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Mini-Review

Emerging Technologies for Monitoring Plant Health in Vivo

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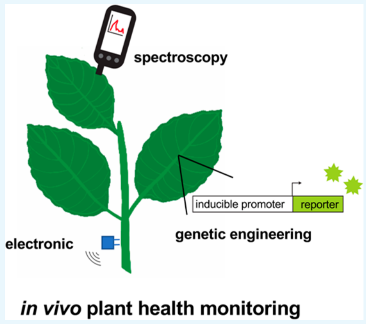
ABSTRACT: In the coming decades, increasing agricultural productivity is all-important. As the global population is growing rapidly and putting increased demand on food supply, poor soil quality, drought, flooding, increasing temperatures, and novel plant diseases are negatively impacting yields worldwide. One method to increase yields is plant health monitoring and rapid detection of disease, nutrient deficiencies, or drought. Monitoring plant health will allow for precise application of agrichemicals, fertilizers, and water in order to maximize yields. In vivo plant sensors are an emerging technology with the potential to increase agricultural productivity. In this mini-review, we discuss three major approaches of in vivo sensors for plant health monitoring, including genetic engineering, imaging and spectroscopy, and electrical.



1. INTRODUCTION

There is a critical demand for more sustainable agriculture practices to increase crop yields to meet the demand for a rapidly growing population. The UN estimated that by 2050 the global population is expected to reach 9.8 billion people.[1] However, farmers are facing many obstacles, such as extreme temperatures, soil degradation, and drought that are expected to worsen as the climate changes. Increased sustainable agricultural practices are needed to ensure high yields that utilize minimal inputs and are minimally destructive to the land.

Plant health monitoring is one such method to increase yields and decrease environmental impact. Using low-cost, in-field methods, water level, soil quality, and presence of pathogens and pests could be constantly monitored. Expensive agrichemicals and water can be used in a directed manner for optimal plant growth. Pathogen detection would allow for immediate corrective action to prevent disease from spreading. There are many agricultural practices and technologies currently employed by farmers to maximize yields, such as crop rotation to improve soil health, use of genetically modified seeds, or monitoring plants for presence of pathogens and pests by planting non-native plants, or sentinel plants.[2] There are also many diagnostic technologies employed to detect disease. However, current laboratory-based techniques for plant diagnostics are not adequate for point-of-use plant monitoring. There are several point-of-use technologies that have been developed, such as lateral flow devices or portable devices for in field use.[3] However, these types of devices require harvesting and processing plant tissue, which is not conducive to continuous monitoring.

Nanotechnology in plants is an emerging field in the past decade that has the potential to create more productive systems of agriculture. The use of nanotechnology has been extensively studied for applications in human health, medicine, pharmaceuticals, and wearable devices. Even implantable sensors for continuous monitoring in humans are possible.[4] Nanotechnology has the potential to improve agriculture in several ways, including formulation of nanofertilizers and agrichemicals, novel delivery mechanisms for agrichemicals, nanosensors for disease detection, nanodevices for genetic modification, and postharvest crop management. For a thorough review of plant nanotechnology, refer to Giraldo et al.[5] Here, we solely focus on emerging technologies for in vivo plant sensors for monitoring plant health.

2. GENETIC ENGINEERING APPROACH

2.1. Synthetic Biology. One class of in vivo plant sensors, phytosensors, were developed using synthetic biology. Liu and Stewart comprehensively reviewed the major applications of synthetic biology to plants, including phytosensors.[6] Phytosensors are plants that report plant pathogens, toxins, or nutrients. Plants have an innate, inducible defense mechanism to protect against pathogens, toxins, and nutrient deficiencies. Phytosensors are created by fusing reporter genes, such as fluorescent proteins, to synthetic inducible plant defense promoters. By fusing reporter genes to plant stress promoters, plants sense pathogens at a molecular level and quickly have a visible-to-the-naked-eye read out. This allows for rapid

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detection, as there is often several days or weeks from the point of infection to presentation of visible symptoms. Since plants naturally sense biotic and abiotic changes and alter biochemical and gene expression patterns, phytosensors hold a lot of promise as a modular, easily modified biosensor. This type of sensor is feasible for on-the-ground, in-field detection or could be used on a larger scale to monitor fields via satellite images with image detection software. There are several proof-of-concept studies. Mazarei et al. used elements from the promoter regions of pathogen-inducible genes and genes responsive to plant defense signal molecules such as salicylic acid, jasmonic acid, and ethylene.[7] They used *Arabidopsis* and tobacco as their model hosts and transformed them with the pathogen-inducible synthetic promoters fused with reporter gene, GUS. Phytohormone and plant elicitor treatment showed that the expression of GUS was increased compared to that with the control (Figure 1). Transformed tobacco plants had an increased expression of GUS when infected with Alfalfa Mosaic Virus but not Tobacco Mosaic Virus, demonstrating that different promoters could be used to detect different targets. In another study, Fethe et al. transformed four pathogen-inducible promoter elements fused to orange fluorescent protein into *Arabidopsis* and tobacco.[8] They tested the robustness and predictability of the transgene by monitoring the transgenic tobacco throughout two field seasons. They found 3 of 4 transgenic lines maintained the expected fluorescence signal. In particular, one line was specifically induced by bacterial phytopathogens and showed an increase in fluorescence only 48 h post-infection, much sooner than visible symptoms. These studies demonstrate the feasibility of phytosensors in live plants and in field settings. There are many innate plant responses that could be used in the design of phytosensors, though the degree of specificity and sensitivity would vary greatly among each promoter and element and would require widespread studies.

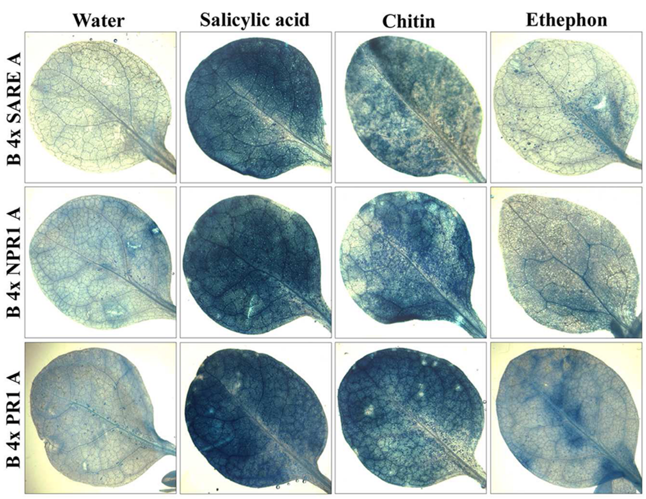

Figure 1. Histochemical analysis of GUS expression in transgenic tobacco plants exposed to salicylic acid, chitin, or ethephon treatments for 24 h. Adapted with permission from ref 7. Copyright 2008 Multidisciplinary Digital Publishing Institute.

3. IMAGING AND SPECTROSCOPIC APPROACHES

Another method of rapid diagnostics is through imaging and spectroscopy.[9] Molecular methods that use spectroscopy, such as real-time PCR and ELISA, are common methods for plant disease diagnostics but are highly invasive. They will not be covered in this mini-review. Imaging includes techniques such as thermography, RGB imaging, fluorescent imaging, and hyperspectral imaging. Spectroscopy techniques included in this mini-review are Raman spectroscopy, X-ray spectroscopy, and mass spectrometry.

3.1. Imaging. Thermography imaging detects heat emitted by objects; it is often used to survey large stretches of land at once. Changes in plant temperature can be attributed to a number of factors including pathogen response, such as closing stomata, or abiotic stress. While this method is ideal for monitoring large fields and is noninvasive, it is an indirect and nonspecific detection method.

RGB imaging utilizes digital cameras to measure any changes in transmittance. Simple digital images and videos have been used for monitoring a diverse set of plants in a field. It can be used for single plants, such as with a smartphone sensor, or used with drones to monitor large fields. Notably, machine learning algorithms are being designed to detect patterns that indicate disease. A comprehensive review by Mahlein points out several uses of RGB imaging.[10] Since RGB imaging relates changes in color to changes in plant health, it is an indirect method and cannot always provide specific insight into factors effecting the plant.

Fluorescent imaging is similar to RGB imaging; however, it often includes a laser, in addition to a camera, in order for fluorescent excitation. The most common use of fluorescent imaging is chlorophyll fluorescence imaging, where the fluorescence of a leaf or plant is compared to surrounding plants or to a baseline value. Chlorophyll naturally fluoresces when excited by certain light. Several studies have utilized this occurrence by relating fluorescence to the activity of photosynthesis. Bolhàr-Nordenkamf and colleagues used chlorophyll fluorescence to determine the photosynthetic activity of leaves collected from areas with different ambient air pollution and different agrichemical treatments.[11] These different factors altered the chlorophyll fluorescence, indicating some interruption in photosynthetic activity. This study also outlined several possibilities for portable in-field devices. Since chlorophyll is fluorescent under intense sunlight, a simple fluorimeter can be used to take measurements in the field. Though this method is noninvasive, nondestructive, and easily adaptable to in-field use, it is nonspecific and unable to diagnose specific abiotic or biotic stressors. Leaf fluorescence fluctuates often and in response to multiple biotic and abiotic factors. For a comprehensive review on chlorophyll fluorescence, refer to Mohammed et al.[12]

Hyperspectral imaging is a technique that analyzes light across the electromagnetic spectrum to evaluate changes that are not always visible in RGB images. Though it can detect more nuanced changes than visual or fluorescence images, it can only be used to detect general changes in plant surfaces. With further studies, hyperspectral patterns can be attributed to specific conditions. For example, Zhang et al. analyzed hyperspectral features of yellow rust disease and, after statistical analysis, were able to differentiate yellow rust from nutritional deficiencies.[13]

In the following studies, polydiacetylene (PDA) polymer and DNA-functionalized single-walled carbon nanotubes (SWCNTs) were incorporated into leaves before imaging. Both techniques were solely carried out in a lab setting, though both show promise of potential in-field applications that incorporate materials directly into live plant leaves for

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diagnostics. In order to measure the amount of water output from individual stomata, Seo et al. developed a PDA-based brush-on sensor with a hydrochromic PDA system.[14] Diacetylene monomers were brushed on the abaxial side of the leaf and photopolymerized. Fluorescence microscopy was used to detect the change in moisture, as the polymer undergoes blue to red transition in response to changes in moisture coming from individual stomata. With fluorescence microscopy, open stomata can be detected to see possible environmental effects (temperature, wind, or humidity) on stomata activity. This is a small-scale, lab-based application but has the potential to be used for in-field diagnostic methods. Wu et al. developed a hydrogen peroxide sensor based on functionalized SWCNTs and near-infrared fluorescent imaging.[15] Hydrogen peroxide is generated in response to plant stresses. In this study, the effects of UV-B, high light, wounding, and pathogen-related stresses were tested, in addition to direct application of hydrogen peroxide. The SWCNTs were functionalized with the aptamer sequence that binds to hemin, which catalyzes hydrogen peroxide to produce hydroxyl radicals. The reactive hydroxyl radicals then quenched SWCNTs' fluorescence in the near-infrared range (Figure 2). In conditions of direct hydrogen peroxide

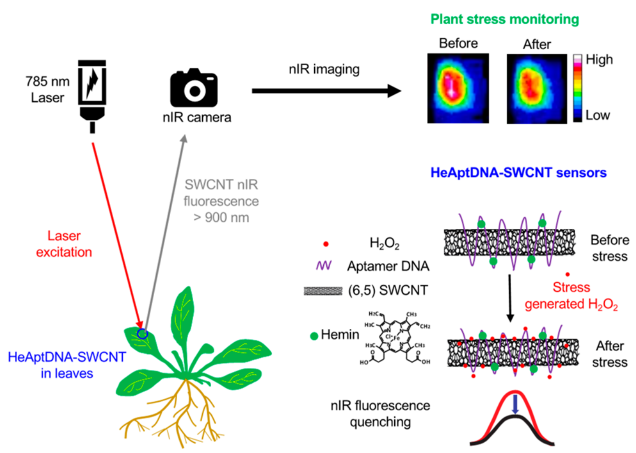

Figure 2. In vivo monitoring of plant health by SWCNT sensors for H_2O_2. SWCNTs functionalized with a DNA aptamer that binds to hemin (HeAptDNA-SWCNT) quench their nIR fluorescence upon interaction with H_2O_2 generated by the onset of plant stress. The spatial and temporal changes in nIR fluorescence intensity in leaves embedded with HeAptDNA-SWCNT sensors are remotely recorded by a nIR camera to assess plant health status. Adapted from ref 15. Copyright 2020 American Chemical Society.

application and in stress conditions, fluorescent emissions were reduced. This nanosensor is able to provide early signs of stress and could be optimized for precision agricultural practices and monitoring of plant health. SWCNTs can be functionalized using varying methods for detection of a wide variety of analytes.[16]

3.2. Spectroscopy. Raman spectroscopy detects vibrational frequencies of molecules; it can be used to determine the chemical footprint of a structure in order to identify molecules. Simply, a sample is illuminated with a monochromatic laser. The light interacts with the sample, and the resulting shift in energy gives insight into the molecules contained within a sample. Raman spectroscopy is nondestructive and biochemically safe for detection of molecules in highly complex samples.

Altangerel et al. developed a portable Raman spectroscopy instrument and used coleus lime as their model organism.[17] Two photosynthetic pigments, anthocyanins and carotenoids, were the target molecules for the Raman study. Carotenoids are a first line of defense against reactive oxygen species (ROS), and anthocyanins block harmful irradiation. Both increase biosynthesis in response to several environmental factors. Four methods of abiotic stress were applied: light irradiation, cold, drought, and saline stress. Using both a Raman microscope and the portable Raman instrument, the relative concentration of carotenoids and anthocyanins, which are indicative of abiotic stress, were determined 2 days after light, cold, drought, and saline stress were applied. The concentration of carotenoids and anthocyanins indicated the presence of stress in the plant before physical symptoms arose (Figure 3). Both results were confirmed with chemical

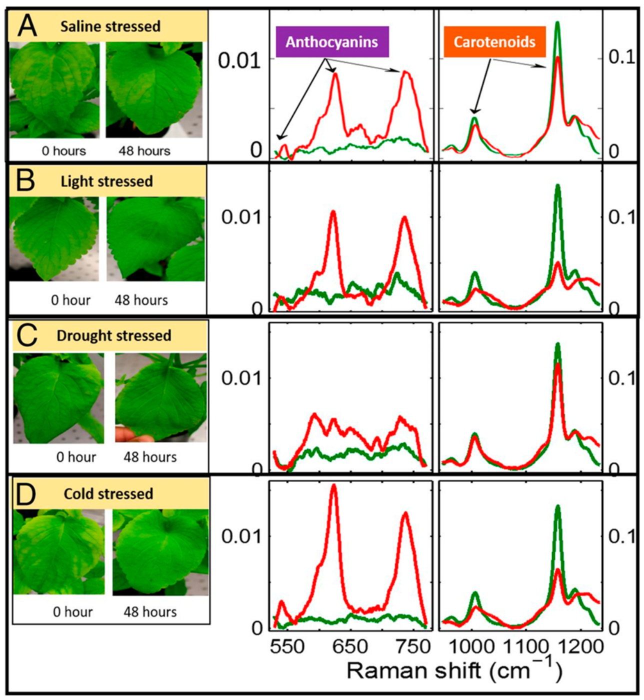

Figure 3. The Raman spectra of unstressed plants (green curves) and stressed plants at 48 h after stress (red curves) of (A) saline, (B) light, (C) drought, and (D) cold. Insets: Photos of coleus leaves for unstressed (left) and stressed (right) plants. Adapted with permission from ref 17. Copyright 2017 National Academy of Sciences.

analytical extractions. The changes to these pigments over time showed that Raman spectroscopy was a method to accurately measure these molecules and indicated there was a functional relationship between the molecules and response to excessive ROS during abiotic stress. The portable Raman instrument had limitations; it was unable to detect anthocyanins. However, further optimization could expand the capabilities. Gupta et al. developed a portable Raman leaf clip sensor that can distinguish between nitrogen-rich and nitrogen-deficient plants.[18] Raman spectroscopy has also been shown to detect pathogens and pests that live within host seeds[9] and the presence of chemical pesticides.[19]

X-ray fluorescence (XRF) spectrometry is a nondestructive method used to determine the chemical composition of many sample types. In XRF, an X-ray beam interacts with the sample and the fluorescent X-rays produced can be used to identify the

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elements in the sample. Montanha et al. used XRF along with an infrared gas analyzer to elucidate the uptake kinetics of aqueous Zn and Mn in soybean leaves and stems for 48 h.[20] The authors also monitored elemental distribution changes in plants in order to see the effect of localized X-ray exposure on live plant tissue. Typical XRF did not cause visible damage, dehydration, or elemental redistribution in live plants, though the long-term effects of low-dose X-ray exposure have not been studied.

Mass spectrometry is a method used to determine the mass-to-charge ratio of ions; there are several different types depending on the sample to be analyzed. Ambient ion mass spectrometry allows for mass spectrometry analysis without typical sample manipulation, such as a high vacuum environment. Low-temperature plasma (LTP) can be used to ionize samples at ambient air. LTP is a relatively gentle method of ionizing. Martínez-Jarquín et al. demonstrate that LTP mass spectroscopy is gentle enough to be used to analyze nicotine biosynthesis in live tobacco plants.[21]

3.3. Combination Approaches. There is a recent influx of methods that combine two or more imaging or spectroscopy methods for more accurate diagnostics and more sensitive detection.

A method by Crawford et al. allows for in vivo monitoring of genomic targets by integrating plasmonic nanoprobes and three complementary techniques to image and sense the probes: surface-enhanced Raman scattering (SERS), XRF, and plasmonic-enhanced two-photon luminescence (TPL).[22] This study used plasmonic-active silver-coated gold nanostars functionalized with double-stranded DNA, which changes conformation in the presence of a specific biotarget.[22] These probes were used to detect miR156, an miRNA in *Arabidopsis*, but they could be used to sense a wide variety of biotargets. The technique was validated in *Arabidopsis* using SERS tags to verify agreement among imaging modalities. Then, nanoprobes to detect miR156 were used. Raman imaging only detects the probe when it binds to its target. TPL and XRF detect the probe regardless of interaction with the target. The XRF signal was used to normalize the signal from Raman spectroscopy, allowing for quantification, an important aspect of biosensing. Not only can this method be used to track changes over time of a given target, but it can be used for diagnostics of plant pathogens. In other studies, thermal imaging and fluorescence imaging were complementary to each other in monitoring for plant stress.[23]

4. ELECTRICAL-BASED APPROACHES

Lastly, there are many studies using an electrical components for in vivo plant monitoring. While this requires external equipment, the use of nanotechnology allows for devices that can be integrated into plants.

4.1. Microneedle Electrodes. A study by Jeon et al. looked at measuring salinity, an important factor in plant health and crop yield.[24] They developed a real-time monitoring system to detect salinity in a nondestructive manner through electrical conductivity inside the stems of tomato plants. They designed a self-contained unit, including a microneedle electrode and electrode pad, that can be inserted into the stem of a tomato plant. This device was tested in greenhouse conditions and in field conditions. In field conditions, there was a decrease in signal noise and a decrease in electrical conductivity measurements, though the authors believe that decreased signal can be fixed by redesigning the electrical components to make it more practical for in-field use. A similar methodology, employing a thermal microneedle probe, was used to measure xylem sap movement in tomato stems.[25] Daskalakis et al. used maize as a model system to develop a similar microneedle leaf sensor.[26] However, their device takes canopy temperature measurements that can be used for water stress measurements. It can be calibrated for any plant, soil type, and relative humidity. It is powered by solar and emits data wirelessly through an antenna.

4.2. Organic Electrochemical Transistor-Based Sensors. An organic electrochemical transistor sensor (OECT) has been explored for use in biosensing. Simply, a conductive polymer film or channel is placed in direct contact with an electrolyte and electrodes. There are a source and drain electrode connected to the channel and a gate electrode that establishes electrical connection to the electrolyte. A common OECT sensor is made using the conductive polymer poly(3,4-ethylenedioxythiophene) (PEDOT) doped with various side groups.

Coppede et al. developed an OECT sensor for continuous monitoring of plant health based on changes to solutes in sap.[27] This study used tomato as their model organism, as commercially grown tomato requires optimization of conditions throughout its cropping cycle and yield and quality is largely variable. Here, OECT sensors are integrated into plant stems using cotton fibers. These sensors are highly biocompatible and commonly integrated into textiles to detect sweat. Commercial cotton fiber was functionalized by soaking in the conductive polymer and letting it dry in the oven. Functionalized cotton was inserted into the tomato stem and cut so it protruded from each end of the stem. Thin metal wire was attached to either end of the cotton thread, and a third thin wire was introduced as the gate electrode (Figure 4). A time constant and resistance (based on voltage across sensor) were measured. These can be used to deduce the physiological state of the plant. While this is an indirect measurement, it can be used to continuously monitor over a prolonged period. Recently, their group demonstrated the use of this sensor for drought detection in tomato plants. Using a bioristor sensor, drought stress was detected only 30 h from withholding of

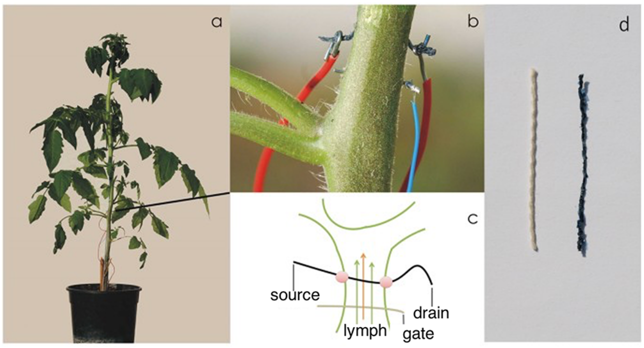

Figure 4. (a) A bioristor integrated in a tomato plant. (b) Detail of the textile device implantation and the silver gate connected through the plant stem. (c) Sketch of the proposed biosensor device showing the electrical connections. Green lines: sketch of plant stems. Black line: textile thread. Grey line: gate electrode. Arrows: lymph flow. (d) Cotton thread untreated (left) and functionalized with PEDOT:PSS (right). Adapted with permission from ref 27. Copyright 2017 Nature Publishing Group.

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Table 1. Overview of In Vivo Plant Sensors

ref	category	method	plant condition/disease of interest	target	range of detection or time to detection
7	synthetic biology	synthetic plant defense promoters fused to reporter genes and used to transform tomatoes	general plant stress	plant defense hormones,	24–72 h postinfection
15	nIR fluorescent imaging and functionalized SWCNT	SWCNT functionalized to detect H_2O_2	general plant stress	H_2O_2	50 min post-H_2O_2 addition, detection from 1 μM to 1 mM H_2O_2
25	electronic	microneedle sensor inserted into tomato stem	plant response to light, humidity, and soil water content	sap flow	in vivo sensor values were within 10% of values measured with control method
28	electronic	OECT sensor inserted though tomato stem	drought	ion concentration (Na^+, K^+, Mg^{2+}, Ca^{2+})	detect onset of drought within 30 h of withholding of water
29	electronic	OECT sensors inserted into xylem of aspen trees	photosynthesis	sucrose and glucose	100 μM to 1 mM

water.[28] Diacci et al. also utilized OECT sensors to measure the glucose and sucrose levels in xylem sap of aspen trees.[29]

5. CONCLUSION

There are a diverse set of needs for better plant diagnostic technologies. The best technology for a given farmer will depend on the size of land they are farming, the specific needs of their crops, and the natural, social, and economical environment they are in. Developing an array of sensors and innovative technologies is important in meeting agricultural demands of a larger population. Current technology for measuring plant health or diagnosing disease is expensive, invasive, and often requires sending samples to central facilities for processing. Nanotechnology and advanced spectroscopy techniques are emerging technologies for diagnosing plant disease and detecting plant distress, all with the common goal of increasing yield in a sustainable way. Table 1 illustrates the diversity in sensor type and target. Current challenges of these technologies include implementing them in field settings. Many of these studies are proof-of-concept demonstrations and would require further investigations to determine the efficacy in the field. Factors important to consider for a successful in vivo sensor include, but are not limited to, accuracy, specificity, sensitivity, durability, cost, ease of use, and environmental impacts. These sensors could allow for precision agriculture, where expensive resources are used in a directed manner and crop yield is maximized. Moreover, making these technologies affordable and accessible to large-scale and small-scale farmers alike is vital, as both are important in increasing agricultural production.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

Biographies

Jenna M. Roper completed her Bachelor's degree in Bioengineering from the University of California, Riverside, in 2017. In 2017, she began to pursue a Ph.D. at the same institution under the guidance of Dr. Hideaki Tsutsui. Her research interests include polydiacetylene-based biosensors for plant disease detection.

Jose F. Garcia is an undergraduate student in the Bourns College of Engineering at the University of California, Riverside. He will graduate with a B.S. in Bioengineering in 2022. Garcia has a strong interest in the field of biotechnology and biosensor development.

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