HARNESSING THE POTENTIAL OF BIOMARKERS AS POWERFUL TOOLS FOR PLANT PERFORMANCE

Abstract

Biomarkers have emerged as invaluable tools in the realm of plant biology, offering a deeper understanding of plant performance and providing crucial insights into various physiological and biochemical processes. This chapter explores the potential of biomarkers as performing tools for plants, encompassing their role in plant growth, stress response, and overall plant health assessment. We delve into the diverse range of biomarkers utilized in plant research, including molecular markers, metabolomic profiles, and physiological indicators. Furthermore, we discuss the application of biomarkers in plant breeding, crop improvement, and precision agriculture. By elucidating the intricate relationship between biomarkers and plant performance, this chapter illuminates the promising avenues for harnessing biomarkers as indispensable tools for optimizing plant productivity and sustainability in the face of global agricultural challenges.

Keywords: Physiological indicators; Molecular markers; Metabolomic profiles; stress response; Plant breeding; Crop improvement; sustainability.

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I. INTRODUCTION

Plants play a crucial role in global ecosystems, serving as the primary producers and providers of food, fiber, and various other resources. Understanding and enhancing plant performance is of paramount importance for sustainable agriculture, environmental conservation, and the well-being of human societies. Biomarkers, as versatile tools, hold significant potential for unravelling the mysteries of plant biology and aiding in improving plant performance. Biomarkers are measurable biological indicators that provide information about an organism's physiological, biochemical, or molecular status. In the context of plants, biomarkers offer valuable insights into various aspects of plant health, stress responses, and overall performance. They encompass diverse molecules, including proteins, nucleic acids, metabolites, and phytohormones, which can be identified, quantified, and analyzed to assess plant physiological processes and their interactions with the environment.

The potential of biomarkers lies in their ability to provide a snapshot of plant performance and response to environmental factors. Researchers can decipher the intricate mechanisms underlying plant growth, development, and adaptation by identifying specific biomolecules that serve as indicators of particular physiological states or stress conditions. For example, the levels of certain proteins or metabolites can reveal the metabolic pathways activated in response to stress or the efficiency of nutrient utilization. Biomarkers also enable non-invasive monitoring of plant health and the assessment of stress tolerance, enabling timely interventions to mitigate the negative impacts of abiotic or biotic stressors. Furthermore, biomarkers offer great promise in precision agriculture and crop improvement. By analyzing the expression profiles or abundance of specific biomolecules in different genotypes or under varying environmental conditions, researchers can identify genetic traits or environmental factors that contribute to enhanced plant performance. This information can be harnessed to develop stress-tolerant crop varieties, optimize crop management practices, and maximize agricultural productivity while minimizing resource inputs and environmental impacts. The potential of biomarkers as performing tools for plants is immense. They provide valuable insights into plant physiology, stress responses, and adaptive mechanisms. By harnessing the power of biomarkers, researchers can unravel the complexities of plant biology, enhance our understanding of plant-environment interactions, and ultimately contribute to developing sustainable agricultural practices and conserving natural ecosystems. The integration of biomarker-based approaches in plant research and crop improvement holds great promise for unlocking the full potential of plants and ensuring a resilient and productive future.

II. BIOMARKERS AND PLANT RESPONSES

1. **Environmental Stress and Biomarker Indicators:** Environmental stress poses significant challenges to plant survival and productivity. In response to adverse conditions such as high temperatures, drought, salinity, or pollutants, plants activate various defence mechanisms to mitigate the negative impact on their growth and development. Biomarker indicators offer valuable insights into plants' physiological and biochemical responses to environmental stressors, providing a means to assess their adaptive strategies and overall health. One common group of biomarkers used to monitor plant environmental stress is reactive oxygen species (ROS) and antioxidant enzymes. ROS, including superoxide radicals, hydrogen peroxide, and hydroxyl radicals, are

produced as by products of cellular metabolism and are known to increase under stress conditions. Elevated ROS levels can cause oxidative damage to cellular components. However, plants have evolved antioxidant defence systems, including enzymes like superoxide dismutase, catalase, and peroxidase, which scavenge and neutralize ROS. By measuring these enzymes' activity or expression levels, researchers can gauge the degree of oxidative stress and the plant's ability to counteract it.

Another group of biomarkers related to environmental stress is osmolytes or compatible solutes. Low-molecular-weight compounds accumulate in plants under stress conditions to maintain cellular osmotic balance and protect cellular structures. Examples of osmolytes include proline, betaine, sugars, and polyols. Quantifying the levels of these osmolytes can serve as indicators of plant response to water stress, salinity, or extreme temperatures. Higher osmolyte concentrations imply an adaptive response to the stressor, promoting cell turgor and protecting cellular integrity.

Stress-responsive proteins are also commonly used as biomarkers for environmental stress. For instance, heat shock proteins (HSPs) are molecular chaperones that help refold denatured proteins and prevent protein aggregation under heat stress. The abundance or induction of specific HSPs can be used to assess the severity of heat stress and the plant's capacity to cope with it. Similarly, other stress-responsive proteins such as dehydrins, late embryogenesis abundant (LEA) proteins, or pathogenesis-related (PR) proteins can serve as biomarkers for drought, cold, or pathogen-induced stresses, respectively.

- **Water Stress:** The responsiveness of the biomarkers used to identify genotypes resistant to water scarcity should take into account a variety of environmental factors and responses to water scarcity triggers. According to Shao *et al*. [1], a lack of water can reduce the quantity and size of stem cells, causing them to accumulate closer together and prevent water loss but restrict initial growth. The plasticity of leaf area is essential for efficiently managing crop water use [1]. The leaf area was chosen as a biomarker to guide the early selection of eucalyptus clones that can withstand dieback, a physiological condition linked to water scarcity [2]. Silva *et al*. [3] reported that eucalyptus clones subjected to water deficit had less leaf area. Because a decrease in leaf area also results in a decrease in water loss, these authors see it as the first line of defence against water scarcity.N, K, Ca, Mg, S, Cu, Zn, Mn, and B are recognised as suitable biomarkers for early selection among the evaluated nutrients while selecting water deficit-tolerant genotypes. In addition to being crucial for plant growth and development, nutrients have been shown by Waraich *et al*. [4] to help plants respond to abiotic stresses like drought by minimising their severe effects. These authors state that a key factor in plants' ability to withstand a lack of water is their nutritional status. This problem was successfully addressed by Müller *et al*. [5], who linked water deficit conditions to the effectiveness of nutrient absorption. Nitrogen (N), despite having one of the lowest heritability of the chosen biomarkers, showed a notable genotype x treatment interaction and high accuracy. N has been identified as a biomarker linked to the early selection of tolerant eucalyptus clones because of its role in enhancing tolerance to dieback, a physiological condition occurs more severe due to water scarcity [2].The interconnected plasticity and interrelation of traits expected from biomarkers that support water deficit tolerance are supported

by these reports. On the other hand, physiological characteristics like photorespiration influence nitrate assimilation significantly [6], increase with water deficit stress [7], and contribute to water deficit tolerance [8]. As a result, N has significance in distinguishing between genotypes that are water-tolerant.

Stress-sensitive clones show decreased absorption efficiency, decreased root formation effectiveness, and increased absorption efficiency in leaf formation in the presence of drought stress, whereas stress-tolerant clones typically have higher absorption rates despite having lower nutrient utilisation rates. It is expected that the probable impacts of stress on tolerant and susceptible genotypes [5], as well as their interactions with stress intensity/duration [9] and nutrient availability [10], will further enhance the interconnected adaptability and interdependency of biomarkers linked to water deficit tolerance. Among nutritional biomarkers, potassium (K) stands out for having a favourable impact on physiological and structural characteristics, such as osmotic adjustments and reduced membrane damage [11], which helps eucalyptus adapt to water deficit [12,13]. K is associated with reduced leaf growth and greater osmotic adjustment under water-scarce conditions, the latter of which helps to improve leaf turgor during dry spells.K might serve as a junction between morphological, nutritional, and metabolic responses to water deficiency.

However, during the rapid stress response, calcium (Ca) participates in the signal transduction pathway, playing a critical role in plant metabolism and significantly assisting in maintaining the integrity of the cell wall [14]. Calcium (Ca) plays a significant role in the recovery of plants under water stress due to its role in maintaining cellular structure through the stimulation of ATPase enzyme activity, which is essential for the restoration of nutrients lost during stressful times [4]. Magnesium (Mg) emerged as a notable biomarker for water deficit tolerance and displayed the highest heritability among the chosen markers, playing a role similar to calcium (Ca) in cell wall composition [15]. A large number of plant enzymes, including ATPase, ribulose-1,5-bisphosphate (rubisco), carboxylases, RNA polymerase, and protein kinase, all contribute to different photosynthesis-related processes [4, 6], including the decrease in CO_2 fixation where magnesium (Mg) is essential for the activation of these enzymes.

The amount of phenols and flavonoids, a class of metabolites known to protect cells against oxidative stress, increased in wheat flag leaves that had been subjected to water stress and treated with soil-applied Zn fertilization [16]. Additionally, Zn has been shown to have important advantages for root development [4] and plant photosynthetic efficiency under water stress [16].

Positive correlations exist between manganese (Mn) and tolerance to water deficit. Several antioxidant enzymes, including ascorbate peroxidase, SOD, and CAT, which scavenge free radicals and are essential for plant growth in stressful situations, work better with Mn as cofactors [17]. Although elevated SOD, CAT, and peroxidase activity have also been linked to excess Mn [18]. According to Mattiello *et al*. [19], a B deficiency decreases the plasma membrane's permeability and water flow. B also plays a important role in the cell wall's basic structure [20]. B deficiency also affects the development of the xylem, cellular cortex hypertrophy and modifications, and

stoma deformation [20]. These structural changes are expected to directly affect plant susceptibility to water stress by reducing hydraulic conductivity, transport, and nutrient availability. These interpretational methods are stated by Barros Filho [21] and support the notion that B is a biomarker for the ability to tolerate water deficits.

- **Temperature Stress:** Temperature stress, a key environmental factor, has a major effect on the growth, development, and productivity of plants. Biomarkers have an indispensable role in understanding the physiological and molecular responses of plants to temperature stress, enabling researchers to develop strategies for improving plant tolerance and resilience. Biomarkers associated with temperature stress responses can be detected at various levels, including cellular, biochemical, and molecular. At the cellular level, changes in membrane integrity, ion leakage, and cellular ultrastructure serve as biomarker indicators of temperature stress. These biomarker signatures give additional information on how temperature affects the physiology of plant cells and can help assess the degree of stress experienced by plants.

  Biochemical markers offer a deeper understanding of temperature stress responses by revealing metabolic changes induced by temperature fluctuations. For instance, measuring antioxidant enzyme activities, such as superoxide dismutase, catalase, and peroxidase, provides insights into the plant's defense mechanisms against oxidative stress caused by temperature extremes. Metabolomic profiling identifies and quantifies specific metabolites associated with temperature stress, providing valuable information about metabolic shifts and stress adaptation strategies. Molecular markers, including gene expression patterns and transcriptomic analyses, help unravel the genetic basis of temperature stress responses in plants. These biomarker signatures allow researchers to identify key genes involved in temperature tolerance, heat shock proteins, and regulatory networks governing stress responses. Understanding the molecular mechanisms underlying temperature stress responses provides opportunities for developing crop varieties with enhanced heat tolerance and resilience. By leveraging biomarkers associated with temperature stress responses, researchers can gain insights into plants' physiological and molecular changes under temperature stress conditions. This knowledge contributes to developing effective mitigation strategies, such as breeding for heat-tolerant varieties or implementing management practices to alleviate the impact of temperature stress on crops. Additionally, biomarker-based assessments enable the monitoring of temperature stress levels and can aid in optimizing cultivation practices to minimize temperature-related crop losses.

- **Salinity Stress:** Salt stress can cause several physiological and molecular changes, which in turn restrict plant growth by preventing photosynthesis and lowering the amount of resources available. Salt stress regulates the transition of photosynthesis's state and has an impact on how the light-harvesting complex forms [22]. The integrity of photosynthetic pigments, stomatal function, and other physiological and metabolic processes in plants are all negatively impacted by salt stress [23,24]. As a result, plants utilise a number of mechanisms to prevent the deposition of Na^+ and Cl^- in their tissues. Pea Calzada *et al.* (2022) [25] found that the net photosynthesis rate (A), transpiration rate (E), and stomatal conductance (gs) of soybean plants decreased

when they were subjected to salinity levels of 50 and 100 mmol L^{-1} of NaCl. According to a report, plants that are under salinity stress close their stomata to lower down the absorption of Na^+ and Cl^- by their roots [26]. As a result, studies showed that soybean plants decreased the E flux to prevent the buildup of NaCl. Amino acid supplementation also decreased the harmful effects of salt stress on soybean gas exchange. The crucial function of AAs (amino acids) in protecting proteins and photosystems is likely related to this buffering effect of AA mixtures against the detrimental effects of salt stress on A. Similar outcomes to those seen here, for instance, were obtained by [27], in which the authors demonstrated how incorporating arginine to sunflower plants improved the ability of photosynthesis. Additionally, AAs can function as critical osmolytes to regulate ion transport, stomata opening, and the balance of cellular osmotic potential [28]. For instance, it has been demonstrated that tryptophan applied exogenously increases the A, E, and gs of numerous crops [29]. Stomatal pores are essential for both plant gas exchange and the ability of leaves to cool off. Stomatal pore opening is the primary mechanism by which plants regulate the temperature of their leaves.

As a result, when stomata are open, it makes it easier for water vapour and heat to be removed from leaves and transferred to the atmosphere, which cools down the plant canopy [30]. Pea Calzada *et al*. (2022)[25] noted that soybean plants exposed to 50 and 100 mmol L^{-1} of NaCl hadelevated leaf temperatures in comparison to the plants which did not get any NaCl treatment. This increase in temperature of leaves has the potential to damage photosynthesis by approaching the optimal photosynthesis temperature or exceeding the maximum photosynthesis temperature [30]. At high temperatures, photosynthesis is a very delicate process, and PSII is thought to be the part of the photosynthetic apparatus that is most sensitive [22]. As a result, soybean leaves exposed to warmer and salt-stress conditions may sustain more severe damage. According to Pea Calzada *et al*. (2022) [25] data, when plants were treated with AAs under salinity conditions, the leaf temperature decreased. This is probably because AAs have mitigating effects on gs and E. The detrimental effects of salt stress can be further exacerbated by raising the leaf temperature of plants growing in salinity [31,24]. The findings in wheat [32] and cauliflower[33] suggested that exogenous administration of AAs, such as arginine, may be an effective tactic to increase tolerance to abiotic stress. For the photosynthetic system to operate properly, chlorophyll is a crucial pigment. Indicators of plant health under abiotic stress conditions are frequently used, most notably the monitoring of leaf chlorophyll levels and changes in leaf colour [32]. In the presence of NaCl, the leaf chlorophyll index decreased; nonetheless, the reduction was alleviated through the foliar administration of mixes containing AA. Abiotic stress has an adverse effect on plant growth and causes the breakdown of numerous organelles, including the endoplasmic reticulum, mitochondria, and chloroplast cell membrane. [34]. Photochemical efficiency measures both the potential for photosynthetic activity as well as the maximum efficiency of the photochemical process within photosystem II. It typically reveals values between 4 and 6 mol electrons per square metre per second [33]. Within the range of 0.75 to 0.85, photosystem II quantum efficiency is considered to be satisfactory. Both quantum and photochemical efficiencies decreased in soybean plants under salt stress. Plants treated with AA application, however, exhibited a lesser impact. This advantageous effect of AA mixtures is probably due to

their ability to inhibit ROS, which reduces the oxidative damage caused by salt stress to the photosynthetic machinery [35,36]. Additionally, it was discovered that arginine and glycine increase the amount of photosynthetic pigments in maize [37], sunflower [38], and *Pereskia aculeata* [39]. Plants were under osmotic stress, as evidenced by the observed RWC decline under salt stress in this study [40,41]. As expected, RWC reduced as the NaCl concentration increased. However, using AA mixtures reduced the impact of this effect. Along with the control of A, E, and gs, the increase in K^+ content and decrease in Na^+ can be used to explain this effect. Conversely, the foliar application of AA mixtures can control water relations because ion transport is controlled [42]. These findings support earlier research on *Ocimumbasilicum* [43] and tomato [31], which suggested that glycine and tryptophan play a role in enhancing the iWUE of plants. High levels of Na^+ are accumulated by plants under salt stress, which affects the homeostasis of other elements like K^+ and NO_3^- and causes other physiological issues and ion imbalances [40,44].In spite of the fact that plants exposed to 50 and 100 mmol L^{-1} of NaCl, accumulated a significant amount of Na+, the external introduction of AAs caused a decrease in Na^+ levels and a concomitant rise in K^+ levels within plant tissues. Cell desiccation and ionic and osmotic imbalance are brought on by a variety of abiotic stresses, including salinity. Plants build up compatible osmolytes like sugars, proline, AAs, or proteins in response to these occurrences. [45]. The requirement of quick improvement in plant metabolism following stress may be influenced by the relationship between the incorporation of AAs into plant tissues and the storage of protein synthesis precursors [46]. Additionally, recent studies [47, 48] have emphasised the significance of AAs in the control of cellular ionic homeostasis. Under conditions of salt stress, Malondialdehyde (MDA) content increased. The main cause of peroxidative damage is the oxidative degradation of unsaturated fatty acids within membranes caused by ROS, such as hydrogen peroxide, which is present within the cellular environment. NaCl causes lipid peroxidation of cell membranes. [49]. This increase in lipid peroxidation is regarded as signalling the beginning of oxidative cell damage. The synthesis of some proteins is differentially inhibited in cultures that have been exposed to salt stress. Additionally, the oxidation of nucleic acids, protein denaturation, and lipid peroxidation caused by ROS can result in the loss of cell viability and irreversible metabolic damage, which has a significant negative impact on the performance and productivity of cultures. [40]. Because of the increased activity of antioxidant enzymes, the application of AAs reduced the MDA content as a result of lipid peroxidation brought on by salt stress [50]. This supports the role of AAs as protective molecules against oxidative damage. Under conditions of extreme salinity, such as proline, plants accumulate large amounts of osmolytes as a response to osmotic stress [23,42]. One of the main osmoprotectants known for regulating salinity tolerance in plants, safeguarding membrane integrity, and stabilising enzymes and proteins is proline, a low molecular weight cyclic AA [51,52]. Our findings indicate that AAs improved plant adaptation and osmotic adjustment by raising the proline content in the leaves and roots of salt-stressed soybean plants. Other cultures that used AAs to lessen the effects of salinity have provided evidence of this fact [53, 54]. AAs play a significant role in protein synthesis and other vital cellular processes as plant metabolites. According to some research, glycine and arginine are involved in physiological processes like photosynthesis [50]. Additionally, AAs can function as critical osmolytes to regulate ion transport, stomata opening, and the balance of

cellular osmotic potential [42]. Similar findings were made by [38], where the authors demonstrated that arginine application enhanced the photosynthetic activity of sunflower plants.Additionally, it has been demonstrated that tryptophan applied exogenously can raise the A, E, and gs of numerous crops [37]. Stomatal pore opening is the primary mechanism by which plants regulate the temperature of their leaves. The removal of heat and water vapour from the leaf to the atmosphere is thus made easier when the stomata are open. Plants treated with AA mixtures under salinity conditions had lower leaf temperatures, probably as a result of the AA mixtures' mitigation effects on E and gs.

- **Wind Stress:** When exposed to various biotic and abiotic stimuli, flavonoids act as stress markers and accumulate in significant amounts throughout different plant tissues [55, 56–57], which promotes the removal of harmful free radicals [58]. Flavonoids have also been found to be reliable markers of environmental pollution, particularly in relation to O_3 contamination [59]. Flavonoids, a broad and common category of plant phenolics, have over 5000 unique variations divided into six main subclasses [60]. Plants have the capacity to alter the synthesis, production, secretion, and storage of secondary metabolites in response to abiotic stress factors [61]. Environmental stress may be the cause of R-adrenaline being present in plants found in industrial areas. In fact, previous research by Hughes and Wilson [62] suggests that adrenaline has antioxidative properties and can be shielded by flavonoids. Additionally, Cetinkaya *et al.* [61] noted that flavonoid levels rose in response to unfavourable circumstances. Additionally, a study by Cannac *et al.* [63] found that over the course of three months, *Pinus laricio* produced significantly more total phenolic compounds. Because of this, it is possible that total phenolic compounds can be used as bioindicators to evaluate how pine needles will respond in the short term to controlled burning. Therefore, the presence of pollution that causes stress may be the cause of the abundance of total phenolics and flavonoids seen in plants within the industrial zone [64].

    At industrial site, NOx can infiltrate cells and give rise to toxic nitrite ions (NO_2) at high concentrations, as well as nitrate ions (NO_3) that participate in nitrogen metabolism as if they were taken up by the roots. Prior research has shown that exposure to pollutant gases, particularly SO_2, causes stomata to close, protecting the leaves from additional pollutant penetration but also reducing photosynthesis [65]. An investigation by Nanos and Ilias [66] found that cement dust contamination, which has been associated to heavy metal toxicity, can harm plants' photosystems. Additionally, although transpiration is not significantly impacted, the accumulation of dust particles on the lower leaf surface may lessen the leaf's capacity to conduct water vapour and the movement of CO_2. Heavy metals can be directly taken up by leaves from the surrounding air, contingent upon factors like the leaf's adsorption capability, physical attributes, and the specific plant species. (67). *B. glabra* plants may be useful for phytoremediation in addition to their use as bioindicators due to their substantial uptake of pollutants [68]. Using plant species with a high capacity to accumulate pollutants to treat the environment are known as phytoremediation. Additionally, a number of studies have shown that specific plant species, including Cupressus sempervirens and *Pinus halepensis*, are particularly effective at monitoring atmospheric pollutants [69, 70]. These plants are useful as indicators of

environmental pollution due to their ability to absorb and accumulate xenobiotics [71].

2. **Nutrient Availability and Biomarker Signatures:** Plants require a diverse range of essential nutrients for their growth and development. The availability and uptake of these nutrients significantly impact plant health and productivity. Biomarker signatures related to nutrient availability provide valuable insights into plant nutrient status and can help optimize fertilizer management strategies. Biomarkers associated with nutrient availability can be detected at various levels, including physiological, biochemical, and molecular. At the physiological level, indicators such as leaf chlorophyll content, nutrient content ratios, and growth parameters reflect the plant's response to nutrient availability. Changes in these biomarker signatures can serve as early warning signals for nutrient deficiencies or imbalances, allowing for timely corrective measures. Biochemical markers offer a deeper understanding of nutrient availability by revealing specific metabolic processes related to nutrient uptake, transport, and assimilation. For instance, the analysis of enzyme activities involved in nitrogen assimilation pathways, such as nitrate reductase and glutamine synthetase, can indicate nitrogen availability and utilization efficiency in plants.

   Molecular markers, including gene expression patterns and metabolite profiling, provide further insights into the molecular mechanisms underlying nutrient availability. Transcriptomic analyses can identify genes involved in nutrient transporters, metabolic pathways, and regulatory networks, enabling a more comprehensive understanding of nutrient responses in plants. Metabolomic profiling allows the identification and quantification of specific metabolites associated with nutrient availability, providing a holistic view of plant nutritional status.

   By leveraging biomarker signatures related to nutrient availability, plant scientists and agronomists can optimize nutrient management strategies. These biomarkers can aid in determining the appropriate timing and dosage of fertilizers, facilitating precise nutrient application and minimizing environmental impacts. Furthermore, biomarker-guided approaches can help identify nutrient-efficient plant genotypes, contributing to the development of crop varieties with enhanced nutrient uptake and utilization efficiency.

3. **Disease Resistance and Biomarker Identification:** The interaction between hosts and pathogens is still poorly understood in terms of metabolites, more research is required to understand how stress affects the plant system. In addition to the significance of secondary metabolites in the fight against pathogens, the role of primary metabolism must also be considered because it controls defence responses in plants in the presence of potential pathogens or pathogen-derived elicitors in addition to serving as an energy source [72]. Primary metabolism is crucial to produce energy, but it also plays a role in producing secondary metabolites, PR protein building blocks, and elements of the defence signalling cascade [72].Especially, it was found that the expression of genes linked to mechanisms for energy production increased and the expression of genes linked to assimilatory processes decreased [73]. Specific Arabidopsis genes involved in primary metabolism were found to be regulated in distinct ways by Less *et al.* (2011) [73] in response to biotic and abiotic stresses. Changes in primary compounds were discovered by Chitarrini *et al*. in 2017 [74], with proline showing an especially intriguing

modulation. Salicylic acid-mediated resistance in *Arabidopsis* includes both proline supply and catabolism, which contributes to cell death in response to *Pseudomonas*[75, 76]. Proline can currently be identified as a putative biomarker, but its function in the *Bianca grapevine* variety after *P. viticola* infection needs to be clarified with additional research [74].A group of substances known as lipids are distinguished by their diverse structural makeup and complex makeup. They are essential parts of plant cell membranes and offer the necessary energy to power metabolic processes. After *P. viticola* infection, Chitarrini *et al*. (2017) [74] observed changes, with some unsaturated fatty acids declining more quickly at 24 hpi.Ceramides may be important as signalling molecules in the initiation of plant programmed cell death linked to defence mechanisms, according to earlier studies [77, 78]. Ceramide accumulation began in infected samples earlier than in control samples, and it continued for up to 96 hours after biotic stress induction. The pathogen then had a stronger impact on secondary metabolism, changing the volatile compounds between 48 and 96 hpi and phenolic compounds at the latest at 96 hpi. The resistant cultivar Regent can be distinguished from the susceptible *Trincadeira* by a number of phenolic substances, including phenylpropanoids and flavonoids [79]. Langcake and Pryce (1977) [80] discovered transresveratrol production in infected grapevine leaves. Trans-resveratrol has been shown to be a precursor of phytoalexins, which are fungal toxins produced by grapevine leaves in response to biotic and abiotic stress and can be used by the grapevine as a marker of pathogen resistance [81]. According to Martinez (2012) [82], benzaldehyde is regarded as a growth inhibitor, spore inhibitor, and has activity against *Botrytis cinerea* even at low concentrations. Additionally, benzaldehyde promotes the accumulation of salicylic acid, triggers the expression of PR proteins, and enhances tobacco's resistance to TMV [83]. A biomarker for preventing the growth or spread of *P. viticola*, benzaldehyde's elevated concentration in infected *Bianca* samples (roughly 1.5 times higher than the control) suggests its potential utility [74].

4. **Growth and Development Monitoring through Biomarkers:** Monitoring the growth and development of plants is essential for understanding their physiologicalprocesses and optimizing agricultural practices. Biomarkers serve as valuable indicators of plant growth and development, offering insights into various stages of plant life cycles, from germination to flowering and fruiting. Biomarkers associated with growth and development can be detected at different morphological, physiological, and molecular levels. At the morphological level, biomarkers such as shoot and root length, leaf area, and plant biomass directly measure plant growth. These biomarker signatures help assess plants' overall vigor and productivity, enabling comparisons between different genotypes or treatments. Physiological biomarkers contribute to a deeper understanding of growth and development processes by reflecting the plant's metabolic activities. For instance, measurements of photosynthetic parameters, such as chlorophyll fluorescence, stomatal conductance, and carbon assimilation rates, offer valuable insights into plant energy production and utilization.

Changes in these biomarker signatures can indicate stress responses, nutrient limitations, or growth abnormalities, allowing for timely interventions.Molecular biomarkers, including gene expression patterns and hormone profiling, provide a molecular perspective on growth and development processes. Transcriptomic analyses can identify genes involved in cell division, elongation, and differentiation, shedding

light on the molecular pathways governing plant growth. Hormone profiling helps unravel the intricate regulatory networks controlling growth and development, as hormones play critical roles in modulating plant growth responses. By utilizing biomarkers for growth and development monitoring, researchers and farmers can make informed decisions regarding crop management. Biomarker-based assessments enable the identification of optimal growth conditions, the evaluation of treatment effects, and the selection of superior genotypes with desirable growth traits. Furthermore, biomarker-guided interventions can assist in mitigating growth limitations, optimizing resource allocation, and improving crop yields.

III. TYPES OF BIOMARKERS

1. **Biochemical Markers: Unveiling Plant Metabolic Pathways:** Biochemical markers have emerged as invaluable tools for unravelling the intricate metabolic pathways within plants. These markers, which encompass a wide range of biomolecules such as proteins, nucleic acids, metabolites, and phytohormones, provide essential insights into the dynamic nature of plant metabolism. By identifying and quantifying specific biochemical markers, researchers can decipher the interplay between various metabolic pathways and comprehensively understand plant physiological processes. Proteins are fundamental components of plant metabolism and serve as key players in catalyzing biochemical reactions. Biochemical markers, such as enzymes and transcription factors, can be used to elucidate specific metabolic pathways. For instance, the presence or absence of specific enzymes can indicate the activation or suppression of particular metabolic processes. By monitoring these markers' activity or expression levels, researchers can identify critical steps within metabolic pathways and assess their regulation. Metabolites, on the other hand, provide a snapshot of the metabolic state of a plant. These small molecules serve as intermediates or end products of biochemical reactions and can be analyzed to infer the activity of specific metabolic pathways. By quantifying metabolites using techniques like mass spectrometry or nuclear magnetic resonance spectroscopy, researchers can map out metabolic networks and identify key metabolites associated with specific plant responses, such as stress tolerance or nutrient uptake.

   Phytohormones, including auxins, cytokinins, gibberellins, and abscisic acid, regulate various aspects of plant growth, development, and stress responses. Biochemical markers can be employed to measure the levels of these phytohormones, providing insights into their biosynthesis, transport, and signaling pathways. Researchers can unravel the complex regulatory mechanisms underlying plant physiological processes by tracking the dynamics of phytohormone levels in response to different environmental stimuli or genetic modifications.

2. **Molecular Markers: Revealing Genetic Variation and Expression Patterns:** It is crucial to have knowledge of genetic diversity in order to optimise conservation and utilisation strategies. With the help of new molecular tools, it may be possible to identify the genes responsible for a variety of traits, including adaptive traits and polymorphisms that result in functional genetic variation (QTN, or quantitative trait nucleotides). A fundamental evaluation of the functional gene variants present in a specific person or population is provided by phenotypic characterization. Examining polymorphisms using unidentified molecular genetic markers is one of the quickest and most affordable ways to

measure genetic diversity. These techniques are used to supplement existing data or in the absence of precise phenotype and QTN (Quantitative Trait Nucleotide) data. Assuming that distinct populations with a specific evolutionary history at the neutral markers (for example, because of ancient isolation or independent domestication) are likely to carry particular variants of functional variations, anonymous markers are likely to provide indirect information on functional genes for significant traits. Molecular techniques have proven useful in examining species' origin, domestication, and subsequent movements, identifying regions of intermingling among populations with different genetic backgrounds, and drawing evolutionary connections through phylogenetic trees. Molecular methods may offer a promising alternative for complex, difficult-to-observe characteristics like adaptation and disease resistance. Molecular markers have eliminated the limitations of morphological, chromosomal, and protein markers, and they also have special genetic characteristics that make them more useful than other genetic markers. They are numerous and widely dispersed across the genome.

Genetic diversity is a fundamental component of biological diversity and has importance for conservation efforts [84, 85]. Genetic diversity in plants affects levels of overall biodiversity. Global populations would struggle to adapt to and withstand environmental changes in the absence of genetic diversity. Genetic studies are useful for preserving germplasm, identifying populations and varieties, and finding alleles that might help an organism adapt to changing environmental conditions. The most popular genetic markers are called molecular markers, and they include a variety of DNA molecular markers that can be used to analyse genetic variations. These markers can contain expressed and unexpressed sequences and are inherited for dominance and codominance. The genetic variations within populations have been studied using these markers, which have been used and are ideal [86]. Using various molecular markers, variations in DNA sequences within and between plant species have also been discovered [87].

It would be effective to resolve the genomic differentiation patterns that morphological-dependent taxonomic classifications could reveal by systematically analyzing the molecular genetic data in the germplasm. The breeders can benefit from the information provided by the molecular genetic variation data sets on the allelic richness, population structure, and diversity parameters of germplasm, which can help them use genetic resources more efficiently while reducing the number of pre-breeding activities for cultivar growth and improvement. Germplasm characterization based on molecular markers has grown significantly in importance recently [88] as a result of the high quality and speed of data generated.

IV. DETECTION AND QUANTIFICATION OF BIOMARKERS

1. **Traditional Methods for Biomarker Detection:** Abiotic stress is the main cause of decreased plant productivity, so it is essential to quickly identify and treat the stress to prevent long-term damage to plant health and productivity. The limitations of conventional agricultural methods are brought on by expensive equipment and time-consuming sample processing. Initially, paper chromatography or thin-layer chromatography were combined with a bioassay, which was used as a reporter system, in order to detect plant biomarkers [89]. This strategy, though, is not very specific. In earlier studies, after substances were separated using paper chromatography or thin-layer

chromatography, specific colour reactions were used to detect substances like IAA and its related compounds [90]. A combination of these methods and high-performance liquid chromatography (HPLC) or high-performance thin-layer chromatography (HPTLC) is now preferred to the use of single methods alone for detecting IAA. Endogenous auxin has been measured using immunological methods such as radioimmunoassay [91] and immunocytochemistry [92]. However, due to their unreliable test results and expensive antibody requirements, these techniques are not widely used. Other phytohormones like cis-jasmone and indole-3-butyric acid (IBA) have also been analysed using electrochemical methods [93, 94, 95, 96] The oxidation behaviour of plant hormones in suitable media is the basis for the detection principle. However, there are some limitations to the use of electrochemical sensors for the detection of plant biomarkers. The materials used for electrode modification lack effective electrocatalytic properties, some biomarkers have low or no electrochemical activity, and the electrode surface is vulnerable to contamination from biomarker electrooxidation and electropolymerization [97,98,99, 100, 101, 102].

2. **Advanced Techniques: Metabolomics and Proteomics:** Metabolomics and proteomics are powerful analytical approaches that have revolutionized the field of plant research by providing comprehensive insights into the biochemical and molecular composition of plants. These advanced techniques offer unique advantages in understanding plant metabolism, identifying biomarkers, and unravelling complex biological processes. Metabolomics focuses on the comprehensive analysis of small molecules, including metabolites, in a biological system. By employing techniques such as mass spectrometry and nuclear magnetic resonance spectroscopy, metabolomics enables the identification and quantification of a wide range of metabolites present in plants. This holistic approach allows researchers to capture the dynamic metabolic profiles and pathways in response to various environmental cues, stress conditions, or genetic modifications. Metabolomics offers invaluable information on the physiological status of plants, metabolic shifts, and the impact of external factors on plant metabolism. Proteomics, on the other hand, aims to characterize the entire complement of proteins expressed in a given biological system. Through high-throughput techniques such as liquid chromatography and mass spectrometry, proteomics enables the identification, quantification, and functional analysis of proteins. By examining protein expression patterns, post-translational modifications, and protein-protein interactions, proteomics provides insights into the molecular mechanisms underlying plant responses to different stimuli. It facilitates the discovery of novel proteins, identification of protein networks, and understanding of protein functions in diverse biological processes, including stress responses, growth, and development.

Both metabolomics and proteomics complement each other in unravelling the complex interactions between genotype, phenotype, and the environment. Integrating metabolomics and proteomics data allows for a comprehensive understanding of plant biology and the identification of key biomarkers or molecular targets associated with specific traits or responses. These techniques have been instrumental in identifying biomarkers related to stress tolerance, nutrient utilization, and developmental processes. Moreover, metabolomics and proteomics provide essential insights into the metabolic pathways and molecular networks that can be targeted for crop improvement, precision agriculture, and the development of sustainable farming practices.

V. BIOMARKER DISCOVERY AND ADVANCEMENTS

1. **Omics-based Approaches: Expanding the Biomarker Repertoire:** Omics-based approaches, including genomics, transcriptomics, and proteomics, have revolutionized the field of biomarker discovery in plants. These comprehensive and high-throughput techniques have significantly expanded the repertoire of biomarkers available for studying plant biology and understanding their responses to various environmental conditions and stressors. Genomics, the study of an organism's entire set of genes, provides a foundation for identifying genetic markers associated with specific traits or responses. Through techniques such as DNA sequencing and genome-wide association studies (GWAS), genomics enables the identification of genetic variations or polymorphisms that are linked to desirable traits, such as stress tolerance, disease resistance, or high yield. These genetic markers serve as valuable biomarkers for marker-assisted selection and breeding programs, facilitating the development of improved plant varieties. Transcriptomics, on the other hand, focuses on studying the complete set of RNA transcripts in a given organism. This omics approach, using techniques like RNA sequencing, allows researchers to identify and quantify gene expression levels in response to different stimuli or developmental stages. By comparing transcriptomic profiles, researchers can identify differentially expressed genes that serve as biomarkers for specific biological processes or stress responses. Transcriptomics also aids in understanding the regulatory networks and molecular pathways underlying plant development and stress adaptation.

Proteomics, the study of all proteins expressed in a cell or tissue, provides insights into the functional aspects of genes and gene products. Using techniques such as mass spectrometry, proteomics allows for the identification and quantification of proteins and their post-translational modifications. By comparing proteomic profiles under different conditions, researchers can identify protein biomarkers associated with stress tolerance, metabolic pathways, and other important biological processes. Proteomics also sheds light on protein-protein interactions and signalling cascades, providing a deeper understanding of the molecular mechanisms underlying plant responses to environmental cues.These omics-based approaches offer complementary insights into the plant's molecular processes, bridging the gap between genotype and phenotype.

By integrating genomics, transcriptomics, and proteomics data, researchers can uncover key biomarkers, regulatory networks, and molecular mechanisms that govern plant responses and traits. This expanded biomarker repertoire enhances our ability to monitor and manipulate plant responses to optimize growth, stress tolerance, and agricultural productivity.

Omics-based approaches have greatly expanded the biomarker repertoire in plant research. Genomics, transcriptomics, and proteomics provide comprehensive insights into the genetic, transcriptional, and protein-level dynamics within plants, enabling the discovery of biomarkers associated with specific traits or responses. These techniques enhance our understanding of plant biology and offer valuable tools for improving plant performance, breeding, and the development of sustainable agriculture practices.

2. **Emerging Technologies in Biomarker Research:** Nuclear magnetic resonance (NMR), gas or liquid chromatography (L/GC), mass spectrometry (MS), and other cutting-edge, high-throughput separatory techniques are used in plant metabolomics analysis to detect, identify, and assess the complex plant metabolome. The two most popular techniques are LCMS and GCMS because of their excellent sensitivity, selectivity, robustness, and reproducibility. High-performance liquid chromatography (HPLC) and ultra-high-performance liquid chromatography (UHPLC), which offers high selectivity and resolution along with competent mass spectrometry, have become the recognised standard for metabolomics studies, metabolite profiling, and analysis [103]. Furthermore, secondary metabolites like phenolics, vitamins, and glucosinolates are compatible with LCMS-based metabolite profiling, along with higher-molecular-weight, polar, and thermo-labile compounds [104, 105]. Although the preparation of samples for primary metabolites such as organic acids, amino acids, sugars, and low-molecular-weight hydrocarbons frequently requires chemical derivatization, GCMS is an excellent tool for identifying volatile and thermally sensitive compounds [104, 105]. However, NMR gives information on the structural components of unidentified metabolites, is highly accurate, only requires small sample volumes, and is therefore simple to prepare [106, 107]. It is also non-destructive and doesn't require chromatographic or hyphenation techniques to be separatory. The coverage of the plant metabolome is less than MS due to the low resolution and sensitivity of this method. However, NMR-based metabolomics is a practical, quick, and highly effective tool for plant metabolomics in identifying similar samples and mapping biological pathways [104, 108]. The sample and target metabolites (polar/nonpolar, volatility) under investigation or the analytical tool's accuracy, selectivity, and sensitivity typically determine the choice of a metabolomics approach. High-performance thin-layer chromatography (HPTLC), direct-infusion mass spectrometry (HPTLC), Fourier transform ion cyclotron resonance mass spectrometry (FI-ICR-MS), and capillary electrophoresis-mass spectrometry (CE-MS) are additional methods in metabolomics [104, 105, 109, 110]. It is now possible to observe plant responses and metabolic changes in response to both biotic [111, 112] and abiotic stress using these techniques [113, 114].

VI. IMPLICATIONS OF BIOMARKERS IN PLANT BREEDING AND AGRICULTURE

1. **Precision Agriculture and Biomarker Applications:** The advancement of precision agricultural techniques has become crucial for addressing the current issues with food security. By 2050, this technology can reduce the demands of the expanding population by increasing agricultural production by 70% [115, 116]. Precision agriculture necessitates using sensors that can convey details about crop health, ideally on an individual level [116], to achieve high-quality monitoring [117, 118]. However, spectral imaging techniques have dominated the field of implantable sensors in crops, frequently requiring the pre-implantation of nanomaterials like carbon nanotubes and determining the changes in fluorescence [119]. These sensors typically cannot detect complex reactive oxygen species (ROS), such as H_2O_2 or nitroaromatics [120], and must use expensive optical equipment to image the implanted nanoparticles, which limits their usefulness in real-world settings.

Electrochemical technologies have made it possible to create implantable sensors that can continuously monitor biomarkers in plants at a low cost. These techniques could be applied to a wide variety of analytes and applications, such as the monitoring of hormone fluxes in roots using self-referenced microelectrodes made of carbon nanotubes [121]. Transistor-based devices have recently made it possible to monitor the relative concentrations of some leaf biomarkers, including glucose and fructose [122]. Due to the dearth of appropriate materials for their detection, the detection of single ions like H^+ still poses a challenge in the field of implanted sensors in plants.

A promising strategy to deal with the early diagnosis of plant diseases is to measure the pH inside plant directly stems. Increase in plasma cell acidity have been seen in Arabidopsis in consequence of infections caused by *Fusarium oxysporum*[123]. According to research on tomato plants, xylem pH may change as a result of transpiration [124], nitrogen sources [125], and a slight increase in nighttime extracts as compared to daytime extracts [126]. As a result, xylem pH represents a potent multimodal biomarker that may be used to track the physiological status of plants.Cost-effective sensors enable accurate plant disease diagnosis by combining *invivo* pH data with environmental parameters, such as temperature, humidity, and other elements. Given their high sensitivity, rapid response, and long lifetime, metal oxide-based sensors have attracted much attention in recent years for pH measurement [127]. Due to their ability to preferentially interact with H^+ ions and produce a near-Nernstian potentiometric reaction, iridium and ruthenium oxide substances have attracted attention in recent years [128]. One of the most popular pH-sensing materials today is iridium oxide, which also exhibits good biocompatibility and is suitable for in vivo studies [127, 129]. Additionally, the equipment needed for the fabrication, characterization, and testing in practical applications is frequently quite expensive, which restricts its applicability in low-resource environments. As a result, innovative methods for the creation of affordable and reliable pH sensing devices are required to make it possible to monitor plant health for intelligent agricultural applications.

2. **Sustainable Farming Practices Enhanced by Biomarkers:** Biomarkers play a pivotal role in enhancing sustainable farming practices by providing valuable insights into plant health, resource management, and environmental impact. These powerful tools enable farmers and researchers to optimize agricultural practices, reduce resource wastage, and minimize the use of agrochemicals, promoting environmentally friendly and economically viable farming methods. Biomarkers related to plant health and stress responses contribute to sustainable farming by enabling early detection and prevention of diseases, pests, and nutrient deficiencies. By monitoring biomarker signatures associated with plant vigor, nutrient status, and stress tolerance, farmers can make informed decisions regarding the application of fertilizers, irrigation, and pest management strategies. This targeted approach minimizes unnecessary resource use, reduces environmental pollution, and maximizes crop productivity. Additionally, biomarkers facilitate precision agriculture, a key component of sustainable farming. By analyzing biomarker data, farmers can map spatial variability in crop growth, nutrient distribution, and water needs within a field. This information allows for site-specific management interventions, such as variable-rate fertilization and irrigation, tailored to the unique needs of different crop zones. Precision agriculture optimizes resource allocation, reduces input costs, and minimizes the environmental impact of agricultural practices.

Biomarkers also contribute to the development of resilient and climate-smart crop varieties. By identifying biomarkers associated with stress tolerance, such as drought, heat, or salinity, researchers can select and breed crops with enhanced resilience to adverse environmental conditions. This leads to increased crop productivity and stability in the face of climate change, reducing the reliance on unsustainable agricultural practices and mitigating food security risks. Furthermore, biomarkers enable the monitoring and assessment of soil health and microbial activity. Biomarker-based analyses can evaluate soil fertility, nutrient cycling, and the presence of beneficial or harmful microorganisms. This information guides sustainable soil management practices, such as organic amendments, cover cropping, and conservation tillage, which promote soil health, biodiversity, and carbon sequestration.

VII. STRATEGIES TO MITIGATE ABIOTIC STRESS

Abiotic stresses on plants include low and high temperatures, salinity, and drought. The genetic potential of plants is inhibited by these abiotic stresses, which leads to a significant decline in crop productivity, a reduction in yields of vital crop plants of more than 50%, and an imbalance in the sustainability of agriculture. They affect the physio-morphological, molecular, and biochemical makeup of the plants as well as alter their normal metabolism, making them a major contributor to decreases in crop productivity. These modifications to plant systems also aid in reducing abiotic stress situations. When a plant is under stress, its sensor molecules detect the external stress signal and start a signalling cascade that sends a message and activates nuclear transcription factors to cause a particular gene to express. Plants have a number of mechanisms for avoiding, adapting, and acclimating to abiotic stress. In addition to these, plants have a number of tolerance mechanisms for dealing with stress conditions. These involve ion transporters, osmoprotectants, proteins, and other transcriptional control factors. Signalling cascades are also stimulated to counteract the biochemical and molecular changes brought on by abiotic stress. The capacity to react to a stress stimulus, to produce a signal, and to initiate the necessary biochemical and physiological changes is essential for plant growth and survival. Primary signals for ion toxicity detection, low proline and chlorophyll content, low CO_2 assimilation, and osmotic effects, among other things, are present in the cells of plants under abiotic stress. The complicated secondary effects of these abiotic stresses include oxidative stresses that damage different cellular components like nucleic acids, proteins found in membranes and lipids, and metabolite malfunction. As a result, various abiotic stresses produce distinct and overlapping signals [130]. Water potential homeostasis and ion distribution are impacted by salt stress and drought at the cellular and molecular levels. Growth inhibition, molecular harm, and even demise can result from changes in water and ion homeostasis [130]. Some cellular reactions are triggered by primary stress signals, while the remainder are triggered by secondary stress signals. The hyperosmotic signal raises phytochrome and abscisic acid levels in plants, providing protection against a variety of abiotic stresses like salt stress and drought [130]. When under cold or chilling stress, plants initially show changes in cell membrane architecture that have an effect on their growth. These changes then result in protein or protein complex instability and decreased ROS scavenging enzyme activity. These mechanisms result in severe membrane damage, reduced photosynthesis, and photoinhibition [130–131]. Stress also activates gene expression and protein synthesis by causing the formation of secondary RNA structures [132]. All of these aspects of plant activity are essential for stress tolerance in order to reduce internal damage in the new stress

environment, allowing for the restoration of homeostatic conditions and growth, albeit at a slower rate [133]. Plant proteomes, transcriptomes, and metabolomes change as a result of altered gene expression brought on by the recognition of a stressful environment. The way that plants react to various forms of abiotic stress is not a straightforward process; rather, it is a complex integrated circuit made up of a number of pathways, precise tissue and cellular compartments, and interactions with other cofactors, as well as signalling molecules for managing a specific response to a given stimulus. Thiourea (TU), a synthetic plant growth regulator with 36% nitrogen and a 42% sulphur composition, has attracted much attention for its role in plant stress tolerance. Some of the pathways involved in plants' resistance to abiotic stress are modulated by thiourea. Crop production under stress conditions may be increased by comprehending the processes that take place during TU-induced tolerance [134]. E3-ubiquitin ligases regulate positive or negative abiotic stress responses. Furthermore, the specific target protein and the outcomes of UPS-mediated breakdown, activity control, or relocation are determined by the involvement of plant ubiquitin ligases-E3 enzymes in the response to abiotic stress. In order to better understand stress responses, it is important to clarify and define the goals of ubiquitin ligases [135]. These complex mechanisms involve many steps including sensing, signal initiation, transcription, transcript processing, translation, and posttranslational modifications. Recent advances in our understanding of these mechanisms' molecular intricacies in plants' adaptation to abiotic stress situations have highlighted the complexity these mechanisms. Crop production and agricultural sustainability are improved by increasing knowledge of and using various strategies, including genetic, chemical, and microbial techniques [136].

VIII. CONCLUSION

The exploration of biomarkers as powerful tools for studying plant performance has demonstrated their immense potential in unravelling the complexities of plant biology. By identifying and quantifying specific biomolecules, researchers can gain valuable insights into plant physiology, stress responses, and adaptation mechanisms. Biomarkers offer non-invasive means of monitoring plant health, assessing agricultural interventions, and optimizing crop production. While significant progress has been made in biomarker discovery and analysis techniques, challenges remain in terms of identification, validation, and implementation. However, with ongoing technological and research advancements, biomarkers are promising for advancing plant research, breeding programs, and precision agriculture. Integrating biomarkers into plant science can revolutionize agricultural practices, leading to improved crop productivity, enhanced stress tolerance, and sustainable food production. As we continue to unlock the potential of biomarkers, their application in the field of plant biology is expected to expand, ultimately contributing to a greener and more resilient future.

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