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Enhancing agricultural sustainability through plant Nanobionics: Innovations, challenges and future Directions

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Abstract
Plant nanobionics, a rapidly growing interdisciplinary field situated at the convergence of nanotechnology and plant biology, aims to leverage the capabilities of nanoparticles in enhancing various aspects of plant functionality. The focus of this abstract is to delve into the process of integrating artificial nanoparticles into different plant organelles, with a specific emphasis on chloroplasts, to elevate the performance of essential physiological mechanisms such as photosynthesis and the ability to sense environmental cues. By incorporating nanoscale materials like single-walled carbon nanotubes (SWNTs) and nanoceria, the overall efficiency of photosynthetic processes is notably boosted, presenting a multitude of promising opportunities for the domains of sustainable agriculture and applications related to renewable energy sources. Furthermore, the introduction of nanoparticles equips plants with the capacity to not only identify but also counteract environmental stressors like drought and soil contamination, thereby aiding in soil restoration efforts and augmenting agricultural output. In addition to these benefits, the continuous progress in the realm of plant nanobionics has paved the way for the creation of innovative biosensors that possess the ability to independently recognize alterations in the surrounding environment, particularly in terms of water scarcity. The deployment of intelligent dust particles, which consist of miniature sensors dispersed throughout agricultural landscapes, plays a pivotal role in offering real-time information regarding soil conditions, thereby empowering farmers with valuable knowledge to optimize land utilization strategies. This underscores the profound transformative capacity inherent in plant nanobionics, illustrating its potential to revolutionize not only agricultural practices but also environmental surveillance measures and the management of sustainable resources.

Keywords: Plant nanobionics, nanoparticles, carbon nanotubes, photosynthesis, environmental sensing, soil remediation, biosensors, smart dust, sustainable agriculture

Introduction
The emergence of innovative biotechnological materials often originates in the field of nanobionics, where the exploration of live cell structures and biological processes is made feasible through the application of nanomaterials (Kozhukharov and Machkova 2013) [21]. Nanobionics, focusing on the electrical interactions at the nanoscale within biological systems, has expanded significantly, enabling the modeling and simulation of biological molecules. Another interpretation of nanobionics, as noted by Mansoori (2017) [25], involves developing technical systems, particularly electronic ones, inspired by biological systems.
Breaking down the term "nanobionics" involves "nano," referring to scientific principles applied at the nanoscale, typically ranging from 1 to 100 nanometers, and "bionics," which encompasses the study of biological systems to inspire design and engineering. Plant nanobionics, an emerging field at the intersection of nanotechnology and plant biology (Siddiqui *et al.*, 2015) [37], focuses on utilizing nanoparticles to enhance plant functions and capabilities, potentially revolutionizing agriculture, biosensors, defense, and other sectors. The integration of nanotechnology with biological systems has birthed novel approaches to enhancing the world, exemplified by plant nanobionics, which aims to imbue plants with unprecedented abilities (Strano).

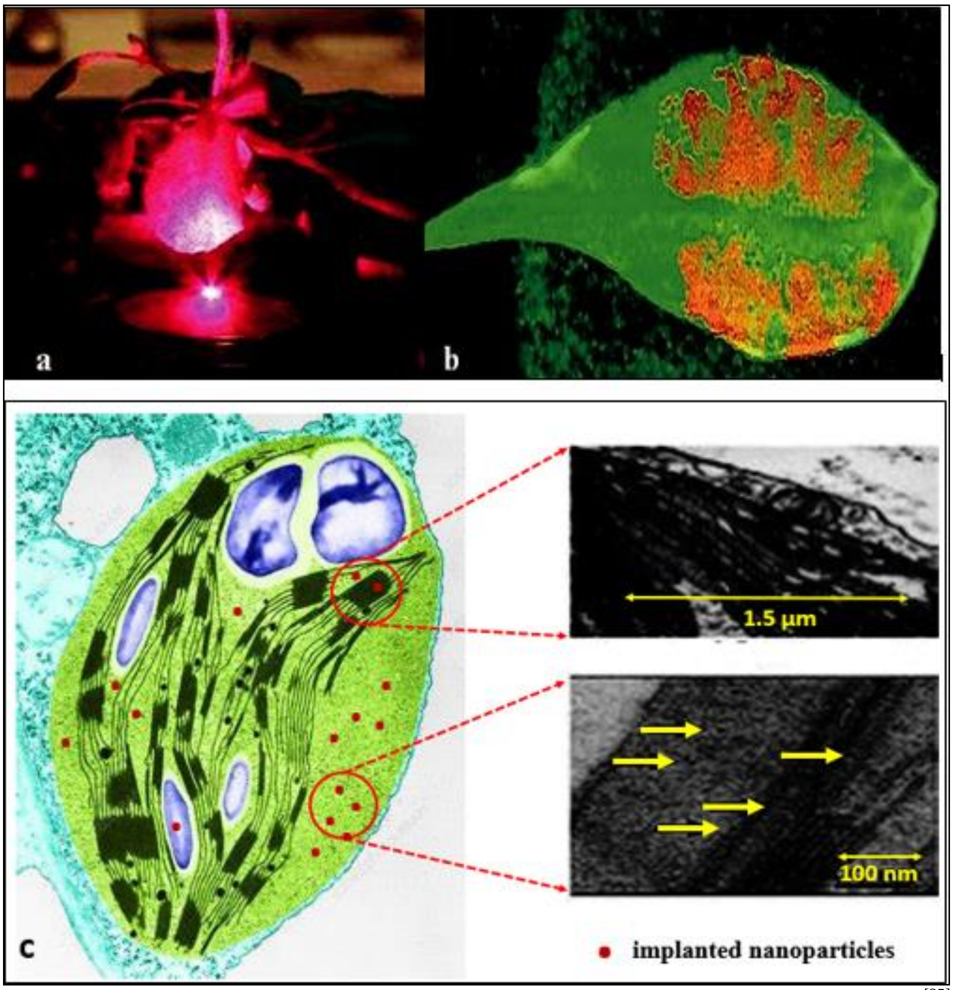
Bionics, a subfield of mechanical engineering synonymous with biomimetics, involves studying biological systems as models for designing materials and machines, while biomimetics encompasses developing human-made systems that mimic natural processes (Bar-Cohen, 2005; Neville, 2007) [3, 27]. The application of nanotechnology in agriculture holds promise for increasing productivity and managing plant diseases innovatively (Khan and Rizvi, 2014; Ghorbanpour and Fahimirad, 2017) [20, 13]. This interdisciplinary approach, spanning nanotechnology, robotics, artificial intelligence, medicine, and military sectors, underscores the significance of biomimetics in driving technological advancement.

Enhancing photosynthesis through plant nanobionics

Meeting the increasing demand for food production has been a significant challenge, prompting researchers to explore innovative solutions. Recent studies indicate that genetically modified plants, designed to utilize light more efficiently, have shown a notable increase of 20% in output, potentially revolutionizing global food production. Integration of nonbiological nanostructures with plant organelles offers a promising avenue to enhance their functionality. For instance, when single-walled carbon nanotubes (SWNTs) are introduced into chloroplasts, they become embedded within the chloroplast's lipid coat, significantly accelerating the rate of electron transport and enhancing photosynthesis by approximately threefold (Boghossian *et al.*, 2011, 2013) [4]. Moreover, the incorporation of nanoceria, tiny cerium oxide particles, into chloroplasts serves as potent antioxidants, effectively neutralizing harmful reactive oxygen species and bolstering photosynthetic efficiency (Wu *et al.*,2013) [2].

Plant nanobionics holds immense potential for developing biomimetic materials capable of efficiently harvesting light for photosynthesis and sensing biological changes. Studies have shown that nanoscale antioxidants, such as dextran-wrapped nanoceria, at optimal concentrations, effectively reduce oxidative stress while preserving chloroplast photoactivity, thereby facilitating sustained photosynthetic function (Boghossian *et al.* 2013) [5]. Researchers have also investigated the use of nano anatase TiO_2 treatment to enhance photosystem II (PS II) particles in spinach chloroplasts, leading to accelerated electron movement and oxygen production, as evidenced by spectroscopic analysis (Hong *et al.*,2005) [18].

Additionally, the utilization of metal nanoparticles, such as water-dispersible gold nanoparticles (GNPs), coupled with various molecules, has been shown to enhance photosynthesis in chloroplasts, as demonstrated in simulated models of photosynthesis (Das *et al.*, 2017) [6]. These advancements underscore the potential of plant nanobionics in augmenting photosynthetic processes at the molecular level, offering promising avenues for sustainable agriculture and renewable energy applications.



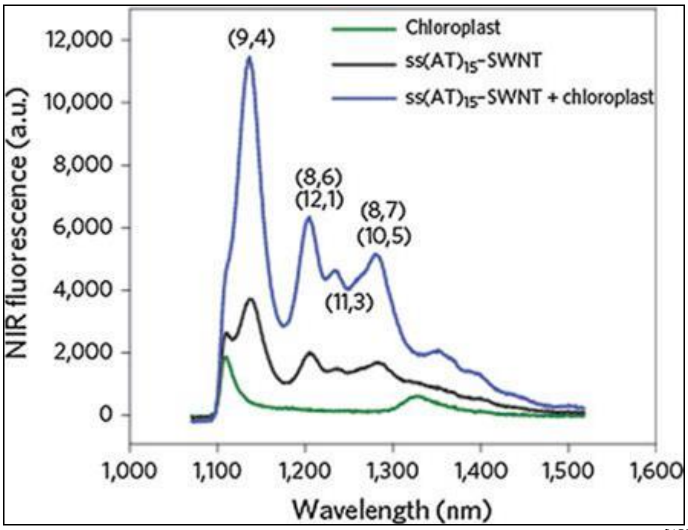
Sharma and Kar (2019) [35]

Fig 1: Improved photosynthesis with the use of nanobionic plants. a) Enhanced photosynthesis in bionic plants. As shown in the near-infrared fluorescence, carbon nanotubes appear orange in (b). (c) Chloroplasts with implanted nanoparticles for free-radical scavenging. Higher photosynthetic activity, as seen by faster electron transport, photophosphorylation, and oxygen evolution, has been linked to the presence of GNPs, which have been demonstrated to boost photon absorption in light-harvesting molecular complexes.

Plant nanobionics for environment monitoring using smart dust: Several effective practices are employed in agriculture, including the application of pesticides and fertilizers to manage weeds, insects, and diseases, soil composition analysis using sensors, and the use of fertilizers to enhance plant growth and productivity.

Enhanced ROS defense capabilities through plant nanobionics: In a study by Krichevsky A *et al.* (2010) [22], it was demonstrated that nanosensors based on single-walled carbon nanotubes (SWNT) could effectively monitor the dynamics of free radicals within chloroplasts at the single-molecule level. This monitoring aimed to optimize environmental parameters crucial for photosynthesis, such as light and CO_2 levels. However, a significant limitation of utilizing chloroplasts for solar energy generation is their susceptibility to decomposition due to light and oxygen-induced damage to photosynthetic proteins.

To address this challenge, Giraldo *et al.* (2014) [14] conducted research where cerium oxide nanoparticles, also known as nanoceria, were combined with a highly charged polymer. This approach aimed to enhance the resilience of chloroplasts against oxidative stress, thereby improving their longevity and efficiency in solar energy conversion.



Ghorbanpour and Fahimirad (2017) [13]

Fig 2A: Near-infrared Imaging for Fluorescence enhancing ROS defence in Chloroplast

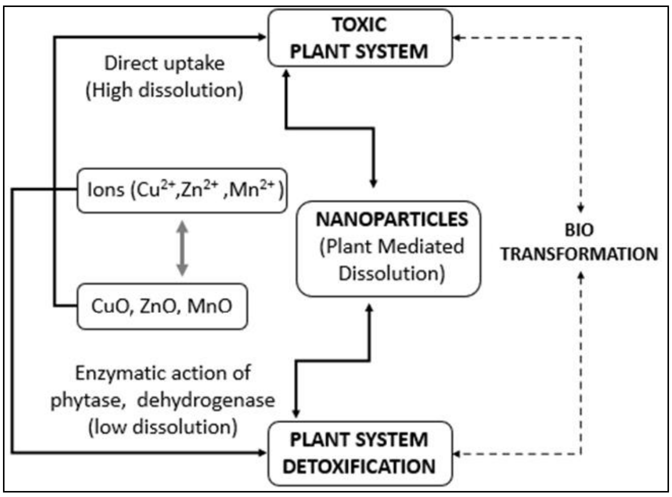
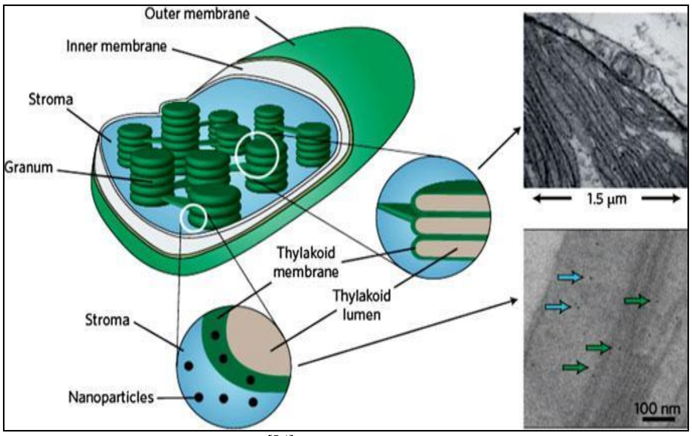


Fig 2B: Mitigation of Chloroplast Auto fluorescence in NIR Imaging and its mechanism

In Figure 2A and B, the interference caused by chloroplast auto fluorescence in near-infrared images was mitigated by employing an 1100 nm filter composed of polyacrylic acid. This filter selectively permitted the transmission of light through the outer membranes of chloroplasts, reaching the stroma while effectively safeguarding the photosystems from damage by neutralizing reactive oxygen species present within the chloroplast. Moreover, this methodology facilitated the real-time monitoring of free-radical species and environmental pollutants utilizing nanosensors integrated into living organisms and tissue samples, as illustrated in Figure 3 (Siddiqui *et al.*, 2015) [37]. Additionally, the chlorophylls within two key pigment-protein complexes, namely photosystems I and II (abbreviated as PSI and PSII), play pivotal roles in capturing solar energy.



Scholes and Sargent (2014) [34].

Fig 3: Natural and nanobionic chloroplasts

The majority of the photosynthetic machinery resides within the thylakoid membranes of chloroplasts, which are flattened and organized into grana, as observed in a micrograph of the cryptophyte alga *Proteomona sulcata* (top right). Nanoparticles are distributed in both the thylakoid membrane and the stroma, as indicated by the green and blue arrows in the micrograph (Scholes and Sargent, 2014) [34]. These nanoparticles undergo conversion processes to produce electrochemical energy, which is then utilized for ATP and NADPH production, critical for CO_2 fixation. Photosystem II (PSII) drives the light-induced oxygen evolution reaction, transferring electrons from water to plastoquinone within the membrane. Conversely, Photosystem I (PSI) generates reducing power by utilizing electrons from PSII, subsequently reducing ferredoxin and NADPH. Demonstrated that combining nanomesoporous silica compound (SBA) with photosystem II (PSII) maintained the efficient oxygen-evolving capacity of *T. vulcanus* PSII, even when confined within silica nanopores. This activity persisted for over three hours during moderate illumination/dark cycles. The utilization of PSII-SBA conjugates, alongside mediator recycling systems, shows promise in mitigating the adverse effects of electron acceptors and light-induced radicals, making them attractive candidates for photo sensors and artificial photosynthetic systems.

Plants with soil remediation abilities

Nanoparticles hold the potential to confer extraordinary capabilities upon plants, particularly concerning soil remediation. Plant nanobionics involves modifying plant structures by incorporating nanotubes, enabling them to gather soil data. This innovation presents an opportunity to develop plant species capable of identifying pollutants, offering valuable solutions to significant environmental challenges (Jha and

Pudake, 2016) [19]. Bioremediation, a method to address soil pollution, involves modifying ecological conditions to encourage the growth of microorganisms capable of decomposing pollutants. Integrated sensors in plants facilitate efficient monitoring of pesticide use and detection of infections caused by microbial toxin production. Plant nanobionics can detect metal and metalloid contamination in soil (Fig. 4) and possess the ability to remove pollutants. Plant nanobionics offers several distinctive features suitable for soil detoxification, including the utilization of metal transporters, enhancement of enzymes involved in sulfur metabolism, and production of metallothioneins and phytochelatins, which act as chelators, effectively detoxifying metals in soil. This approach is environmentally friendly and cost-effective. Transgenic plants generated through plant nanobionic strategies facilitate phytoextraction of metals like Cd, Pb, and metalloids such as As and Se from soil. Nanotechnology aids in gene transfer in plants using nanoparticles to devise strategies for soil detoxification (Mukhopadhyay and Kaur, 2016) [26].

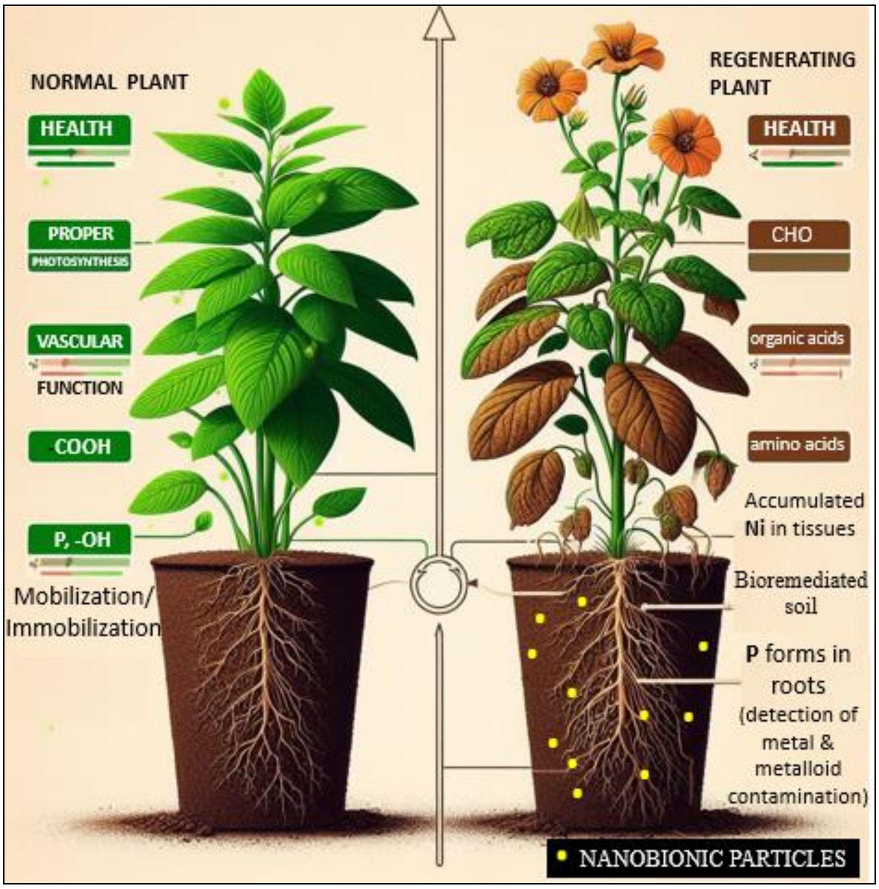
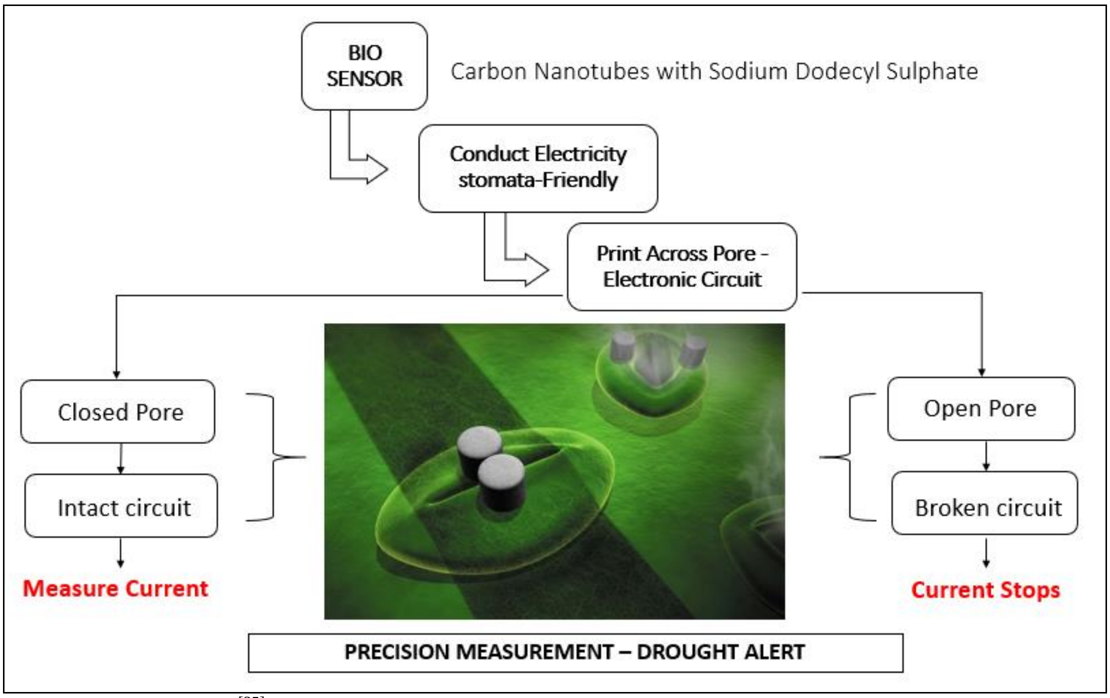


Fig 4: Utilizing plant nanobionics for soil metal and metalloid contamination detection to regenerate plant capabilities to restore altered soils.

Nanobionic plants - drought biosensors

Research has revealed that plants respond to osmotic or drought stress by closing their stomata and transmitting this signal to nearby unstressed plants (Falik *et al.*, 2012) [11]. Nanotechnology finds diverse applications, including gene transfer in plants using nanoparticles to develop insect-resistant varieties, food processing and storage, as well as the creation of nano feed additives to prolong product shelf life. Engineers at MIT have devised innovative sensors designed for implantation into plant leaves. Saxena *et al.* (2016) [33] suggested the use of these sensors to detect water scarcity and identify drought-affected plants, providing early warnings to farmers about drought conditions. This ground breaking innovation is credited to Michael Strano, the Carbon P. Dubbs Professor of Chemical Engineering at MIT. The insights gleaned from this technique are invaluable and unattainable through other means. Sensors can also be deployed in soil or satellite imaging and mapping to highlight drought conditions. However, it's important to note that different plants possess varying abilities to perceive water potential, and the importance of water levels may vary depending on individual preferences and requirements. According to nanobionics empowers plants to autonomously detect water scarcity and convey this information to farmers, enabling them to take timely measures to address the issue (Figure 5). Recent research indicates that nanoparticles can transform wild-type plants into pre-biohybrids. A novel nanobionic-plant system has been developed using spinach plants and single-walled carbon nanotubes (SWCNTs) as nanoparticles. The integration of single-walled carbon nanotubes (SWCNTs) into plant systems offers numerous possibilities in the realm of robot-plant biohybrids. Additional functionalities could be incorporated to facilitate the assessment of a plant's physiological condition or the presence of environmental indicators such as water scarcity or drought (Skrzypczak *et al.*, 2017) [38].

These sensors, when attached to plant leaves, can detect and signal water scarcity in plants. The effects of yttrium-doped γ-Fe_2O_3 nanoparticles were investigated in terms of their potential as a plant fertilizer and their enzymatic activity in alleviating drought stress. Palmqvist *et al.* (2017) [28] observed a reduction in hydrogen peroxide and lipid peroxidation levels in Brassica napus plants when γ- Fe_2O_3 nanoparticles were administered through soil irrigation following a period of drought.



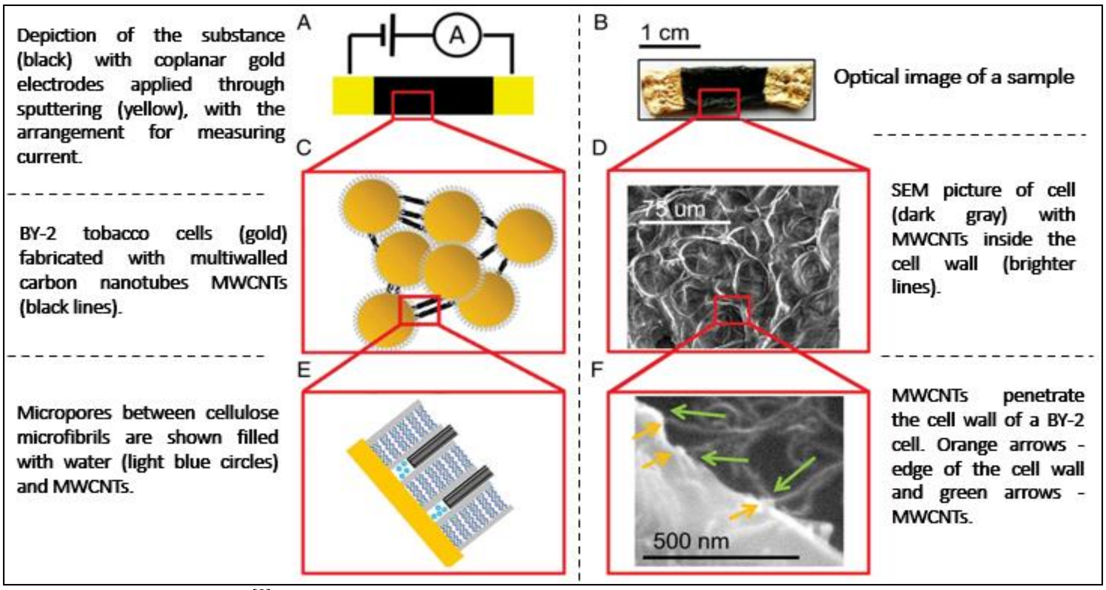
Sharma and Kar (2019) [35].

Fig 5: Nanobionic plants used as biosensors for detecting drought

Michael Strano, the creator of this ground breaking technology, suggests that it holds promise in addressing the issue of neglected household plants and offering early alerts to farmers regarding potential water scarcity risks for their crops. This early warning system would empower farmers to devise strategies to mitigate the impact of drought on their plants. Researchers could contribute to developing innovative methods for engineering plants with heightened drought resistance, enabling them to withstand periods of water scarcity. Research findings indicate that the application of nanoscale TiO_2 concentrations can enhance both the morphological and physiological characteristics of plants under water scarcity conditions, resulting in improved plant performance. Studies conducted by Hatami *et al.* (2014) [16] and Aghdam *et al.* (2016) [1] have demonstrated that the application of nano-TiO_2 particles at appropriate concentrations can enhance the drought tolerance of flax plants.

Plant nanobionic material-enhanced temperature responsiveness

Throughout Earth's history, nature has continuously inspired innovative ideas across various domains, including machinery, pharmaceuticals, and materials. Scientists at the Department of Mechanical and Process Engineering at the Swiss Federal Institute of Technology (ETH Zurich), Switzerland, have developed a temperature sensor inspired by the mechanisms observed in temperature-sensitive plants. However, instead of directly mimicking plant properties, they have engineered a hybrid material incorporating both plant cells and synthetic components. According to Professor Chiara Daraio from the Department of Mechanics and Materials, this approach leverages natural processes to achieve our objectives.



Di Giacomo *et al.* (2015) [8].

Fig 6: Innovative application of plant nanobionic material with enhanced temperature responsiveness

The researchers have successfully developed an exceedingly sensitive temperature sensor, an electronic component capable of adjusting its conductivity in response to temperature changes, without compromising the material's activation energy. This sensor exhibits an unprecedented level of sensitivity to minor temperature fluctuations, leading to substantial alterations in conductivity. According to Di Giacomo *et al.* (2015) [8], a key figure in Daraio's research group, our sensor demonstrates a responsiveness at least 100 times greater than that of the most advanced sensors currently available. The remarkable ability of plants to detect and respond to subtle temperature changes by adjusting the conductivity of their cells is well-documented. In this regard, plants outperform existing artificial sensors. Professor Di Giacomo conducted experiments using tobacco cells in a cell culture system and highlighted the challenge of transferring temperature-sensitive cell properties into inert, desiccated material while retaining these characteristics (Figure 6).

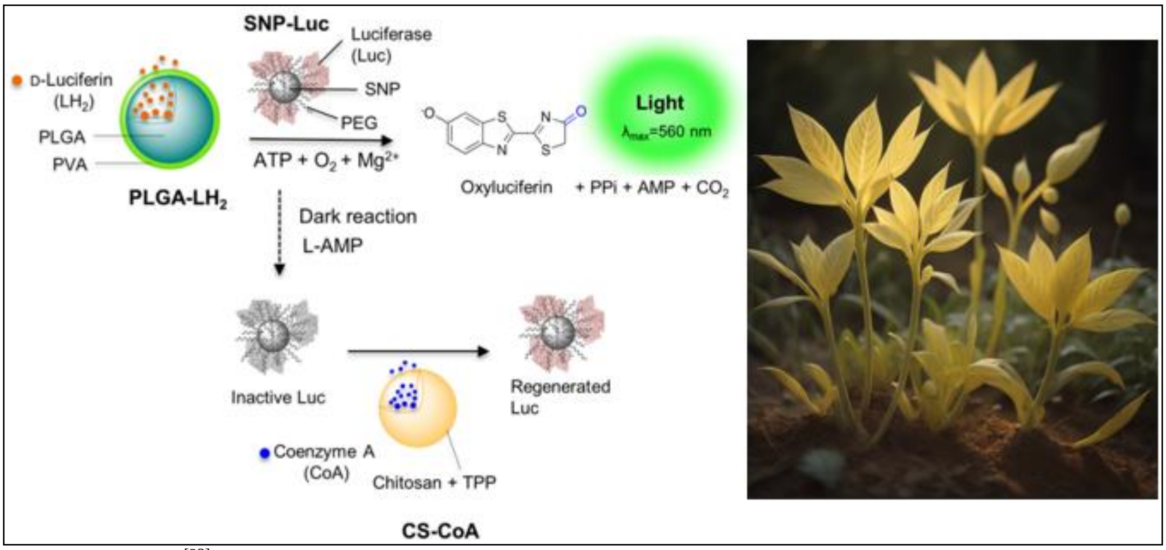
The cells were cultured in a carbon-containing medium, where conductive carbon nanotubes formed an electrical network connecting the tobacco cells and effectively penetrated the plant cell walls. Upon dehydration of the nanotube-treated cells, Professor Di Giacomo discovered a rigid material, dubbed "cyberwood," exhibiting temperature-dependent electrical conductivity akin to living tobacco cells. Pectins and charged ions play crucial roles in maintaining temperature sensation in both living plant cells and cyberwood. Pectins, heteropolysaccharides present in the primary cell walls of terrestrial plants and certain intercellular tissues can form a gel through cross-linking at different temperatures. Calcium and magnesium ions are essential components within this gel. As temperature rises, pectin links break, resulting in a softer gel and increased ion mobility, leading to high electrical conductivity maintained even at elevated temperatures. This ground breaking discovery has been submitted for patent application, with ongoing efforts aimed at adapting the sensor to operate independently of plant cells, relying primarily on pectin and ions. The primary goal of their research is to develop a biocompatible sensor capable of detecting extremely high temperatures, expected to be cost-effective. The development of thermal sensors will open up opportunities for their application in various fields, including biomedical devices and consumer products.

Plant nanobionics, a modern technique involving the integration of synthetic nanoparticles into plant organelles to enhance physiological processes like photosynthesis, has gained attention. While higher plants possess the ability to detect temperature changes accurately, this capability is limited to their living state. Recently, scientists have proposed using carbon nanotubes (CNTs) to stabilize the temperature response of isolated plant cells. Materials with a high-temperature coefficient of electrical resistance (TCR) can be employed to construct interconnecting cells. These biosensors exhibit significantly higher sensitivity compared to existing sensors. A pectin-based structure resembling an egg box, situated between cellulose microfibrils enclosing metal ions, is pivotal for cellular response to elevated temperatures. Carbon nanotube networks enhance material stability and conductivity, enabling detection of high temperatures without reducing activation energy. A polymer nanocomposite temperature sensor, utilizing multi-walled carbon nanotubes (MWCNTs) as nano-fillers, has been designed to demonstrate an increase in resistance with rising temperature and decreasing MWCNT content. Alamusi *et al.* (2013) [2] successfully developed this highly sensitive temperature sensor, an electronic module capable of adjusting its conductivity in response to temperature changes. According to Raffaele Di Giacomo, a postdoctoral researcher in Daraio's group, previous sensors have not exhibited the same precision in detecting minor temperature fluctuations. The sensor demonstrated responsiveness at least 100 times greater than that of the most advanced sensors currently available.

Plants' ability to detect environmental smart dust

The use of nanotechnology in agriculture, encompassing applications such as enhanced plant growth through fertilizer usage, soil quality monitoring via sensors, and pest and disease management using pesticides, has become increasingly prominent. Nanotechnology aims to amplify the effectiveness of various agricultural practices, reducing effort while increasing yield and minimizing waste. Micro-electromechanical systems (MEMS), including sensors and robots capable of sensing light and temperature, are collectively termed smart dust. Smart dust, comprising very small wireless sensors referred to as motes, possesses sensing, computing, and wireless communication capabilities and remains suspended in the environment like dust particles. A wireless network connection is required to operate these smart dust particles, enabling detection and sensing of their presence. Over the past decades, advancements in technology have revolutionized farming, transitioning it from a primitive industry to one that leverages technology in virtually every aspect of grain production systems. US farmers are at the forefront of this agricultural revolution, bridging the gap between global distributors and food manufacturers to ensure a more secure and sustainable food supply. However, there is limited attention paid to emerging technologies like smart dust and smart contacts, which extend current communication systems into new realms beyond traditional person-to-person communication.

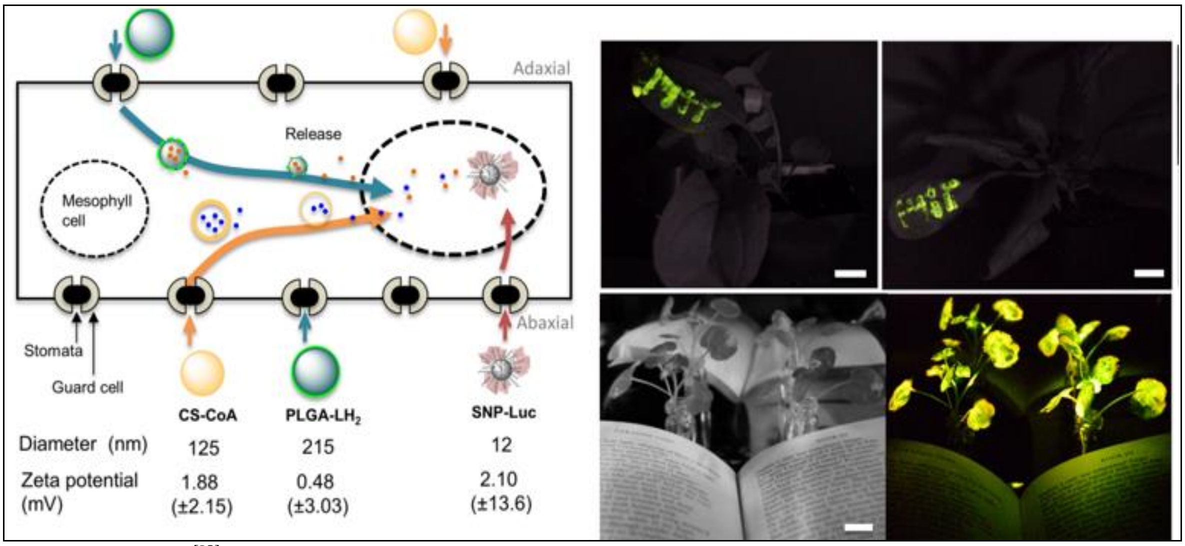
Smart dust, envisioned by Kris Pister at UC Berkeley in the 1990s, comprises numerous tiny sensors smaller than rice grains scattered across the Earth's surface. These "smart dust" particles function as electronic nerve endings for the planet, equipped with computer processors, wireless radios, and extended battery life, capable of generating real-time data about people and the natural environment. Smart dust is designed to detect a range of environmental parameters, including soil temperature, moisture content, and chemical composition, providing farmers with detailed field information. Data collected from smart dust particles will offer farmers valuable insights into soil characteristics, allowing them to optimize land use and potentially monetize information rights associated with land attributes like water and minerals. This real-time data will surpass the validity and significance of existing information technologies, enhancing our understanding of plant life's intricacies. Silica nanoparticles were utilized to carry the luciferase enzyme, while larger particles composed of (poly lactic-co-glycolic acid) (PLGA) polymers were employed for transporting the luciferin substrate. The luciferase enzyme itself doesn't produce luminescence; instead, it facilitates the oxidation of luciferin, leading to light emission as it reverts to its ground state (Figure 7). To mitigate the potential interference of reactant by-products on luciferase activity, coenzyme A was incorporated into the nanoparticles.



Kwak *et al.* (2017) [23].

Fig 7: Innovative application of nanobionics in engineering plants to emit light, transforming them into glowing organisms.

To administer nanocarriers into plant leaves, nanoparticles were suspended within a solution, and then plant leaves were submerged in this solution. Through the application of elevated pressure, the nanoparticles diffused through the stomata, which are tiny pores present on the leaf surface. Subsequently, these nanoparticles discharged molecules that were absorbed by the plant cells. The design of these nanoparticles was customized to concentrate luciferin and coenzyme A in the extracellular space of the mesophyll layer, whereas smaller nanoparticles containing luciferase were able to penetrate the mesophyll cells.



Kwak *et al.* (2017) [23].

Fig 8: A fascinating concept that merges plant biology with nanotechnology and bioluminescence.

The PLGA nanoparticles release luciferin, which is then absorbed by plant cells. Inside these cells, luciferase catalyzes the reaction with luciferin, resulting in the emission of light (Fig. 8). Previous attempts to create light-emitting plants involved expensive and labor-intensive genetic engineering methods. The emitted light produced by these processes is typically faint and observable only in specific plants with extensively studied genomes (Kwak *et al.*, 2017) [23]. The current approach in nanobionics offers increased brightness and operates through a simpler mechanism. Researchers view this technology as a promising alternative for energy-efficient lighting. It is conceivable that shortly, plants could replace table lamps and trees could replace streetlights along roads.

Conclusion

Plants play a crucial role in supporting life on Earth, contributing significantly to various aspects of human existence. Mythical tales often depict the existence of magical trees and plants capable of fulfilling any desire. Genetic engineering has enabled the creation of plant products with enhanced features, while horticultural strategies have played a role in realizing dreams by developing hybrids with unique traits. As children, such stories seemed fantastical, but with maturity, we recognize their potential within the realm of reality. Biotechnology has facilitated the introduction of novel attributes in both plants and animals, blurring the lines between fantasy and reality.

Plants are now not only utilized for their nutritional value but are extensively employed in nanobionics to mimic various biological processes, resulting in the creation of fascinating and innovative characteristics. They are used as bomb detectors, illuminating study lamps, pollution detectors, and more. Nanobionics is achieving remarkable outcomes by integrating nanotechnology with biological processes. Nanoscale particles, like carbon tubes, are integrated into plants to confer new abilities. Plants are imbued with extraordinary capabilities through nanotube integration, enabling them to function as sensors detecting environmental changes. Plant nanobionics, an emerging field, involves the insertion of nanoparticles into living plant cells and organelles, such as chloroplasts, to alter plant behavior. Plant nanobionics aim to enhance plants with new functionalities, such as environmental imaging or light emission, alongside their natural functions like photosynthesis, making them versatile for various applications.

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