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RECENT DEVELOPMENTS IN ELECTROCHEMICAL BIOSENSORS FOR CHLOROPHYLL AND PHOTOSYNTHESIS-RELATED METABOLITE DETECTION

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Abstract

Photosynthesis underpins primary productivity, food security and the global carbon and oxygen cycles, and the molecules that drive or report on it chlorophylls, the photosystem II reaction centre, plastoquinone, reactive oxygen species, reduced nicotinamide cofactors, soluble sugars, phytohormones and carotenoids constitute a uniquely informative panel of physiological biomarkers. Conventional analysis of these analytes relies on chromatography, spectrophotometry and fluorometry, which are accurate but laboratory-bound, destructive and ill-suited to continuous or in-field monitoring. Electrochemical biosensors offer a compelling alternative: they are intrinsically sensitive, rapid, low-cost, maintainable and amenable to in vivo and wearable deployment. This review surveys recent progress in electrochemical biosensing of chlorophyll and photosynthesis-related metabolites, organized around the molecular targets of the photosynthetic apparatus. We first outline the fundamentals of electrochemical transduction relevant to these analytes, including voltametric, amperometric and impedimetric methods and the performance metrics that govern analytical quality. We then examine direct voltametric detection of chlorophylls and pigments; whole-cell, thylakoid and photosystem II based bioreceptors that exploit the herbicide-sensitive electron-transport chain; and sensors for the key downstream metabolites hydrogen peroxide, NADH/NADPH, glucose and sucrose, the phytohormones indole-3-acetic acid and salicylic acid, and carotenoids. A dedicated section addresses the nanomaterials and electrode-engineering strategies graphene and laser-induced graphene, carbon nanotubes, MXenes, noble-metal and metal-oxide nanoparticles, metal-organic frameworks and conducting polymers that have driven the most significant gains in sensitivity and stability. Representative analytical figures of merit, recognition elements and detection limits are tabulated. We close by discussing applications in precision agriculture, environmental monitoring and

food quality control, and by identifying the principal challenges long-term in vivo stability, selectivity in complex matrices, calibration and standardization, and integration with wireless data platforms that must be resolved for these devices to mature from laboratory demonstrations into deployable tools.

INTRODUCTION

Photosynthesis is the biochemical engine of the biosphere. Photosynthetic organisms produce the chemical energy and fixed carbon that almost all terrestrial and aquatic food webs rely on through the light-driven oxidation of water and the reductive fixation of carbon dioxide. At the same time, they release the molecular oxygen necessary for aerobic life. Therefore, agricultural yield, ecosystem function, and the global carbon balance are closely related to the efficiency and integrity of the photosynthetic process. The capacity to track photosynthetic performance and plant physiological state in real time has become a strategic objective for sustainable food production as the world's population approaches ten billion and climate change increases abiotic and biotic stressors on agricultural systems. [3,4]. Precision farming, environmental monitoring, and our fundamental knowledge of plant stress physiology would all be transformed by instruments that could continually, non-destructively, and in the field report on the condition of the photosynthetic system. [5,6].

A tremendously revealing panel of biomarkers is formed by the molecular components and products of photosynthesis. The two main pigments that absorb light are chlorophyll a and chlorophyll b, and their ratio and concentration are sensitive markers of stress, senescence, nutritional deficit, and nitrogen status. [1]. Many herbicides act at the molecular location of the photosystem II (PSII) reaction center, which has an oxygen-evolving complex and the D1 protein that binds the plastoquinone acceptors. As a result, PSII activity is a direct indicator of both herbicide exposure and photosynthetic capability. [6,11]. Downstream of the electron-transport chain, the cell generates and consumes a suite of small molecules whose dynamics encode physiological information: reactive oxygen species such as hydrogen peroxide signal oxidative stress and pathogen attack [22,24]; the reduced cofactors NADH and NADPH report on redox poise and metabolic flux [26]; soluble sugars produced by carbon fixation are transported through the phloem and xylem and reflect source-sink relationships [27]; phytohormones such as indole-3-acetic acid (auxin) and salicylic acid orchestrate growth and defence [29,32]; and the carotenoids provide photoprotection and antioxidant capacity [38]. Quantifying any of these species in living tissue provides a window onto the state of the photosynthetic machinery.

For these objectives, traditional analytical methods are well-established. Immunoassays, mass spectrometry, ultraviolet-visible spectrophotometry, high-performance liquid chromatography, and chlorophyll-fluorescence imaging provide precise, frequently very exact measurement. However, these techniques have similar drawbacks that limit how they can be used for physiological monitoring. They typically necessitate sample collection, extraction, and laboratory equipment; they are often destructive, making it impossible to measure the same tissue repeatedly; they offer snapshots rather than continuous traces; and their expense, size, and requirement for skilled operators make them unsuitable for dispersed in-field deployment. [3,9]. There is consequently a strong incentive to develop measurement platforms that are sensitive, selective, rapid, inexpensive, portable and capable of operating in or on living systems.

Many of these criteria are satisfied by electrochemical biosensors. They combine minimal instrumental complexity with excellent sensitivity by converting a faradaic reaction or biochemical recognition event into an electrical output. Microelectrodes, microneedles, and screen-printed devices that can be worn on a leaf or implanted into tissue are made possible by electrochemical readout's inherent compatibility with miniaturization and microfabrication [3, 4]. Since the signal is electrical from the start, integrating it with wireless telemetry, portable potentiostats, and data-analytics pipelines is made easier. [41,42]. Detection limits in the nanomolar and even picomolar range are routinely achievable, and the cost of disposable electrodes can be very low. These attributes have made

electrochemistry a natural choice for the emerging fields of plant bioelectronics and wearable phytosensing [2,3].

The marriage of electrochemistry with photosynthesis research has followed two broad strategies. In the first, the analyte is detected directly through its own electroactivity: chlorophylls, carotenoids, NADH, hydrogen peroxide and the phenolic and indolic phytohormones all possess oxidisable or reducible moieties that yield characteristic voltammetric or amperometric signals, often enhanced by catalytic electrode modification [8,10,26]. In the second, the photosynthetic apparatus itself isolated PSII complexes, thylakoid membranes, or whole algal and cyanobacterial cells is immobilised on the electrode and used as the biorecognition element, exploiting the fact that photosynthetic electron transport produces a measurable photocurrent that is modulated by inhibitors and environmental factors [11,12,19]. The first strategy excels at quantifying defined metabolites; the second provides an integrated, functional readout of photosynthetic activity and is especially powerful for herbicide and toxicity sensing.

This review brings these threads together. Our aim is to provide a structured, up-to-date account of electrochemical biosensors for chlorophyll and photosynthesis-related metabolites, organised by molecular target and by transduction strategy, and to highlight the materials innovations that have most advanced the field. Section 2 summarises the electrochemical fundamentals and figures of merit needed to interpret the literature. Section 3 treats the direct electrochemical detection of chlorophylls and the closely related pigments. Section 4 examines PSII, thylakoid and whole-cell bioreceptors and the photocurrent-based sensing they enableThe review's analytical center, Section 5, examines sensors for the main metabolites linked to photosynthesis, including sugars, carotenoids, hydrogen peroxide and reactive oxygen species, NADH and NADPH, and phytohormones. Nanomaterials and electrode engineering are covered in Section 6. Before Section 9's closing remarks, Section 7 examines applications in precision agriculture, environmental monitoring, and food quality. Section 8 outlines the remaining issues and potential paths forward. The conceptual linkages between targets, recognition elements, and transduction schemes are depicted in three figures, and typical performance data is compiled in three tables.

Figure 1. The photosynthetic electron-transport chain and the metabolites targeted by electrochemical biosensors

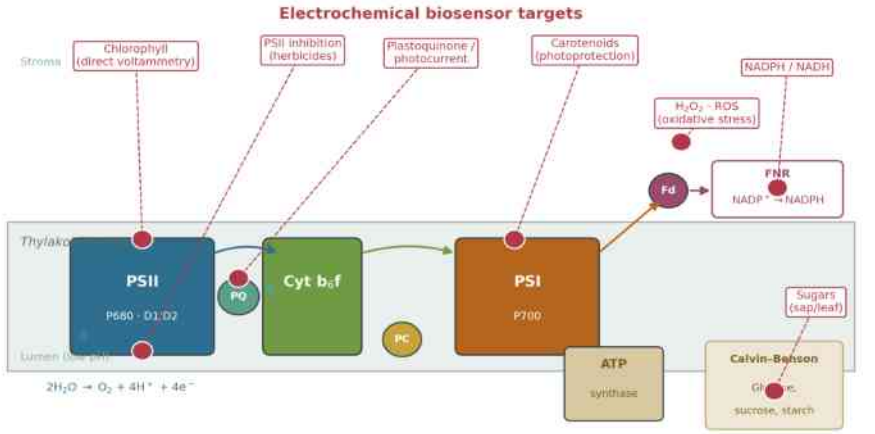


Figure 1. The photosynthetic electron-transport chain and the metabolites targeted by electrochemical biosensors. Light-driven electron flow proceeds from water oxidation at photosystem II (PSII) through the plastoquinone (PQ) pool, the cytochrome b6f complex and plastocyanin (PC) to photosystem I (PSI), ferredoxin (Fd) and the reduction of NADP+ to NADPH, with concomitant proton translocation driving ATP synthesis and carbon fixation in the Calvin-Benson cycle. Red callouts mark the principal analytes addressed in this review and the points in the apparatus to which they relate.

2. Fundamentals of Electrochemical Biosensing

A biological or biomimetic recognition element combined with an electrochemical transducer that transforms the recognition event into a detectable current, potential, charge, or impedance is known as an electrochemical biosensor. Comparing the various devices discussed in the next sections requires an understanding of the primary transduction types and the metrics used to evaluate sensor performance.

2.1 Electrochemical transduction modes

Voltammetric techniques, in which the current is recorded as the applied potential is scanned, are the workhorses of analytical electrochemistry. Cyclic voltammetry (CV) sweeps the potential linearly back and forth and is used chiefly for mechanistic characterisation, revealing the formal potentials, reversibility and electron-transfer kinetics of a redox system. For quantification, pulsed techniques that suppress non-faradaic charging current are preferred. Differential pulse voltammetry (DPV) and square-wave voltammetry (SWV) superimpose potential pulses on a staircase or linear ramp and sample the current at times that maximise the faradaic-to-capacitive ratio, delivering sharp, well-resolved peaks and low detection limits; SWV additionally offers high speed and the ability to resolve closely spaced redox processes [8]. These methods have been used to quantify chlorophylls, carotenoids, NADH and the phenolic and indolic phytohormones through their intrinsic oxidation peaks [8,10,32].

Amperometry measures the steady-state or transient current at a fixed applied potential and is the method of choice for continuous and in vivo monitoring because it provides a direct, real-time current proportional to analyte flux. Chronoamperometric detection of hydrogen peroxide at catalytic electrodes, and amperometric tracking of photosynthetic electron transport at PSII- or thylakoid-modified electrodes, are central examples [13,23,25]. Amperometric operation underlies most microneedle and microsensor implementations, where a constant potential is applied and the current is logged over minutes to hours [23,35].

Electrochemical impedance spectroscopy (EIS) probes the interfacial properties of the electrode by applying a small sinusoidal perturbation over a range of frequencies and measuring the complex impedance. Because the charge-transfer resistance at the electrode-solution interface is exquisitely sensitive to the binding of analytes or the formation of immunocomplexes, EIS is widely used in label-free affinity biosensors, including immunosensors for phytohormones, where antigen-antibody binding increases the interfacial resistance in a concentration-dependent manner [29]. Potentiometric and conductometric modes, and the field-effect transduction of organic electrochemical transistors (OECTs), round out the toolkit; OECTs in particular have proven transformative for in planta sugar sensing because their inherent signal amplification yields large responses from soft, biocompatible, implantable devices [27,28].

2.2 Recognition elements

The selectivity of a biosensor is conferred by its recognition element. Enzymes horseradish peroxidase for hydrogen peroxide, dehydrogenases coupled to NAD+, oxidases for sugars and carotenoids provide catalytic specificity and signal amplification and are the most common biorecognition components [24,26]. Antibodies furnish high-affinity, high-specificity binding for affinity sensors and immunosensors, as exploited for auxin and the herbicide chlortoluron [29,43]. Distinctively for this field, intact photosynthetic machinery serves as the recognition element in a large class of sensors: isolated PSII core complexes, thylakoid membranes and whole microalgal or cyanobacterial cells transduce the inhibition of photosynthetic electron transport into a measurable change in photocurrent, providing a functional rather than a molecular readout [11,12,16]. Finally, many analytes require no biological recognition element at all and are detected directly at bare or nanomaterial-modified electrodes through their own electroactivity; such non-enzymatic and reagentless designs are prized for their robustness and long shelf life [8,30].

2.3 Performance metrics

Several figures of merit govern the analytical quality of a sensor and recur throughout this review. The limit of detection (LOD), conventionally taken as the analyte concentration giving a signal three times the standard deviation of the blank, expresses sensitivity to trace levels; the limit of quantification is the

corresponding ten-sigma value. The linear range defines the span of concentrations over which the response is proportional to analyte level and bounds the working window of the device. Sensitivity, the slope of the calibration curve, is frequently normalised to electrode area. Selectivity describes the ability to discriminate the target from interferents ascorbic acid, uric acid and dopamine are common interferents in plant matrices and is a critical and often limiting property for in vivo measurement. Response time, reproducibility, operational and storage stability, and the capacity for reuse or for disposable single-use operation complete the picture. In the context of plant and environmental sensing, two further considerations are paramount: the degree of invasiveness, since minimally invasive microneedle and wearable formats are increasingly favoured for continuous monitoring [3,4], and the long-term stability of the biorecognition layer under field conditions, which remains a central challenge for enzyme- and photosynthesis-based devices [6].

Figure 2. Building blocks of an electrochemical biosensor for chlorophyll and photosynthesis-related metabolites

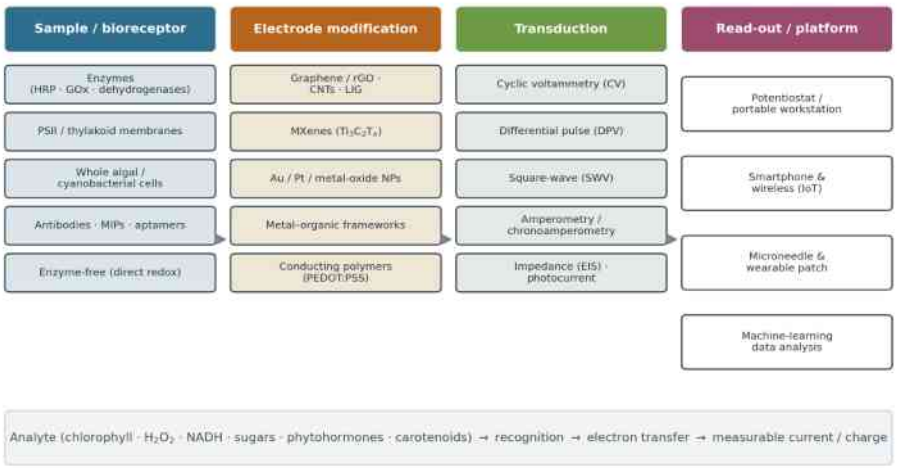


Figure 2. Generic architecture of an electrochemical biosensor for photosynthesis-related analytes. A biological sample (leaf tissue, sap, algal suspension or extract) presents the target analyte to a recognition layer (enzyme, antibody, isolated photosystem II, thylakoid membrane, whole cell, or a bare electroactive interface) immobilised on a modified electrode. The recognition event is converted by a transduction mode (voltammetry, amperometry, impedance or transistor operation) into an electrical signal that is acquired, processed and, increasingly, transmitted wirelessly to a read-out platform.

3. Direct Electrochemical Detection of Chlorophylls and Pigments

Chlorophyll is the most abundant and most diagnostic pigment of the photosynthetic apparatus,

and its direct electrochemical determination is attractive because it dispenses with biological recognition elements and the attendant problems of cost and stability. Structurally, the chlorophylls are cyclic tetrapyrroles (chlorins) chelating a central magnesium ion and bearing a long phytol tail; chlorophyll a and chlorophyll b differ only in a single substituent on ring II (a methyl group in chlorophyll a versus a formyl group in chlorophyll b). This close structural similarity gives them very nearly coincident oxidation potentials, which is the principal analytical obstacle to resolving the two pigments in a mixture [1,8]. Voltammetric detection is based on the extended, electron-rich macrocycle's electroactivity, which can be oxidized to produce a cation radical..

In a thorough description of chlorophyll as a versatile molecule for biosensing, Mandal and Dutta emphasized that the redox activity of the chlorin ring can be used to build chlorophyll-mediated sensing architectures and photo-bioelectrochemical devices in addition to quantifying the pigment itself. [1]. Building on the electroactivity of the macrocycle, Wang, Chen and Compton developed a notable

strategy for the selective voltammetric detection of chlorophyll a and chlorophyll b using a glassy carbon electrode and a semi-circular potential sweep [8]. The key innovation was the use of a non-linear, semi-circular potential waveform that generates large transient scan rates; because the two pigments oxidise at almost identical potentials but with subtly different kinetics, the transient sweep was able to discriminate species that conventional linear voltammetry cannot separate. The method quantified chlorophyll a over the range of approximately 46 to 111 micromolar and chlorophyll b over a comparable range, and was successfully applied to a real spinach extract [8]. This work is significant because it demonstrates that careful waveform engineering, rather than additional chemical recognition, can solve a selectivity problem rooted in molecular structure.

For aquatic and environmental applications, where chlorophyll a serves as the standard proxy for phytoplankton biomass and water quality, fluorometric transduction remains dominant, and the boundary between optical and electro-optical instrumentation is instructive. Zhang and colleagues reported a high-performance, versatile fluorometer for chlorophyll a monitoring that introduced a balanced maximum-entropy modulation scheme and achieved limits of detection in the nanogram-per-litre range for both a fluorescein sodium standard and spinach-extracted chlorophyll a, with configurations suited to both portable and deep-sea deployment [9]. Although fluorometric rather than faradaic, such instruments define the sensitivity benchmark against which electrochemical and electro-optical chlorophyll sensors are measured and motivate the integration of optical excitation with electrochemical read-out in hybrid devices [9,16].

The pigment most closely associated with chlorophyll in the photosynthetic membrane is the carotenoid family, which performs light harvesting and, critically, photoprotection by quenching excess excitation energy and scavenging reactive oxygen species. Carotenoids such as beta-carotene, lutein and astaxanthin are electroactive owing to their extended conjugated polyene chains and are readily oxidised at carbon and gold electrodes. Ziyatdinova and co-workers characterised the voltammetric oxidation of beta-carotene in surfactant-stabilised media, reporting irreversible oxidation peaks and a detection limit in the low micromolar range for raw vegetables and berries [10]. Because carotenoid and chlorophyll signals can overlap in lipophilic extracts, the analytical chemistry of the two pigment classes is intertwined; we return to carotenoid sensing in Section 5.5 in the context of antioxidant capacity. Table 1 collates representative electrochemical and electro-optical approaches to chlorophyll and pigment determination.

Table 1. Representative electrochemical and electro-optical approaches for the detection of chlorophylls and related pigments.

Analyte	Electrode / platform	Technique	Detection limit / range	Reference
Chlorophyll a, chlorophyll b	Bare glassy carbon electrode	Semi-circular sweep voltammetry	Linear ~46–111 µM (Chl a); spinach extract	Wang et al. [8]
Chlorophyll a (aquatic)	Optical fluorometer (modulated)	Fluorometry	~4–5 ng/L; 0–500 µg/L range	Zhang et al. [9]
β-Carotene	Glassy carbon, Triton X-100 media	Voltammetry (CV/SWV)	LOD ~2.5 µM; 10–380 µM	Ziyatdinova et al. [10]

Analyte	Electrode / platform	Technique	Detection limit / range	Reference
Carotenoids (β-carotene, lutein, astaxanthin)	Paraffin-impregnated graphite electrode	Square-wave / stripping voltammetry	Micromolar-range; aqueous media	Čižmek & Komorsky-Lovrić [38]
Chlorophyll (review of strategies)	Various; chlorophyll as redox mediator	CV, DPV, amperometry	Concept and platform overview	Mandal & Dutta [1]

4. Photosystem II, Thylakoid and Whole-Cell Bioreceptors

A distinctive and historically important class of photosynthesis biosensors uses the photosynthetic apparatus itself as the biorecognition element. The rationale is elegant: photosynthetic electron transport, when driven by light, generates a flow of electrons that can be intercepted at an electrode as a photocurrent, and any agent that perturbs that electron flow changes the photocurrent in proportion to its effect. Because a large and economically significant class of herbicides the triazines such as atrazine and simazine, and the phenylureas such as diuron and chlortoluron act precisely by binding the D1 protein of PSII at the QB site and blocking electron transfer from QA to the plastoquinone pool, PSII-based sensors provide a direct, functional assay for these compounds and, more broadly, for any factor that inhibits photosynthesis [6,11]. The result is an integrated readout of photosynthetic competence rather than the concentration of a single metabolite.

4.1 Isolated photosystem II and thylakoid-membrane sensors

The foundational demonstration of this principle was provided by Koblížek and colleagues, who coupled isolated PSII complexes from a thermophilic cyanobacterium to a screen-printed graphite electrode and operated the device in an illuminated flow cell [11]. Herbicide binding reduced the rate of light-driven photoreduction of an artificial electron acceptor, which was registered amperometrically, yielding a reusable sensor with a detection limit of approximately one nanomolar for diuron, atrazine and simazine [11]. This screen-printed, mediated-photocurrent format became the template for an entire family of devices. Touloupakis and co-workers extended it to a multi-biosensor configuration in which four photosynthetic preparations, including thylakoids from spinach and from a herbicide-resistant mutant, were immobilised on separate screen-printed electrodes within an illuminated four-cell array [12]. Because the different preparations exhibit differential sensitivity to individual herbicides, the array provided a degree of pattern-based selectivity and achieved detection limits in the range of roughly fifteen to forty nanomolar for diuron, atrazine, simazine and terbuthylazine in river water [12].

Practical deployment requires storage stability and single-use convenience, and Bettazzi and colleagues addressed this with a one-shot, screen-printed thylakoid-membrane biosensor designed for the measurement of photosynthetic inhibitors in discrete samples, demonstrating that disposable, ready-to-use formats are feasible for thylakoid-based devices [13]. In parallel, efforts to improve sensitivity and reproducibility focused on protein engineering of the recognition element. Giardi and co-workers used computational and biotechnological tools to engineer the D1 protein of Chlamydomonas reinhardtii, generating mutants with altered herbicide-binding properties that improved both the affinity and the dynamic range of the resulting optical and electrochemical biosensors [14]. This convergence of synthetic biology with biosensor design tuning the recognition element at the level of its amino-acid sequence remains one of the most promising avenues for the field.

4.2 Whole-cell algal and cyanobacterial sensors

Whole photosynthetic cells offer a self-renewing, robust alternative to isolated complexes, sacrificing some sensitivity for greatly improved stability and ease of preparation. Husu and colleagues investigated the photo-electrochemical sensing of herbicides driven by intact Chlamydomonas reinhardtii cells, showing that the cellular electron-transport machinery could be coupled to an electrode and used to detect PSII inhibitors [15]. Antonacci and co-workers advanced the whole-cell concept toward practical, low-cost devices with a dual electro-optical biosensor in which Chlamydomonas cells were immobilised on paper-based, nanomaterial-modified screen-printed electrodes; the device simultaneously reported the photosynthetic response through both optical (chlorophyll-fluorescence) and electrochemical channels, achieving very low detection limits for herbicides while exploiting the sustainability and disposability of paper substrates [16]. The same group developed an eco-designed, paper-based algal biosensor for the optical detection of nanoformulated herbicides, underscoring the suitability of these platforms for monitoring emerging agrochemical formulations [17]. A complementary transduction route was demonstrated by Attaallah and colleagues, who used carbon-black-modified electrodes to sense the oxygen evolved by algae as a proxy for photosynthetic activity, detecting atrazine through the suppression of oxygen evolution [18]. Surribas and co-workers, working at the interface of affinity and photosynthesis sensing, reported a disposable electrochemical immunosensor for the phenylurea herbicide chlortoluron, illustrating that the same herbicide targets can be approached either functionally, through PSII inhibition, or molecularly, through antibody recognition [43].

4.3 Thylakoid bioelectrodes and photocurrent harvesting

Beyond herbicide sensing, a vigorous line of research seeks to maximise the photocurrent extracted from thylakoid membranes, both for energy-harvesting biofuel cells and for more sensitive sensing. The efficiency of these devices is limited by the interfacial electron transfer between the photosynthetic membrane and the electrode, and recent work has concentrated on nanostructured electrode materials that improve this junction. Hong and colleagues modified electrodes with two-dimensional ruthenium dioxide nanosheets and reported a substantial enhancement of photosynthetic electron harvesting from thylakoids; proton adsorption and the favourable surface polarity of the nanosheets promoted thylakoid attachment and shifted the electrode potential, increasing the photocurrent severalfold relative to a gold electrode and confirming, through inhibitor studies with DCMU, that the current originated in the photosynthetic chain [19]. Kim and co-workers pursued scalability with an electrosprayed thylakoid-alginate film deposited on a micro-pillar electrode, increasing the electroactive interfacial area and the loading of photosynthetic material to boost the harvested photocurrent in a format amenable to large-area fabrication [20]. Chang and colleagues focused on the formation of supported thylakoid-membrane bioanodes engineered for effective electron transfer and stable photocurrent output, addressing the operational stability that is essential for any practical device [21]. Collectively, these advances in thylakoid bioelectrode engineering not only improve energy-harvesting figures of merit but also raise the photocurrent baseline on which inhibitor-sensing schemes depend, tightening the link between fundamental photo-bioelectrochemistry and analytical application. Table 2 summarises representative photosystem, thylakoid and whole-cell biosensors and bioelectrodes.

Table 2. Representative photosystem II, thylakoid and whole-cell electrochemical biosensors and bioelectrodes.

Bioreceptor	Transducer / electrode	Target	Performance	Reference
Isolated PSII (cyanobacterial)	Screen-printed graphite, illuminated flow cell	Triazine / phenylurea herbicides	LOD ~1 nM (diuron, atrazine, simazine)	Koblížek et al. [11]
Thylakoids (multi-preparation array)	Four screen-printed electrodes, illuminated	Herbicides in river water	LOD ~15–41 nM; pattern selectivity	Touloupakis et al. [12]
Thylakoid membrane (one-shot)	Screen-printed, disposable	Photosynthetic inhibitors	Single-use; discrete samples	Bettazzi et al. [13]
Engineered D1 (C. reinhardtii)	Optical / electrochemical	Herbicides	Improved affinity / dynamic range	Giardi et al. [14]
Whole C. reinhardtii cells	Paper-based nanomodified SPE (dual electro-optical)	Herbicides	Pico-/nanomolar LOD; disposable	Antonacci et al. [16]
Whole algal cells	Carbon-black-modified electrode	Atrazine (via O2 evolution)	Suppression of O2 evolution	Attaallah et al. [18]
Thylakoids	RuO2 nanosheet electrode	Photocurrent harvesting	Several-fold photocurrent increase	Hong et al. [19]
Thylakoid-alginate film	Electrosprayed micro-pillar electrode	Photocurrent harvesting	Scalable; increased loading	Kim et al. [20]

5. Sensors for Photosynthesis-Related Metabolites

Downstream of light capture and electron transport, the photosynthetic cell produces and turns over a suite of small molecules whose concentrations encode rich physiological information. This section surveys electrochemical sensors for the most informative of these analytes hydrogen peroxide and reactive oxygen species, the reduced cofactors NADH and NADPH, the soluble sugars, the phytohormones auxin and salicylic acid, and the carotenoids with emphasis on devices capable of in vivo or near-real-time operation.

5.1 Hydrogen peroxide and reactive oxygen species

Hydrogen peroxide occupies a central position in plant physiology as both a damaging by-product of aerobic and photosynthetic metabolism and a versatile signalling molecule that mediates responses to pathogens, wounding, drought, salinity and heat. Its concentration rises sharply during the oxidative burst that accompanies pathogen recognition, making it a sensitive early reporter of biotic and abiotic stress, and its small size and electroactivity make it an ideal target for electrochemical detection [22,24]. The analytical challenge is to measure sub-micromolar fluxes in living tissue without inducing the very stress one seeks to observe.

A range of catalytic strategies has been developed to detect hydrogen peroxide at low overpotential. Mourzina and colleagues constructed a sensor based on a manganese porphyrin combined with electrochemically reduced graphene oxide, achieving a sub-micromolar detection limit and demonstrating in vivo measurement in plant leaves, with the metalloporphyrin providing biomimetic catalytic reduction of peroxide and the graphene support

enhancing conductivity and electron transfer [22]. For minimally invasive in planta monitoring, microsensor geometries have proven especially powerful. Huo and co-workers fabricated a disposable stainless-steel-wire microsensor modified with gold nanoparticles and used it for continuous in vivo monitoring of hydrogen peroxide in the vein of a tomato leaf, detecting concentrations from ten to one thousand micromolar and resolving the rise in peroxide induced by high salinity within two hours [23]. Singh and colleagues advanced the microneedle concept with a biohydrogel-enabled microneedle sensor, in which horseradish peroxidase was immobilised in a chitosan-reduced-graphene-oxide hydrogel; the device achieved high sensitivity with a detection limit near sixty nanomolar over a wide linear range and was applied to in situ monitoring of reactive oxygen species in tobacco and soybean plants under biotic stress [24]. An alternative, remote-readout strategy was demonstrated by Yan and colleagues, who built a metal-organic-framework biosensor that reports hydrogen peroxide in plant organs through color-to-thermal signal conversion: horseradish peroxidase, a chromogenic substrate and a zeolitic-imidazolate-framework precursor were sprayed in situ on roots, petioles or leaves, and the peroxide-driven oxidation product generated a near-infrared-readable thermal signal sensitive to sub-micromolar peroxide [25]. The catalytic chemistry of peroxide detection overlaps with that of NADH, and Prussian-Blue-based screen-printed transducers, reviewed below, have served both analytes [26].

5.2 NADH and NADPH

The reduced nicotinamide cofactors NADH and NADPH are the universal currency of biological reduction and are intimately tied to photosynthesis: NADPH is the principal reductant generated by the light reactions and consumed in the Calvin-Benson cycle, while NADH reports on respiratory and cytosolic redox status. Electrochemical detection of NADH is analytically important because it is the product of more than three hundred NAD+-dependent dehydrogenases, so that a sensitive NADH transducer can be coupled to a chosen dehydrogenase to create a sensor for the corresponding substrate. The central obstacle is the high overpotential and electrode fouling associated with the direct oxidation of NADH at bare electrodes, which has motivated extensive use of redox mediators and catalytic nanomaterials [26]. Ricci and colleagues systematically developed Prussian-Blue-modified screen-printed electrodes as low-potential transducers for both hydrogen peroxide and NADH, exploiting the electrocatalytic properties of Prussian Blue and related mediators to lower the working potential and suppress interference, and provided design guidance that has been widely adopted for reagent less and dehydrogenase-coupled sensors [26]. The same mediated-transduction philosophy underlies the integration of NADH detection into multi-analyte plant-metabolite platforms.

5.3 Soluble sugars: glucose and sucrose

The soluble sugars produced by carbon fixation glucose, fructose and the transport sugar sucrose are the tangible output of photosynthesis and circulate through the plant to support growth and storage. Monitoring their dynamics in vivo illuminates' source-sink relationships, diurnal carbon allocation and stress responses, but it requires sensors that can operate within the delicate, water-rich environment of plant vasculature. Organic electrochemical transistors have emerged as the platform of choice because their inherent transconductance amplification produces large signals from soft, biocompatible, implantable devices operating at low voltage [2,27]. Diacci and colleagues implanted organic-electrochemical-transistor sensors to achieve diurnal in vivo monitoring of glucose and sucrose in the xylem sap of living plants, tracking the daily rhythm of sugar transport with a minimally invasive device [27]. Armada-Moreira and co-workers extended in vivo glucose monitoring in plants using organic electrochemical transistors, refining the device chemistry and demonstrating the broader applicability of the transistor approach to plant metabolite sensing [28]. As reviewed by Dufil and colleagues, these sugar sensors are part of a wider movement in plant bioelectronics and biohybrid devices that integrates organic electronic and carbon-based materials directly into living plant tissue [2].

5.4 Phytohormones: auxin and salicylic acid

Phytohormones are signalling molecules that coordinate plant growth, development and defence at

vanishingly low concentrations, and their real-time quantification is among the most demanding and rapidly advancing areas of plant electrochemical sensing [3,37]. Indole-3-acetic acid (IAA, the principal auxin) governs cell elongation, tropisms and vascular patterning, while salicylic acid (SA) is a master regulator of systemic acquired resistance; the two hormones engage in extensive crosstalk, so their simultaneous measurement is particularly valuable [32,34]. Both molecules are electroactive IAA through its indole ring and SA through its phenolic hydroxyl enabling direct voltammetric detection, while their low physiological abundance has driven the development of catalytic, immunological and microsensor strategies.

Several complementary designs illustrate the breadth of approaches. Su and colleagues built an electrochemical immunosensor for IAA based on gold nanoparticles and a polyaniline-multiwalled-carbon-nanotube composite, using antibody recognition and impedimetric or voltammetric transduction to achieve high specificity [29]. Liu and co-workers took a non-immunological, catalytic route, exploiting the peroxidase-like activity of a hemin-reduced-graphene-oxide nanocomposite to detect IAA at low potential, with a detection limit near 0.074 micromolar achieved by monitoring the oxygen consumed during catalytic oxidation of the hormone [30]. For in vivo measurement, stainless-steel-wire microsensors have proven especially effective: Li and colleagues developed a disposable stainless-steel microsensor decorated with popcorn-like gold nanostructures, platinum nanoparticles and reduced graphene oxide for the in vivo determination of IAA in soybean seedlings under salt stress, reaching a detection limit of forty-three picograms per millilitre and validating the results against ultra-performance liquid chromatography-mass spectrometry [31].

The simultaneous detection of IAA and SA, which is needed to study their interaction, has been pursued through a series of increasingly sophisticated devices. Sun and colleagues introduced an early multiwalled-carbon-nanotube-modified electrode for the simultaneous electrochemical determination of IAA and SA in pea roots, establishing that the two hormones could be resolved in a single voltammetric scan [36]. Cao and co-workers improved sensitivity with gold-nanoparticle-doped three-dimensional reduced-graphene-oxide hydrogel electrodes for the amperometric determination of both hormones, the high surface area and conductivity of the hydrogel enhancing the analytical signal [32]. Sun and colleagues subsequently demonstrated simultaneous in vivo electrochemical determination of IAA and SA in Arabidopsis thaliana, advancing toward measurement in intact model plants [33]. The microsensor lineage has continued to mature: Tang and colleagues reported continuous in vivo monitoring of IAA and SA in tomato leaf veins with an electrochemical microsensor capable of resolving both hormones over physiologically relevant ranges by differential pulse voltammetry and amperometry [34], and subsequently a disposable stainless-steel microneedle sensor for the real-time, in situ analysis of IAA and SA in tomato leaves infected with the bacterial pathogen Pseudomonas syringae pv. tomato DC3000, directly linking hormone dynamics to a defined infection event [35]. As Bukhamsin, Blilou and Salama have argued, electrochemical sensing offers a uniquely facile route to the real-time assessment of phytohormone signalling dynamics, and continued progress in selectivity and miniaturisation is expected to make such measurements routine [37]. Table 3 collates representative metabolite sensors spanning these analyte classes.

5.5 Carotenoids and antioxidant capacity

The carotenoids, introduced in Section 3 as accessory pigments, merit separate treatment as metabolites because their electrochemistry is intimately connected to the antioxidant status of the cell. Beta-carotene, lutein and astaxanthin are potent lipophilic antioxidants whose conjugated polyene backbones are readily oxidised, and their voltammetric behaviour provides both a means of quantification and a measure of antioxidant power [7,38]. Čižmek and Komorsky-Lovrić characterised the electrochemical behaviour of beta-carotene, astaxanthin and lutein in aqueous media using paraffin-impregnated graphite electrodes and square-wave voltammetry, observing distinct oxidation peaks and establishing an antioxidant-activity ranking among the three pigments [38]. The same authors applied electrochemistry as a screening method for the determination of carotenoids in crustacean dietary

samples, demonstrating the translation of the approach from pigment standards to complex biological matrices [39]. According to a survey by Alam and colleagues, electrochemical techniques such as cyclic, differential-pulse, and square-wave voltammetry and chronoamperometry have established themselves as reliable substitutes for spectrophotometric assays in assessing the antioxidant potential of plant and fruit extracts. These techniques provide rapidity, minimal solvent consumption, and direct mechanistic insight into the redox reactions that characterize antioxidant capacity. [7]. Because carotenoid and chlorophyll oxidation signals can overlap in lipophilic extracts, advances in selective waveforms and modified electrodes benefit both pigment classes simultaneously.

Table 3. Typical electrochemical biosensors for metabolites associated with photosynthesis.

Analyte	Recognition / modifier	Electrode & technique	LOD / range	Reference
H2O2	Mn-porphyrin + ErGO	Modified electrode; amperometry	Sub-µM; in vivo leaf	Mourzina et al. [22]
H2O2	Au nanoparticles	Stainless-steel microsensor; amperometry	10–1000 µM; tomato vein	Huo et al. [23]
ROS / H2O2	HRP in chitosan-rGO hydrogel	Microneedle; chronoamperometry	~60 nM; tobacco, soybean	Singh et al. [24]
H2O2	HRP + ABTS in ZIF-8 (MOF)	Sprayed; color-to-thermal (NIR)	Sub-µM; in situ organs	Yan et al. [25]
NADH, H2O2	Prussian Blue mediator	Screen-printed; amperometry	Low-potential transduction	Ricci et al. [26]
Glucose, sucrose	Enzyme-functionalised OECT	Implantable transistor	Diurnal; xylem sap in vivo	Diacci et al. [27]
IAA (auxin)	Anti-IAA antibody; AuNP/PANI-MWCNT	Immunosensor; EIS/voltammetry	High specificity	Su et al. [29]
IAA	Hemin/reduced graphene oxide	Amperometry (O2 consumption)	LOD ~0.074 µM	Liu et al. [30]
IAA	Popcorn-Au / Pt-NP / rGO	Stainless-steel microsensor	LOD ~43 pg/mL; soybean in vivo	Li et al. [31]
IAA + SA	AuNP-doped 3D rGO hydrogel	Amperometry (simultaneous)	Enhanced sensitivity	Cao et al. [32]

Analyte	Recognition / modifier	Electrode & technique	LOD / range	Reference
IAA + SA	MWCNT-modified microsensor	DPV / amperometry; in vivo	Tomato leaf veins; continuous	Tang et al. [34]
β-Carotene, lutein, astaxanthin	Bare PIGE (intrinsic redox)	Square-wave voltammetry	Antioxidant ranking; µM	Čižmek & Komorsky-Lovrić [38]

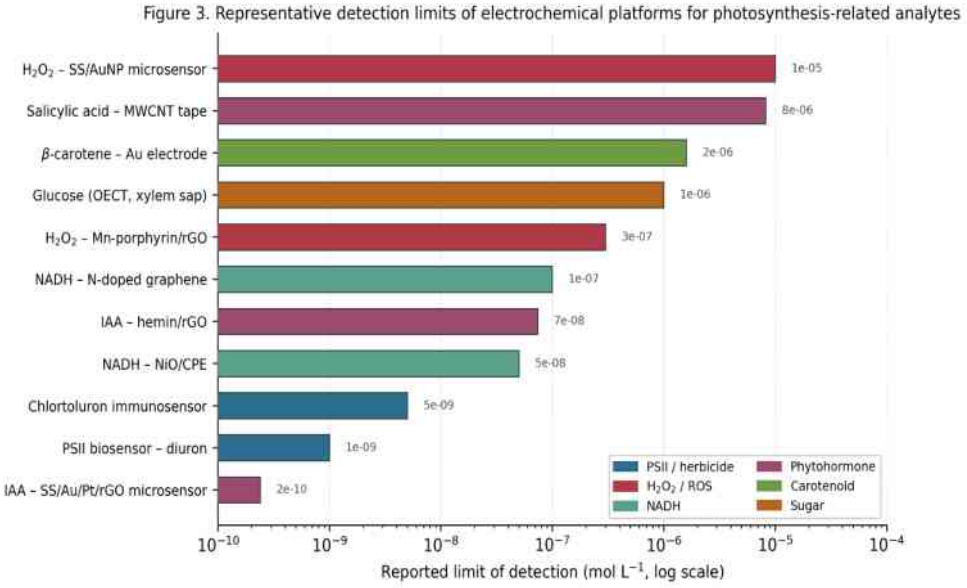


Figure 3. Representative reported limits of detection for electrochemical sensors across the analyte classes discussed in this review, plotted on a logarithmic concentration axis and grouped by target type. Values are indicative of the ranges reported in the cited literature and are intended to convey the relative sensitivity achievable for each class rather than to define definitive benchmarks; exact figures depend on electrode material, technique and matrix.

6. Nanomaterials and Electrode Engineering

The performance gains chronicled in the preceding sections rest, to a remarkable degree, on advances in the nanomaterials used to modify the electrode surface. Nanostructured interfaces increase the electroactive area, accelerate electron transfer, provide catalytic sites that lower overpotentials, and offer biocompatible scaffolds for the immobilisation of enzymes, antibodies and photosynthetic complexes. This section surveys the principal material classes that have driven progress in electrochemical biosensing of photosynthesis-related analytes [2,5].

6.1 Carbon nanomaterials and laser-induced graphene

Carbon nanomaterials are the most widely deployed electrode modifiers in this field. Graphene and reduced graphene oxide combine exceptional electrical conductivity, very high specific surface area and rich surface chemistry for functionalisation, and they recur throughout the sensors described above as the conductive support for metalloporphyrin peroxide catalysts [22], as the matrix for hemin-based auxin

sensors [30], as the three-dimensional hydrogel host for simultaneous auxin and salicylic-acid detection [32], and as a component of the biohydrogel in microneedle reactive-oxygen-species sensors [24]. Carbon nanotubes provide a complementary one-dimensional architecture with excellent charge-transport along the tube axis and have been central to phytohormone sensing, both alone and in composites with conducting polymers and metal nanoparticles [29,36]. Carbon black, an inexpensive and scalable carbon material, has been used to transduce algal oxygen evolution for herbicide detection, illustrating that low-cost carbons can rival more exotic materials in appropriate configurations [18].

A particularly important development for wearable and in-field plant sensing is laser-induced graphene (LIG), a porous graphene network written directly onto polymer substrates by laser scribing. LIG enables the one-step, large-area, low-cost fabrication of flexible electrodes, and Lan and colleagues exploited it to build a flexible, wearable humidity sensor for the real-time tracking of plant transpiration at the leaf interface, demonstrating that LIG electrodes can be conformably integrated with living plant surfaces [41]. The same scalable-fabrication philosophy extends to self-powered systems: Lan and co-workers developed breathable nanogenerators for an on-plant, self-powered sustainable-agriculture system, pointing toward sensor networks that harvest their own operating energy from the plant-environment interface [42]. Together with the thylakoid biofuel cells discussed in Section 4, these efforts sketch a future in which sensing, power and data transmission are co-integrated on the plant itself.

6.2 MXenes and other two-dimensional materials

MXenes, a family of two-dimensional transition-metal carbides and nitrides, have emerged over the past five years as outstanding biosensor materials owing to their metallic conductivity, large and tunable surface area, hydrophilicity and rich, functionalisable surface terminations. As reviewed by Sengupta and Hussain, MXene-based electrochemical biosensors developed between 2020 and 2024 have achieved high sensitivity and rapid response across a broad range of biomolecular targets, and their integration with microfluidic and wearable platforms is advancing quickly [40]. For agrochemical and plant-relevant sensing, fluoride-free synthesis routes have improved the biocompatibility and stability of niobium-carbide MXenes used in enzyme-inhibition biosensors for organophosphorus pesticides, attaining detection limits well below the nanogram-per-millilitre level [40]. The combination of MXenes with graphene and with noble-metal nanoparticles in hybrid architectures further enhances conductivity and analyte affinity, and MXenes are likely to feature increasingly in next-generation photosynthesis-related metabolite sensors.

6.3 Metal and metal-oxide nanoparticles, MOFs and conducting polymers

Noble-metal nanoparticles, especially gold and platinum, are ubiquitous electrode modifiers that provide catalytic activity, biocompatible antibody- and enzyme-immobilisation surfaces, and signal amplification. Gold nanoparticles underpin the stainless-steel microsensors for hydrogen peroxide and auxin discussed above [23,31], the immunosensor for auxin [29], and the hydrogel electrodes for simultaneous hormone detection [32]. Metal-oxide nanomaterials contribute catalytic and charge-storage functions: the ruthenium-dioxide nanosheets that so markedly enhanced thylakoid photocurrent harvesting are a striking example, their supercapacitive character and surface polarity transforming the electrode-membrane interface [19]. Novel transduction techniques, like the in-situ-formed zeolitic-imidazolate-framework biosensor that reports hydrogen peroxide through color-to-thermal conversion, are made possible by metal-organic frameworks (MOFs) due to their very high porosity and chemical tunability. [25]. As demonstrated by the polyaniline-carbon-nanotube composite of the auxin immunosensor [29], conducting polymers, such as polyaniline, offer three-dimensional conductive networks that enhance recognition-element loading and promote charge transfer. Lastly, while being molecular rather than nanostructured, mediator chemicals like Prussian Blue are still essential for low-potential detection of NADH and hydrogen peroxide [26]. To satisfy the unique requirements of each analyte and deployment scenario, the art of electrode engineering increasingly relies on the logical combination of these materials: carbon scaffolds for conductivity and area, metal nanoparticles for

catalysis and bioconjugation, MOFs, and polymers for selectivity and signal transduction.

7. Applications: Precision Agriculture, Environmental Monitoring and Food Quality

Three overlapping application categories demonstrate the practical value of the sensors surveyed here. Data-driven crop management is made possible in precision agriculture by the ongoing, minimally invasive assessment of plant biomarkers. The tomato-leaf microsensors that resolved hormone dynamics during bacterial infection are an example of how wearable and microneedle sensors that track hydrogen peroxide, auxin, and salicylic acid in living tissue can reveal the onset of pathogen infection, salt stress, or drought before visible symptoms appear, allowing for targeted and timely intervention [24,35]. Decisions about irrigation and harvesting can be influenced by complementary data on water status and carbon allocation provided by transpiration and sugar transport sensors [27,41]. The integration of such sensors with self-powered and wireless platforms points toward distributed phytosensing networks for whole-field monitoring [3,42].

In environmental monitoring, the photosynthesis-based biosensors of Section 4 provide rapid, low-cost screening for herbicides and other photosynthetic inhibitors in water and soil. PSII, thylakoid and whole-cell devices detect triazine and phenylurea herbicides at nanomolar levels and integrate the toxic effect of complex mixtures into a single functional readout, complementing chromatographic methods that quantify individual compounds [11,12,17]. Paper-based algal sensors extend this capability to the monitoring of emerging nanoformulated agrochemicals in a sustainable, disposable format [16,17]. The same chlorophyll-fluorescence and oxygen-evolution principles support the assessment of water quality and algal biomass in aquatic ecosystems [9,18].

In food quality and safety, electrochemical determination of chlorophylls, carotenoids and total antioxidant capacity offers rapid alternatives to spectrophotometric and chromatographic assays for assessing the freshness, nutritional value and authenticity of plant-derived foods [7,10,39]. The voltammetric quantification of pigments in vegetable and fruit extracts, and the electrochemical ranking of carotenoid antioxidant power, illustrate how the methods developed for physiological monitoring transfer directly to the food-analysis laboratory [38,39]. Across all three domains, the shared advantages of electrochemical biosensing speed, low cost, portability and the capacity for in-field operation are the decisive factors driving adoption.

8. Challenges and Future Perspectives

Despite the impressive progress documented in this review, several challenges must be addressed before electrochemical biosensors for photosynthesis-related analytes can transition from laboratory demonstrations to robust, widely deployed tools. The foremost is long-term operational stability under real-world conditions. Enzyme- and photosynthesis-based recognition elements are intrinsically labile: isolated PSII complexes and thylakoid membranes lose activity over hours to days, and immobilised enzymes denature under temperature and humidity fluctuations [6,13]. Engineering more stable recognition layers through protein engineering of the D1 protein and other components [14], through the use of robust whole cells in place of isolated complexes [16], and through protective nanostructured and hydrogel matrices [24] is an active and necessary frontier. For in vivo devices, biocompatibility and the avoidance of measurement-induced stress are equally critical, since an invasive sensor that wounds the tissue alters the very signals it measures [3].

Selectivity in complex biological matrices is a second major challenge. Plant saps and extracts contain numerous electroactive interferents ascorbic acid, uric acid, phenolics and other redox-active metabolites whose oxidation potentials can overlap with those of the target analyte. The near-identical oxidation potentials of chlorophyll a and chlorophyll b, and of structurally similar carotenoids and phytohormones, compound the problem [8,38]. Solutions include selective recognition elements such as antibodies and molecularly imprinted polymers, catalytic mediators that shift the working potential away from interferents [26], advanced waveforms that exploit kinetic differences [8], and chemometric data analysis of multi-electrode arrays [12]. Reliable in situ measurement depends on the ongoing development of highly selective, anti-fouling electrode surfaces.

The third area that requires attention is calibration, standardization, and quantitative validation. Many in vivo measurements provide relative changes rather than absolute concentrations, and the translation of a current to a tissue concentration is hampered by living plants' varied, dynamic microenvironments. Rigorous cross-validation against established reference methods, such as liquid chromatography-mass spectrometry, as demonstrated for the soybean auxin microsensor [31], should become routine practice, and the area would benefit from agreed-upon methodologies and reference materials. Looking forward, the integration of sensors with wireless telemetry, low-power electronics and energy-harvesting elements including the breathable nanogenerators and thylakoid biofuel cells discussed above promises autonomous, networked phytosensing systems [42]. The coupling of multiplexed sensor arrays with machine-learning analysis of the resulting data streams offers a route to interpreting the complex, interacting signals of plant physiology, transforming raw electrochemical traces into actionable diagnoses of plant health [3,4]. The convergence of materials science, synthetic biology, microfabrication and data analytics that characterises the most recent work suggests that these challenges, while substantial, are tractable.

9. Conclusion

Electrochemical biosensors have matured into a versatile and powerful toolkit for the detection of chlorophyll and the metabolites that drive and report on photosynthesis. Organised around the molecular architecture of the photosynthetic apparatus, the literature reveals two complementary paradigms: the direct electrochemical quantification of intrinsically electroactive analytes chlorophylls, carotenoids, hydrogen peroxide, NADH and the indolic and phenolic phytohormones and the use of isolated photosystem II, thylakoid membranes and whole photosynthetic cells as functional bioreceptors that transduce the inhibition of photosynthetic electron transport into a measurable photocurrent. Advances in nanomaterials and electrode engineering, from graphene and laser-induced graphene to MXenes, metal and metal-oxide nanoparticles, metal-organic frameworks and conducting polymers, have delivered the sensitivity, stability and form factors needed for sensors that operate not merely in the cuvette but in and on the living plant. Microneedle, wearable and implantable formats now permit minimally invasive, continuous monitoring of physiological biomarkers in real time, with demonstrated applications in precision agriculture, environmental surveillance and food quality control. Significant challenges remain chiefly the long-term stability of biorecognition elements, selectivity in complex matrices, and the standardisation and quantitative validation of in vivo measurements but the rapid, interdisciplinary progress of recent years gives strong grounds for optimism. As sensing, power and wireless data handling converge on the plant itself, electrochemical biosensors are poised to become indispensable instruments for understanding and managing photosynthetic life in an era of intensifying pressure on global food and environmental systems.

Author's Contribution

MU contributed to conceptualization, literature investigation, data collection, and manuscript preparation. ITR contributed to literature investigation, data curation, and manuscript revision. SR contributed to electrochemical sensing, nanomaterials, data organization, and manuscript revision. MF contributed to literature investigation, analysis of sensing mechanisms, and manuscript revision. NA contributed to literature investigation, data synthesis, and manuscript revision. RI contributed substantially to conceptualization, literature investigation, critical synthesis, data curation, preparation of sections, tables and figures, and scientific editing and finalization of the manuscript. All authors reviewed and approved the final manuscript.

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