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NANOSENSORS IN PLANT PHENOTYPING: A NEW FRONTIER IN SUSTAINABLE AGRICULTURE

Sharan S P*and Selvaprakash R

Division of Agricultural Physics, ICAR-Indian Agricultural Research Institute, Pusa, New Delhi - 110012

**Corresponding Author Mail ID: sharansp19@gmail.com*

Introduction

Addressing significant global challenges such as energy and food security requires a robust comprehension of plant biology, including aspects like signaling, physiological characteristics, and the interactions between plants and their environment, commonly referred to as phenotyping. With classical phenotyping being labour intensive, costly and time consuming there is a need for non-destructive and real-time monitoring of plant biology at microscale using advanced approaches introduced in the second green revolution era like nanosensors via optical, wireless or electrical signals. Nanosensors are nanoscale devices capable of identifying specific substances or measuring physical properties at an extremely small scale. At least one dimension of these sensors is less than 100 nm.

This is a developing field within the life sciences that presents significant opportunities for enhancing selectivity, sensitivity, and speed when compared to conventional techniques. Nanosensors contribute towards sustainable development goals by enhancing the efficiency of agricultural practices, thus addressing challenges related to food security.

Types of Nanosensors

Forster resonance energy transfer (FRET) nanosensors are employed to investigate protein interactions, cellular components, and various biophysical parameters by measuring energy transfer between fluorescent molecules. The effectiveness of energy transfer is significantly influenced by the spatial separation between the donor and acceptor fluorophores, typically within a 10 nm range. FRET is ideal for detecting protein conformational changes and interactions, and can be visualized using microscopy techniques. These nanosensors can be genetically encoded or externally applied and enable precise, ratiometric detection of molecular interactions.

FRET-based nanosensors that are genetically encoded consist of two fluorescent proteins exhibiting overlapping spectral properties. These sensors generally generate a ratiometric signal when energy is transferred between the proteins in reaction to the presence of an analyte. Commonly, cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP) are used as a FRET pair. However, one challenge in plants is interference from chlorophyll autofluorescence, though methods to address this issue exist.

Gene silencing in plants has also posed difficulties, but using mutant plants deficient in gene silencing has allowed effective monitoring of metabolites. Externally applied FRET-based nanosensors utilize nanoparticles like gold, quantum dots, and lanthanide-doped particles, which can act as FRET donors or quenchers. These sensors have been applied to detect plant viruses and monitor transgenes. Additionally, nanoparticles have been used to detect adulterants in food products. Surface-enhanced Raman scattering (SERS) is an exceptionally sensitive spectroscopic method capable of identifying analytes at the single-molecule level by enhancing Raman signals from molecules that are adsorbed onto nanostructured metallic surfaces.

Molecules adsorbed on metal nanoparticles experience a signal enhancement of up to 10^{15} fold, making SERS powerful for detecting low concentrations. The enhancement occurs through two mechanisms: electromagnetic (related to surface plasmon resonance) and chemical (involving charge transfer and analyte-surface interactions). SERS is widely used in plant research for detecting biological molecules like hormones and has purposes in food safety as pesticide detection. Electrochemical nanosensors are highly sensitive for detecting biological molecules in plants employing amperometric and voltammetric techniques for qualitative plant sensing.

Nanomaterials like gold and carbon-based materials enhance the active surface area, boosting sensitivity. These sensors can monitor plant hormones, enzymes, and metabolites, as well as reactive oxygen species (ROS), providing insights into plant health and environmental conditions. Additionally, they can analyze soil composition for nutrients and heavy metals. Chemiresistive sensors, which detect changes in electrical resistance due to target molecule adsorption, are also used for gas sensing, such as detecting ethylene in plants. Piezoelectricity is defined as the capability of specific materials to produce an electric charge when exposed to mechanical stress, and, in turn, to alter their shape when an electric field is applied. This property is used in piezoelectric sensors to measure pressure, force, or strain by converting them into electrical signals.

Nanoscale piezoelectric sensors, capable of detecting forces at the nanonewton level, have been applied in studying plant biomechanics, such as in the Venus flytrap. These sensors have also been used in food safety, detecting toxins in apple juice, and in plant disease diagnostics.

Nanosensor based Plant Phenotyping

Invitro and Invivio phenotyping is achieved through nanosensors (Fig 1.). Invitro include plant growth monitoring with flexible strain nanosensor made with chitosan based conductive ink tested on cucumber crop, these sensors are highly sensitive and record the growth as electrical signals produced by stretch in sensor due to plant growth. Real-time tracking of plant signals as affected by environmental condition for plant growth is possible through nanosensors which intuitively represent the minor fluctuations in the water status of the crop.

A Laser induced graphene interdigital electrode (LIG-IDE) coated on polyimide film and casted with graphene oxide will act as long-term humidity sensor when attached to plant leaves. Rapid, on-site monitoring of soil pollutant like arsenic which affects plant growth is achieved through nanobionic sensor made up of fluorescence single walled carbon nano-tubes (SWCNT) which is embedded in plant tissue for monitoring harmful pollutants like arsenic translocation in plants.

In vivo monitoring of plants using traditional approach is difficult due to restriction by optical properties of leaf, quenching the study area. Nanosensors on other hand are well suited for analytes detection when embedded in plant tissues. Oxygen regulates plant growth and development, thus monitoring molecular oxygen which is an important electron acceptor in light dependent reaction of photosynthesis. Oxygen rate in plants were detected using Clark-type polarographic electrode sensors but practical limitations of these electrode like they require extensive sample preparation, invasiveness restrict their use.

With Platinum-coated tips of electrochemical nanosensors, oxygen at the surface of *chara corallina* can be detected (Alova et al., 2020). Adenosine triphosphate (ATP), a nucleic acid which is involved in plant energy transfer produced in chloroplasts and mitochondria was detected using a chimera of enhanced *Renilla luciferace* and a fluorescent protein venus with high bioluminescence resonance energy transfer by FRET mechanism in *Arabidopsis species.* Calcium ions is important in various plant processes and cell signalling is detected with genetically encoded-FRET (cyan flour protein and yellow flour protein) in *Louts japonicue*.

Reactive oxygen species involved in various cell signalling pathways like plant development, cell death is monitored through electrochemical nanosensor modified with platinum nanoparticles. Plant hormones vital for crop growth and development is detected by nanosensors. Auxin responsible for cell enlargement, bud formation is detected utilizing bio-nanocomposite of porous graphene with anti-IAA antibody through amperometric immunoassay by electrochemical nanosensors. Gibberellin hormone characterized by root elongation is quantified with FRET based nanosensor. Detection of salicylic acid, an important plant hormone for mediating host response upon pathogen infection is a challenging one in conventional aptamer-based detection. Employing nanopore thin film sensor is able to detect salicylic acid as low as 0.1 µM concentration in Arabidopsis (Chen et al., 2019).

Non-destructive nutrient detection through nanosensor permit direct communication with plant and growers for controlled fertilizer application. Electrochemical nanosensor can detect heavy metal as well as sodium and potassium nutrients in soil and plant which could be effectively utilized for fertigation. Concerning health risks to living community due to adverse effects of pesticide application in agriculture drives researchers to monitor and control them. Thus, monitoring pesticide uptake and translocation in plants and soil ecosystem is important for reducing overexposure. Surface-Enhanced Raman Spectroscopy (SERS) utilizing gold nanoparticles has been employed for for the real-time observation of pesticide movement within plants tissues.

A single step process to detect pesticide is using SERS sensing films.

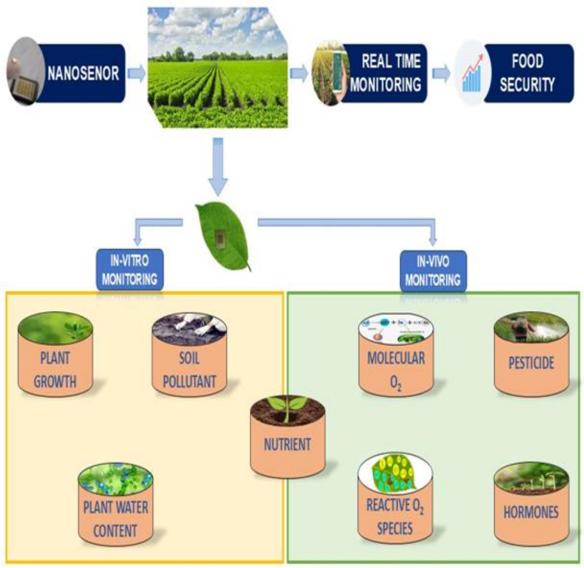

Fig 1. Real time Plant Phenotyping using Nanosensor to enhance food

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Conclusion

The integration of nanosensors into plant phenotyping represents a transformative advancement in precision agriculture, addressing critical challenges in modern agriculture. By offering unprecedented levels of sensitivity, specificity, and real-time data collection, nanosensors enable precise monitoring and analysis of plant health, growth, and environmental interactions at a microscale. Nanosensors such as FRET, SERS, electrochemical, and piezoelectric sensors provide detailed insights into various aspects of plant biology. They facilitate the non-destructive, real-time monitoring of critical factors like plant hormones, metabolic processes, reactive oxygen species, and nutrient levels. This capability significantly enhances our ability to optimize plant growth conditions, improve resource management, and ensure crop health. In addition to advancing our understanding of plant physiology, these technologies support sustainable agricultural practices by enabling precise, data-driven decisions. For instance, nanosensors can detect nutrient deficiencies, soil pollutants, and pesticide levels, thereby contributing to more efficient fertilizer use, reduced environmental impact, and safer food production. The use of nanosensors for real-time monitoring of plant responses to environmental changes further supports adaptive farming strategies that enhance resilience to climate variability. Overall, the deployment of nanosensor technologies in precision agriculture marks a significant leap towards achieving more sustainable, efficient, and productive agricultural systems. These innovations ensure global food security while minimizing environmental impact.

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