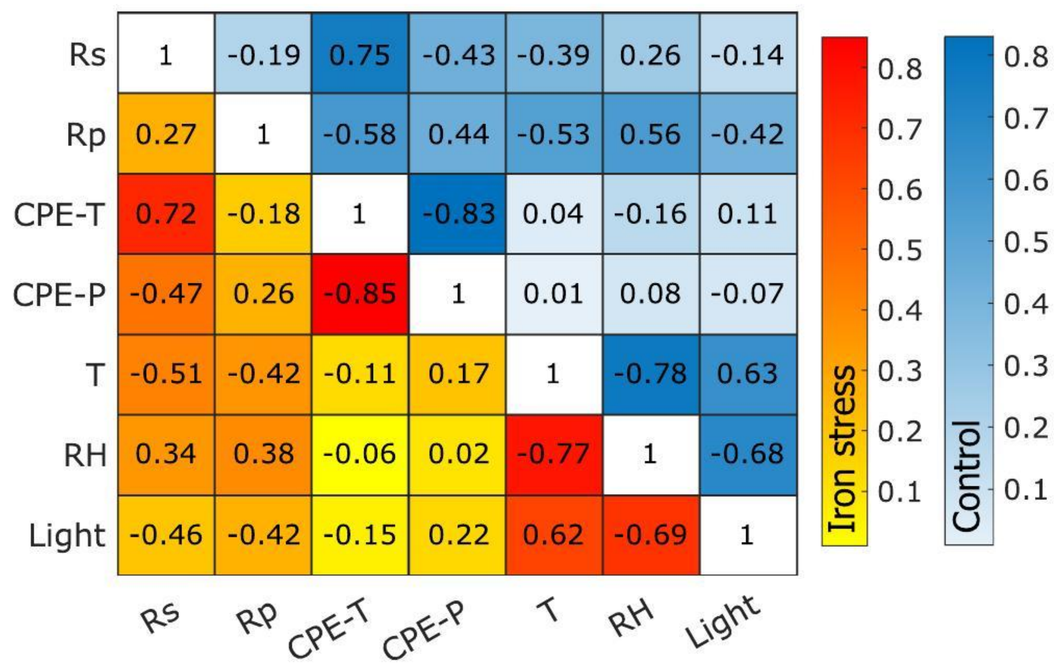

Fig. 3. Correlation matrix of the circuit parameters (R_p, R_s, CPE-T, CPE-P) of the Cole model and the environmental monitoring parameters (temperature T, relative humidity RH, and light intensity). The correlation values for the control plants are in blue, whereas the corresponding values for iron-stressed plants are in red. The legend is presented in terms of absolute values for clarity.

the single dispersion Cole model. Such a model was chosen due to its wide employment as the simplest representation of the flow of current in biological tissues, allowing to reduce the dataset to a smaller set of interpretable variables (i.e., from 200 single-frequency points to 4 circuit component values) while preserving the majority of the representative information about the flow current in the biological tissues. In this model, depicted in Fig. s2 (see Supplementary material), the bioimpedance resistive components (i.e., R_s and R_p) are used to describe the resistance of the extracellular and intracellular mediums, respectively, while the CPE-T element is primarily associated with the pseudocapacitance of the cell membrane and the CPE-P element is related to the heterogeneity originating from variations in the size and shape of the cells [37]. The study of such electrical parameters can consequently be correlated with the plant's physiological responses, thus allowing to monitor the impact of disruptive events such as iron deficiency on the plant structures. For this reason, the obtained electrical parameters were then employed in this study to determine the statistical difference between control and iron-deficient plants. The equivalent circuit model fitting resulted in an average RMSE, obtained by averaging the values of all the considered plant spectra, of $466.3 \pm 68.6\,\Omega$ and $464.5 \pm 454.5\,\Omega$, in terms of impedance magnitude for the control and the iron stress conditions, respectively. Given the low error of the fitting results, it is possible to state that the Cole model is a good approximation of the impedance of the tomato plants, and that is thus possible to employ its resulting extracted parameters in the evaluation of the plants' response to iron stress.

The entire population of plants was exposed to the same environmental conditions, in terms of temperature, relative humidity, and light, as described in the previous section and whose trends are presented in Fig. S1 (see Supplementary material). To exclude the possibility that the evolution of the electrical parameters, commonly dependent on both T and RH, was solely representative of the changes occurring in the plants and not of the environmental conditions, the linear correlation coefficients between the circuit and the environmental parameters were calculated. Fig. 3 presents the correlation coefficient matrix between Cole's circuit parameters and the environmental parameters. Here, it is visible how the capacitive components (i.e., CPE-P and CPE-T) are found to be completely unrelated to environmental parameters, with correlation values close to zero. The correlation values are slightly higher in the case of the resistive components, which could be due to the fact that they are representative of the current flowing in the intra- and extra-cellular medium, which—being composed of ions—is affected by temperature changes. Nevertheless, such correlations are weak (less than 56%) and allow us to assume that the measurements are indicative of a change in the composition of the content within the plant stem, rather than to environmental changes.

Once the dependence on the environmental conditions was excluded, a characterization of the equivalent circuit components overall behavior over time was performed. The graphs in Fig. 4 depict the time-series evolution of the equivalent electrical components R_s, R_p, CPE-T, and CPE-P during the stress time period for control and stressed plants. In addition to the larger variance visible as the plants pass between the two different conditions to which they are subjected, the analysis of the individual circuit parameters allows us to analyze the individual components that can be traced back to physiological phenomena taking place in the plant. A comparable behavior to the one observed in the time analysis of fixed-frequency impedance presented in Fig. 2 is noticeable in the CPE-P component, presented in Fig. 4(c), where a deviation over time between control and iron-stressed plants is clearly visible. Such behavior is potentially due to the metabolic variation of the plant to adapt to iron deficiency [38], which affects the integrity of the cellular structures, and thus, their ability to act as capacitors for the current flowing during the bioimpedance measurement. In addition, from the graphs in Fig. 4(a) and (b), it is evident how the circadian cycle, as expected, is visible only in the trend of the resistive components R_s and R_p, and not on the pseudocapacitive components. This is due to the fact that the fluid in the plant vascular tissues (i.e., the sap flow) is rich in electrically charged ions, which in turn are the main contributors to the observed current flow. Here, from the analysis of the time course of the R_p component, it can be seen that as time progresses and thus the concentration of Fe^+ ions decreases, the variation in resistance amplitude between day and night presents a decreasing trend. This behavior could be traced to a decrease in photosynthetic activity caused by iron deficiency, as reported in other plants [39].

To assess the differences in circuit parameters between stressed and control plants, as well as their changes over time, we used the Cole model parameters and conducted an ANOVA. This approach helped to identify statistically significant variations in the parameters under the two conditions. As illustrated in Fig. 5, the average values of the Cole model electrical parameters for both stressed and control plants are presented, accompanied by the ANOVA findings. In the figure, average values marked with different letters indicate a statistical difference, as determined by the HSD test ($p \leq 0.05$). The statistical evaluation revealed that all the extracted circuit parameters showed significant differences, thus serving as important indicators in differentiating the health conditions of the plants.