



Plants with silver nanoparticles to improve electrical conductivity: an innovative approach in biology

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Abstract

This paper shows the effect of electrical conductivity on the vascular system of plants through the utilization of silver nanoparticles supported by nanocellulose. The plant *Epipremnum aureum*, also known as pothos, was used in the experimentation. The main characteristics of the plant are its vigorous growth, remarkable adaptability to thrive in both soil and aquatic environments, simple propagation, and noteworthy resilience against diseases. The experiment used a polarization voltage ranging from -1.5 to 1.5 V and shunt resistors of 1, 10, 100, and 1000 ohms. The scan polarization rate was 15 mV/s. Methods like environmental scanning electron microscopy and energy-dispersive X-ray analysis were used for chemical and structural analysis. The findings demonstrate that adding silver nanoparticles held together by nanocellulose enhances the vascular system's capacity to conduct electricity. Moreover, the Wilcoxon signed-rank test results revealed no statistically significant difference between the root-stem and stem-leaf output voltage values. These findings imply that silver nanoparticles are distributed throughout the vascular system and act as conductive pathways in plants. Additionally, electrolysis-Faraday's law was used to calculate the recovery of silver material, yielding 40 mg/mm² per hour. These discoveries open new avenues for comprehending and manipulating plant physiological processes, potentially leading to beneficial outcomes in agriculture and contaminant recovery.

Keywords Silver nanoparticles · Nanocellulose · Electrical conductivity in plants · Plant vascular system · Shunt resistor · Contaminant recovery

Introduction

Some algae and higher plants are known for their electrical excitability and signaling, often associated with a swift response to environmental stimuli. These electrical signals, called action potentials (AP), employ ion channels to convey information over significant distances. The assumption

that electrical signals induce physiological changes in common plants, such as rapid leaf movements in plants, has been corroborated in a previous study (Fromm and Lautner 2007). Extensive research has been conducted on root activity and crop adaptation to climatic conditions. Using in-situ measurement of electrical and physiological variables, like electrical capacitance (EC) and electrical impedance (EI), is becoming popular as a way to study how plants grow and how well they can handle stress in a wide range of species (Cseresnyés et al. 2016). Furthermore, there is a quest to validate the effectiveness of non-intrusive methods in detecting specific differences in physiological activities. Electrical impedance spectroscopy (EIS) has examined the electrophysiological responses of plants to various types of abiotic stress (Wu et al. 2017). For instance, Liu et al. (2021) have examined plant roots stress resistance at various temperatures and varying water supplies, from severe drought to well-irrigation.

On the other hand, to perform electrical measurements in plants, the use of probes is required. In the pursuit of

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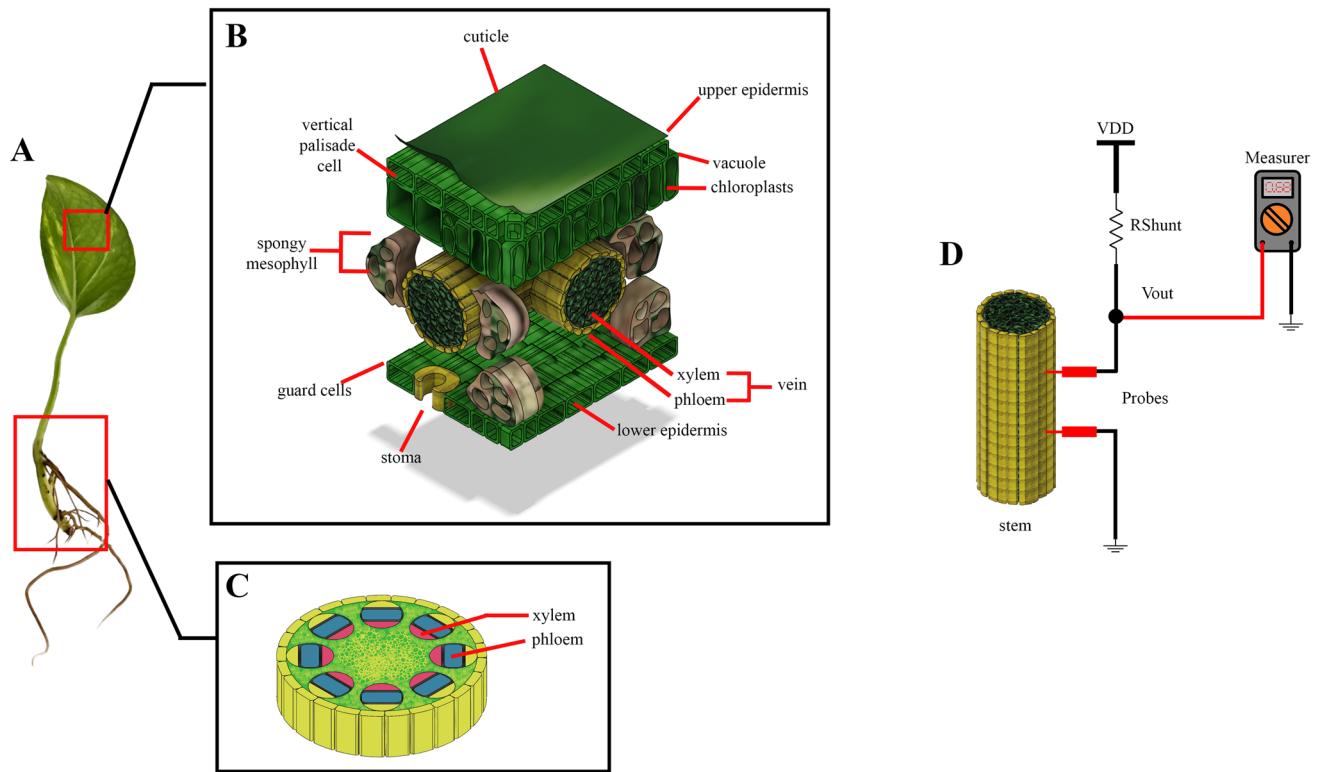


Fig. 1 The fundamental plant physiology and Interconnection are depicted. **A** The Pothos plant. **B** Cross-sectional view. **C** The plant vascular system. **D** Interconnections with the plant stem

innovative probe design, there have been developments in the creation of non-invasive surface electrodes (Meder et al. 2021). Moreover, due to their distinctive electrical and mechanical characteristics, conductive polymers have garnered considerable interest in recent years. They have been extensively explored and utilized across diverse domains, such as bio-electronics, energy storage, and neural interfaces. Incorporating conductive polymers into plants has emerged as an up-and-coming research area, presenting numerous notable advantages and potential applications. The regulation of photosynthesis by hormones and nutrients is one of many fundamental processes necessary for plant growth and operation. Many environmental, physical, and chemical factors affect these processes and the sending and receiving of signals over long distances through the vascular system via the xylem and phloem (Shepherd 2012). Adding conductive nanoparticles from outside sources changes metabolic processes and pathway signals, as demonstrated in Angelini et al. (2016), Stavrinidou et al. (2015). These changes enable

the identification and comprehension of the mechanisms involved in the interaction and communication between these nanoparticles and plants. Moreover, Masarovicová and Králová (2013) and Ouyang et al. (2014), used the electronically conductive polymer poly(3,4-ethylenedioxythiophene) (PEDOT) to investigate various medical and sensor contexts, such as drug administration, regenerative medicine, and neural connections, demonstrating the potential of organic electronic materials.

This advancement in plant biology provides a novel perspective for understanding and manipulating vascular systems and signaling pathways in plants, establishing a technique that allows effective communication with plants and regulation of their functions.

This paper suggests a new way to help electricity flow better by using 10% silver nanoparticles (Ag NPs) and nanocellulose. These ingredients allow electricity to move through the plant's vascular system in the Pothos species, *Epipremnum aureum*. Plant physiology and interconnection are depicted in Fig. 1. Because they are metallic, Ag NPs can carry electrical signals



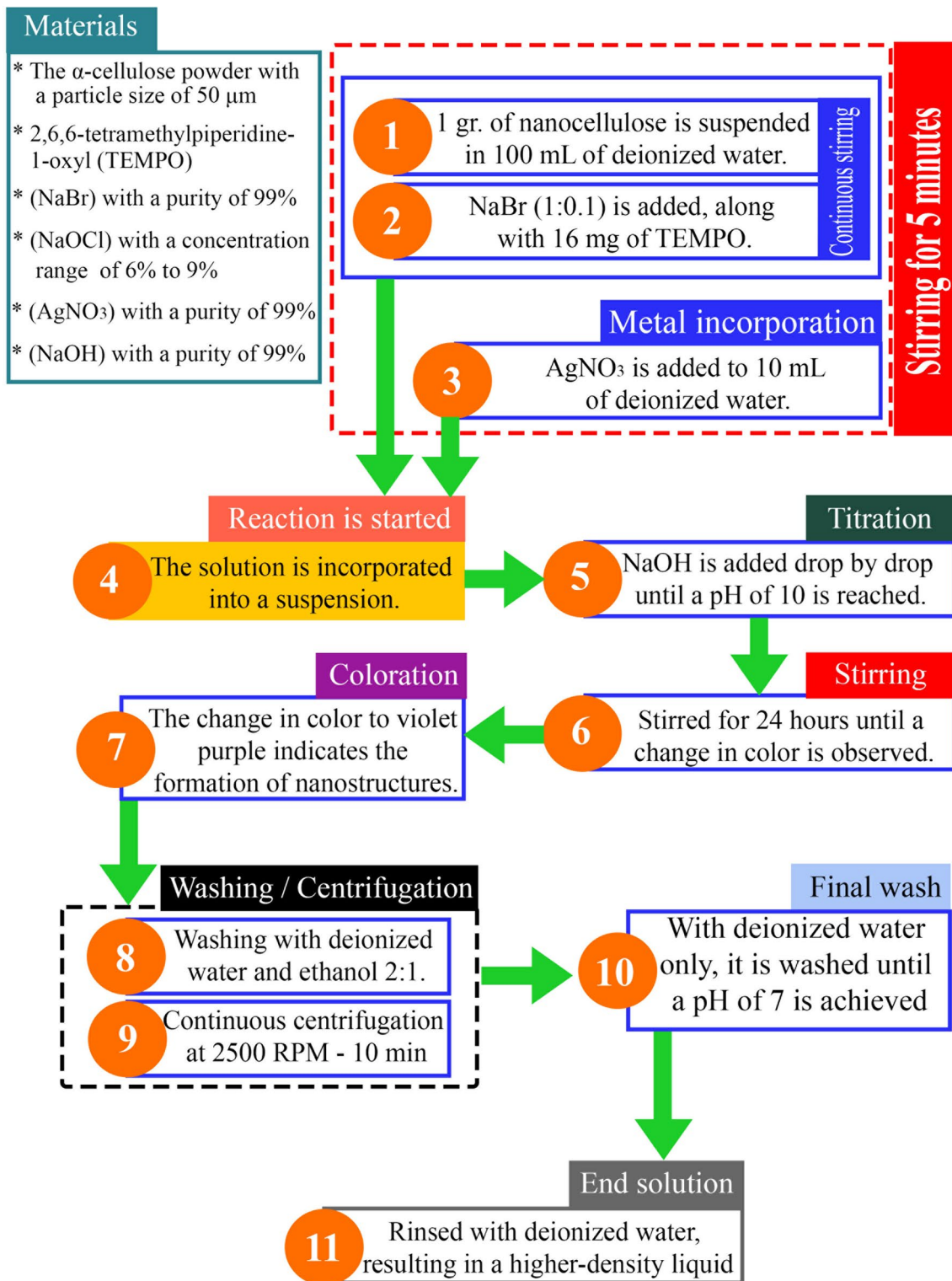


Fig. 2 Flowchart: one-step in situ synthesis of Ag/AgCl nanoparticles supported by nanocellulose, (Macclesh del Pino Pérez et al. 2021)



Fig. 3 Root, stem, and leaf measurements. The root-to-stem (D1) and stem-to-leaves distances (D2) are 5 cm. Additionally, E1, E2, and E3 represent probes' 1cm intervals

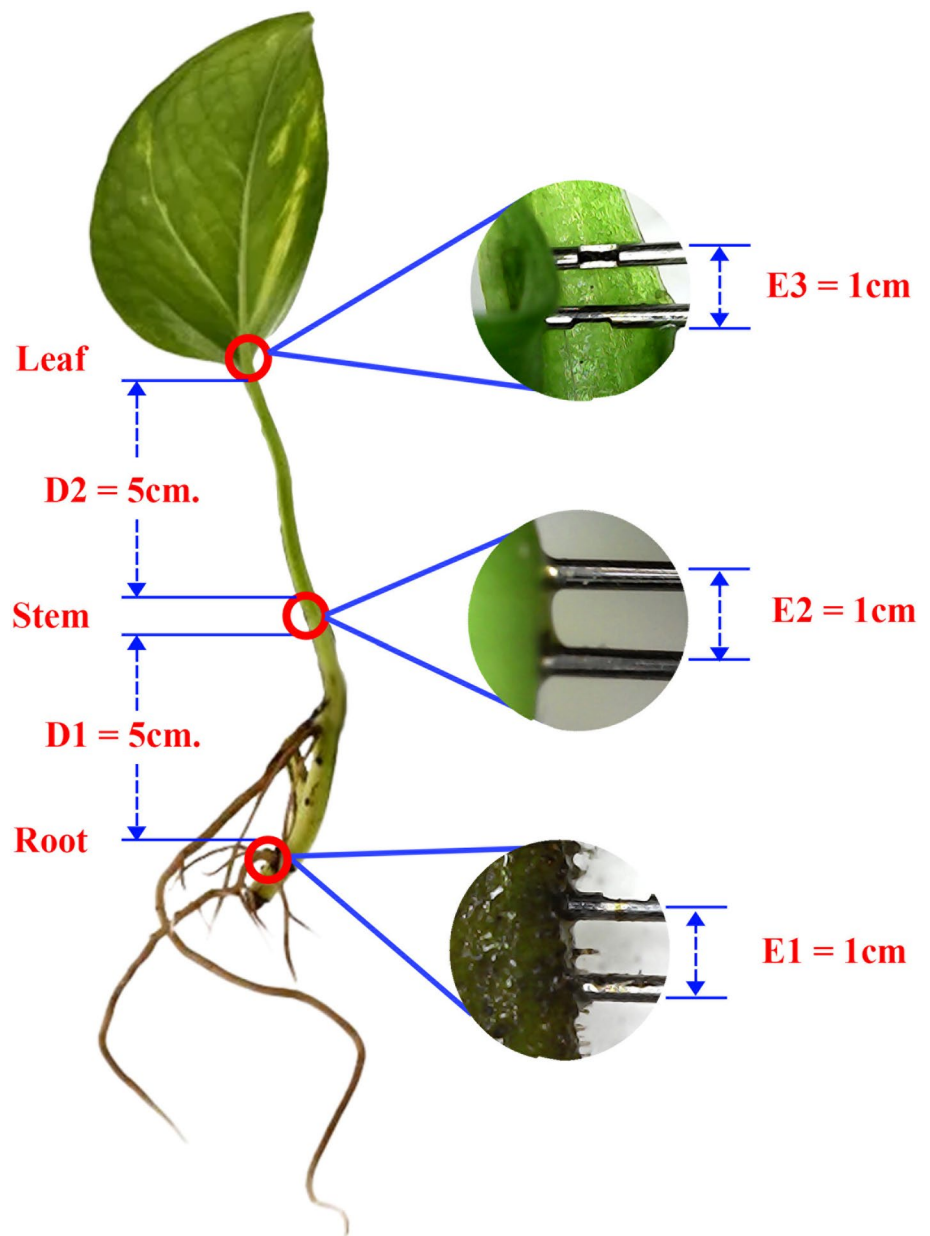


Table 1 Statistical results of the Wilcoxon signed-rank test for root-stem and stem-leaf comparison, with a significance level of %5

	N. samples	Mean rank	<i>p</i> value
Root versus stem (D1 in Fig. 3)			
Negative ranks	358	349.45	6.50E-01
Positive ranks	342	351.6	
Ties	0		
sTotal	700		
Stem versus leaf (D2 in Fig. 3)			
Negative ranks	371	357.21	6.57E-02
Positive ranks	329	342.93	
Ties	0		
Total	700		

The bold numbers indicate a non-significant difference among the two related levels of output voltages between the root-stem (D1) and stem-leaf (D2)

(electrons e⁻ and holes h⁺) and ionic signals (cations A⁺ and anions B⁻) to determine what happens. This property enables the creation of electrical conductors within the plant's vascular system, intensifying the sensitivity of its surrounding environment and allowing perceptible responses from plant species. This approach offers a variety of opportunities to incorporate chemical elements into plant structures selectively. The research was conducted from October 2022 to May 2023 at the TecNM/Instituto Tecnológico de Ciudad Madero and Instituto Politécnico Nacional, CICATA Altamira, México.



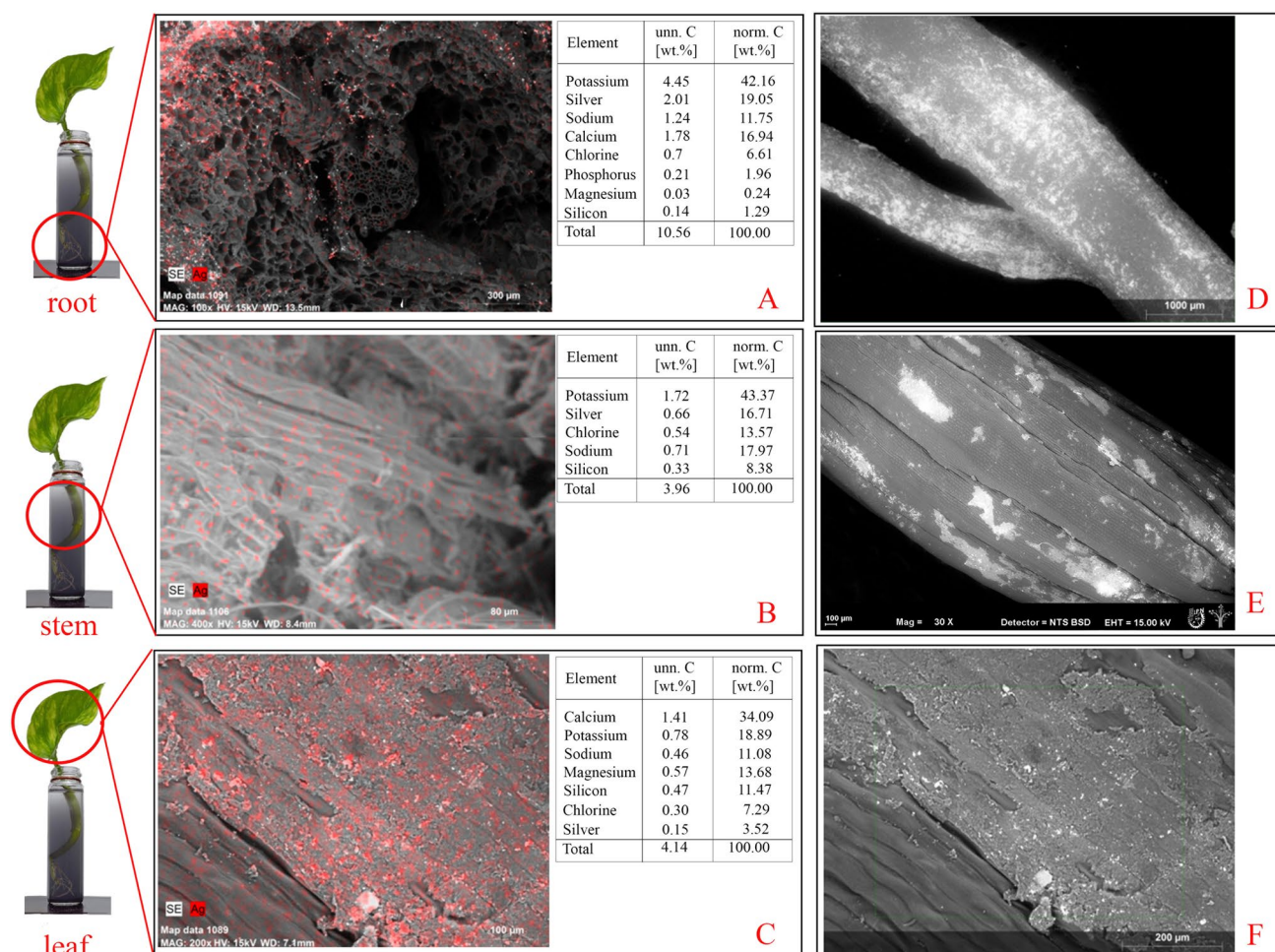


Fig. 4 ESEM and ESEM-EDX results: **A** ESEM on the root. **B** ESEM on the stem. **C** ESEM on the leaf. Notice that ESEM analysis shows red-colored Ag NPs in the cross-section. **D** ESEM-EDX on

the root. **E** ESEM-EDX on stem. **F** ESEM-EDX on leaf. Notice that ESEM-EDX analysis shows Ag NPs clusters highlighted

Materials and methods

Materials

The 50 μm α -cellulose powder and the free radical 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) were bought from Sigma Aldrich. The chemical compounds sodium bromide, NaBr (99%), sodium hypochlorite, NaOCl (6–9%), silver nitrate, AgNO₃ (99%), and sodium hydroxide, NaOH (99%), were acquired from Fermont Company.

Synthesis of Ag nanoparticles (one-step reaction)

The methodology was based on Macclesh del Pino Pérez et al. (2021): A suspension was prepared by adding 1 g of α -cellulose to 100 mL of deionized water. Sodium bromide was then added to the suspension in a ratio of 1:0.1, followed by the addition of 16 mg of TEMPO. The suspension was continuously stirred during this process. The first step in adding metallic components was to dissolve silver nitrate in 10 mL of water. This solution was mixed with the α -cellulose



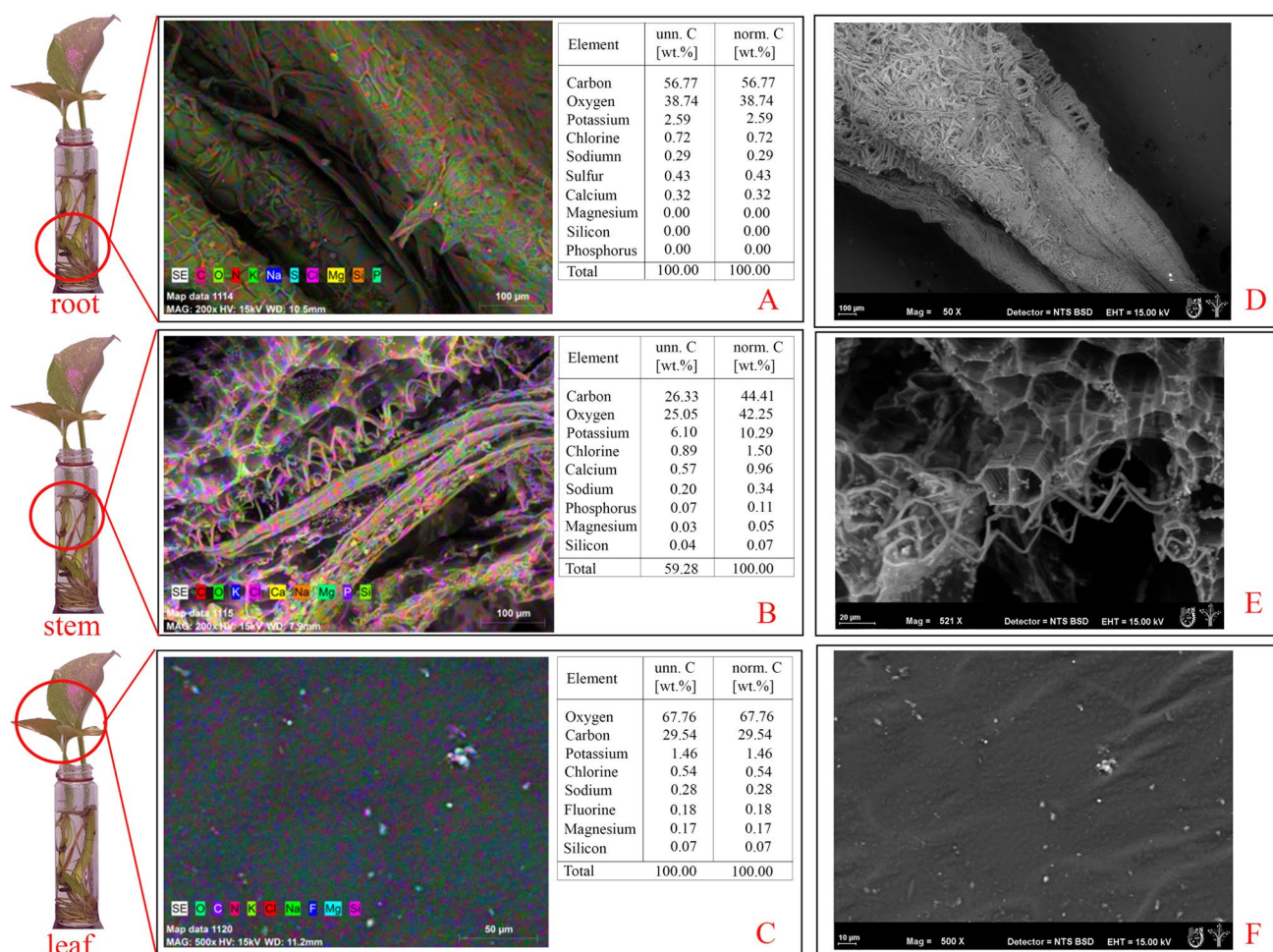


Fig. 5 A–C are ESEM results without Ag NPs on the root, stem, and leaf, respectively. Meanwhile, D–F are ESEM-EDX results without Ag NPs on the root, stem, and leaf, respectively

suspension and stirred for 5 min. In addition, 15 mL of sodium hypochlorite was introduced into the suspension. The utilization of NaOH is imperative for pH level adjustment to a value of 10. Following the introduction of sodium hypochlorite, dropwise additions of NaOH gradually started the reaction. The pH meter was employed to monitor the pH level throughout the reaction, and the effect was deemed to have reached its conclusion when the pH level ceased to decrease. Ultimately, the suspension remained in a state of agitation for 24 h. After this time, a noticeable alteration in the color of the solution was observed. In the initial stage, the substance's color appeared milky-white and subsequently transitioned to a violet–purple hue, thereby serving as a discernible visual indication of the formation of nanostructures. The suspension underwent a washing process using a mixture of deionized

water and ethanol in a ratio of 2:1. This washing was carried out through a series of centrifugation steps at a speed of 2500 revolutions per minute for 10 min. After the pH of the solution reached a value of 7, it was subsequently rinsed with deionized water, acquiring a slightly more concentrated liquid. The Ag/AgCl nanoparticles synthesis steps as depicted in Fig. 2.

Ag NPs conductor pathway formation in *Epipremnum aureum* (Pothos) xylem

The *Epipremnum aureum* (Linden & André) G.S. Bunting plants were meticulously cultivated from cuttings, ensuring their optimal growth conditions under ambient



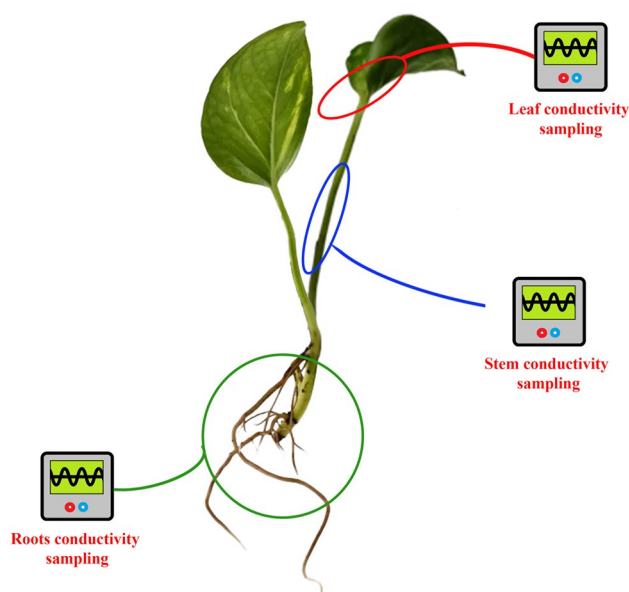


Fig. 6 Plant conductivity sampling

temperature and in deionized water. This careful preparation was crucial for their readiness for use in the experiment.

Young plants were carefully selected, and their roots were immersed in a silver nanoparticle water solution, held in place by 10% nanocellulose. Throughout the experiment, a meticulously controlled humidity level of approximately 73% was maintained, and the ambient temperature was precisely kept around 30 °C. The Pothos plants were immersed in this Ag nanoparticle solution for 12 weeks, allowing the nanoparticles to interact with the plant's vascular system.

Following the conclusion of this exposure period, probe insertions were made in the xylem of the plants at 1 cm to conduct the electrical characterization of the wire formed within the xylem. This analysis provided valuable

information regarding the conductive capacity of the wire formed in *Epipremnum aureum* plants, which is crucial for understanding the plant's ability to transport nutrients and water.

Characterization of xylem conductor pathway

Samples from different parts of the plant (root, stem, and leaves) were assembled into a custom-designed electrochemical cell. Surgical-grade stainless steel probe tips with a diameter of 0.30 mm were employed for the measurements. The electrochemical cell supports were used to move the probes around. The tips of the probes were brought close to the wire, and light pressure was applied to help them go through the xylem and connect with the wire made of silver nanoparticles (Ag NPs) inside.

Measurements were meticulously conducted in three sections of the plant: the root, the stem, and leaves. The study's precision is evident in the fact that the distance between each zone, denoted as D1 and D2, is exactly 5 cm. Figure 3 shows that the probes E1, E2, and E3 were positioned with a 1 cm gap. An asymmetrical power source and the galvanostat potentiostat, BioLogic SP-150, were employed in this study. The polarization voltage from -1.5 to 1.5 V was incrementally varied at a constant scan polarization rate of 15 mV/s.

Moreover, a non-parametric Wilcoxon signed-rank test with a significance level of 5% was carried out. This rigorous trial determines the significance level of the output voltages between the root-stem (D1) and stem-leaf (D2), as shown in Fig. 3. When the estimated *P* value is greater than 0.05, this indicates that the compared output voltage in two sections of the plant does not show statistically significant differences. Table 1 provides a complete summary of the results of this test.

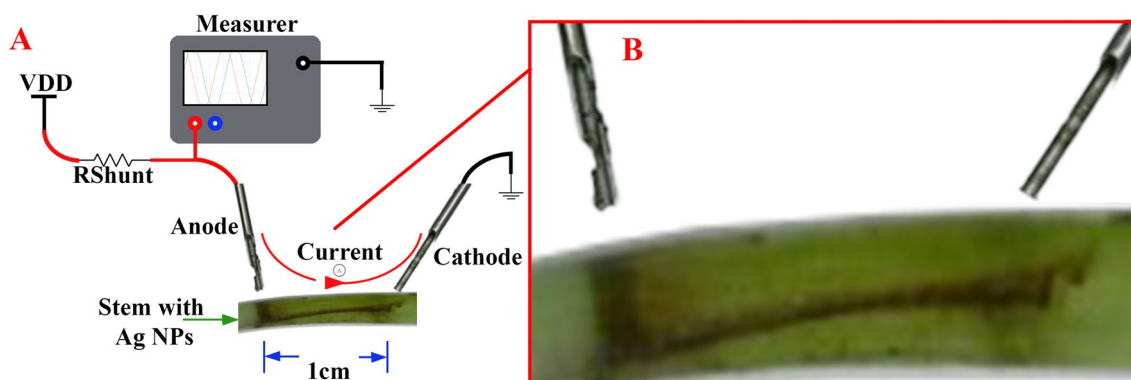


Fig. 7 **A** Experimental setup. **B** Close view of the anode and cathode connection on the plant stem



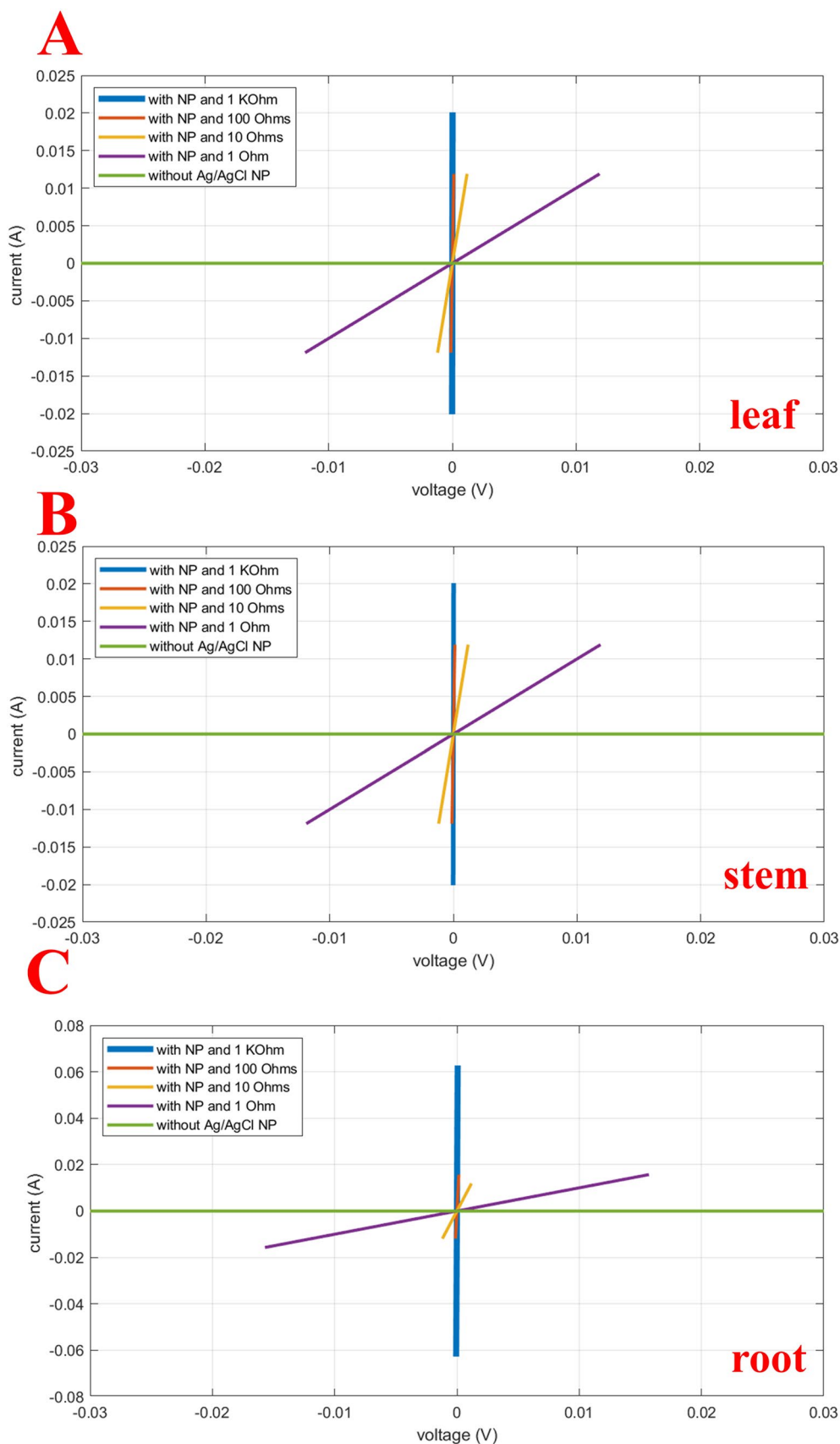


Fig. 8 Measurements with different shunt resistances. Experimentation varying the shunt resistances to 1, 10, 100, and 1000 Ohms. **A** Leaf. **B** Stem; and **C** root. Notice that the highest sensitivity was achieved at 1000 ohms marked in the blue line and non-sensitivity was observed without Ag NPs, line green. All experiments were carried out with the same shunt resistances

Analysis of *Epipremnum aureum* (Pothos) plants using environmental scanning electron microscopy (ESEM)

Detailed analysis of morphological changes in Pothos plants after exposure to 10% wt nanoparticles supported by nanocellulose and suspended in an aqueous solution was carried out using the environmental scanning electron microscopy (ESEM) technique. Firstly, ESEM analysis was performed using a Carl Zeiss microscope, Model EVO LS10 (Germany). Samples were mounted on aluminum stubs and fixed using double-sided carbon conductive tape with an acceleration voltage of 30 kV and a pressure of 80 Pa. A retro-dispersed electron detector (NTS BSD) was used, and the resulting images were captured in grayscale and stored in TIFF format with a resolution of 1024×768 pixels.

Analysis of *Epipremnum aureum* (Pothos) plants environmental scanning electron microscopy (ESEM) coupled with energy dispersive X-ray analysis (EDX)

Environmental scanning electron microscopy (ESEM) in conjunction with energy-dispersive X-ray analysis (EDX) was utilized to conduct a thorough examination of *Epipremnum aureum* (Pothos) plants.

The method employed a retro-dispersed electron detector and an X-ray detector (Bruker, Quantax 200, Germany) to conduct micro-elemental analysis of minerals in the samples. This non-invasive technique utilizes colors to depict the arrangement of chemical elements in a microphotograph taken by an electron microscope. The EDX investigation involved examining the samples using an acceleration voltage of 30 kV and a counting rate ranging from 1000 to 9000 counts per second (cps).

Results and discussion

The *Epipremnum aureum* (Pothos) plant was used as depicted in Fig. 1. From its root extension, the plant was immersed in an aqueous solution of silver nanoparticles

at 10% by weight for 12 weeks. This concentration was chosen based on the observation made by Macclesh del Pino Pérez et al. (2021), who noted that a higher concentration of silver led to the formation of large silver crystals on the surface of the film. These crystals could generate surface imperfections, such as roughness, potentially causing a blockage in the vascular system of the Pothos. Over several weeks, the Pothos cutting underwent its usual life cycle while assimilating the fluid containing silver nanoparticles supported on nanocellulose. After that, the Pothos cutting was removed from the solution and rinsed with deionized water. The outer bark and phloem were removed from the stem's lower, middle, and upper parts to examine the fresh stems. The results of environmental scanning electron microscopy (ESEM) and ESEM coupled with energy dispersive X-rays (ESEM-EDX) confirm that the silver nanoparticles formed conductive pathways, favoring the transfer of electrons within the tubular channels of the xylem, as shown in the images obtained from ESEM (see Fig. 4A–C, inset). The images from ESEM-EDX (Fig. 4D–F, inset) show that the silver nanoparticle in the plant is noticeably bright. In addition, ESEM and ESEM-EDX analyzed a cutting from the same plant, but now without silver nanoparticles. The results can be seen in Fig. 5. This characterization was realized to observe the differences between the plants with silver nanoparticles and those without.

The findings were further validated through a rigorous non-parametric Wilcoxon signed-rank test with a significance level of 5%. This meticulous trial determined the significance level of the output voltages between the root-stem (D1) and stem-leaf (D2), as shown in Fig. 3. Table 1 summarizes the result of this test. According to the results presented in Table 1, there is no significant difference between the output voltage readings in root-stem and stem-leaf. The p value was greater than 5% in both cases, for root-stem with a p value of $6.50E-1$ and stem-leaf with a p value of $6.57E-2$. Silver nanoparticles are found throughout the vascular system as conductive plants.

Discussion

Two surgical-grade stainless steel probes were used to evaluate the conductivity of the pathway formed by the silver nanoparticles, throughout the plant (root, stem, and leaf), as seen in Fig. 6. The overall experimental setup is depicted in Fig. 7A. Notice that the electric current flow



Fig. 9 Electrochemical analysis results: **A** charge density with different shunt resistors. **B** Amount of material recovered in mg/mm^2 per hour

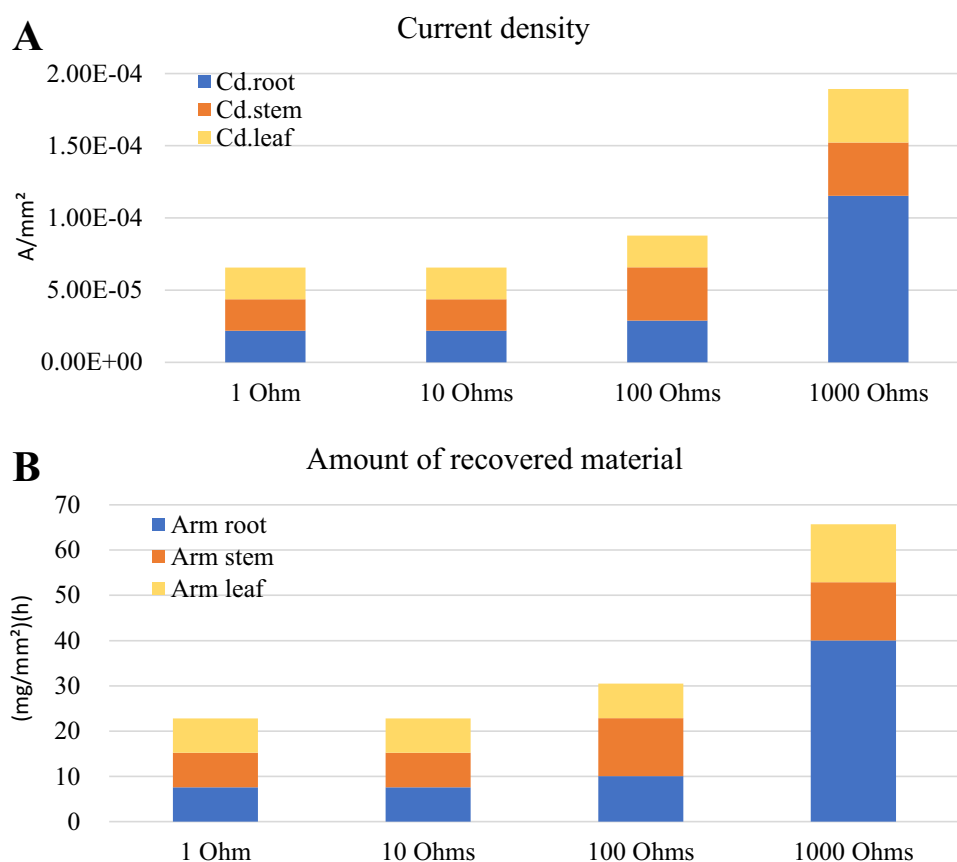


Table 2 Differences between this approach and the most related research

Description	PEDOT poly(3,4-ethylenedioxythiophene) (Stavriniidou et al. 2015)	The approach proposed (silver nano particles, Ag NPs)
Material type	polymer	metal
Electrical properties	semiconductor	conductor
Distance between electrodes	50 μm	1 cm
Plant	Rosa floribunda	<i>Epipremnum aureum</i>
Matrix (structural support)	–	nanocellulosa
Experimental environment	in-vitro	In-vivo
Plant life cycle	brief (5 or 6 days)	It continues its typical life cycle
Recovery material	not feasible	feasible
achievement obtained	Semiconductor device (AND gate)	Conductive plant

generated the dark tone of the conductivity pathway, and the electrode responsible for the anode function exhibited oxidation and degradation by releasing electrons deposited on the cathode Fig. 7B. In the initial stage of the measurements conducted, a polarization voltage from -1.5 to 1.5 V was employed, along with different shunt resistances (1,

10, 100, and 1000 ohms). The experimental results are shown in Fig. 8.

Polarization resistance tests were conducted to understand the underlying electrochemical events and assess the electrolyte's capacity. The theoretical foundation used to analyze the system is based on electrolysis Faraday's law,



which establishes the relationship between the amount of mass deposited on the electrode directly correlates with the magnitude of the electric current flowing through the system. In this context, the goal was to quantify the mass recovered per hour by varying the shunt resistors used in the experiment (Fig. 9).

The 1000- Ω shunt resistance was observed to provide the best response regarding current density in the root, stem, and leaves, with values of $1.15\text{E}-4$, $3.7\text{E}-5$, and $3.7\text{E}-5$ A/mm², respectively. Furthermore, the amount of material recovered throughout the plant in the root, stem, and leaves at one hour was 40.05, 12.84, and 12.84 mg/mm², respectively, as shown in Fig. 6.

On the other hand, the main differences and results achieved between this proposal and the most related research are shown in Table 2.

Conclusion

This study utilized cuttings of the plant *Epipremnum aureum*, commonly referred to as Pothos, to investigate the impacts of silver nanoparticles. The nanoparticles were found in a 10% weight concentration, supported on nanocellulose, and suspended in an aqueous solution. The objective was to assess the influence of these nanoparticles on electrical conductivity. The findings demonstrated the successful integration of silver nanoparticles into various circulatory systems, hence facilitating the transmission of energy through this conductive pathway. The in-situ trials were shown to have no impact on the plant's traditional life cycle. The utilization of environmental scanning electron microscopy (ESEM) and energy dispersive X-ray (EDX) research revealed that silver nanoparticles within the xylem created a channel that conducts electricity. The Wilcoxon signed-rank test reveals that there is no statistically significant distinction between the output voltage values in the root-stem and stem-leaf. These findings indicate that silver nanoparticles are found throughout the whole vascular system of plants and serve as channels for conducting electricity. In addition, an amount of 40 mg/mm² per hour of silver was obtained through the process of electrolysis, following Faraday's law. This study presents innovative opportunities for utilizing silver nanoparticles to enhance electrical conductivity in plant systems. Presently, there are ongoing endeavors to develop this technology for the purpose of monitoring environmental variables through the utilization of natural sensors. This technology has the potential to be utilized in the fields of agriculture and the detection of contaminants.

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Author contributions Conceptualization: HP-V and ABM-C, Methodology: MM-T, ABM-C, and EO-B. Investigation: MM-T, HP-V, ABM-C and EO-B. Visualization: MM-T and HP-V. Supervision: HP-V and EO-B. Writing—original draft: MM-T. Writing—review & editing: HP-V, EO-B and MM-T.

Availability of data and materials All data are available in the main text.

Declarations

Conflict of interest All other authors declare they have no competing interests.

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