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Current and Future Transgenic Whole-Cell Biosensors for Plant Macro- and Micronutrients

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From the soil, plants take up macronutrients (calcium, magnesium, nitrogen, phosphorus, potassium, sulfur) and micronutrients (boron, chloride, cobalt, copper, iron, manganese, molybdenum, nickel, selenium, and zinc). In acidic soils, aluminum can interfere with nutrient uptake. There is a need for improved diagnostic tests for these soil-derived minerals that are inexpensive and sensitive, provide spatial and temporal information in plants and soil, and report bioavailable nutrient pools. A transgenic whole-cell biosensor detects a stimulus inside or outside a cell and causes a change in expression of a visible reporter such as green fluorescent protein, and thus can convert an invisible plant nutrient into a visible signal. Common transgenic whole-cell biosensors consist of promoter-reporter fusions, auxotrophs for target analytes that are transformed with constitutively expressed reporters, riboswitches and reporters based on Forster Resonance Energy Transfer (FRET). Here, we review transgenic plant biosensors that have been used to detect macronutrients and micronutrients. As plant-based biosensors are limited by the requirement to introduce and optimize a transgene in every genotype of interest, we also review microbial biosensor cells that have been used to measure plant or soil nutrients by co-inoculation with their respective extracts. Additionally, we review published transgenic whole-cell biosensors from other disciplines that have the potential to measure plant nutrients, with the goal of stimulating the development of these diagnostic technologies. We discuss current limitations and future improvements needed, and the long-term potential of transgenic whole-cell biosensors to inform plant physiology, improve soil nutrient management, and assist in breeding crops with improved nutrient use efficiency.

Keywords biosensor, nutrient, fertilizer, plant, soil, microbe

I. INTRODUCTION

From the soil, plants take up both macronutrient fertilizers (primary: K, P, N; secondary: Ca, Mg, S) and micronutrient fertilizers (B, Co, Cu, Cl, Fe, Mn, Mo, Ni, Se, Zn), while aluminum (Al) interferes with fertilizer uptake in acidic soils by inhibiting root growth (Mengel and Ernest, 2001). Diagnostic tests for these minerals are used by growers to assist with fertilizer management and by researchers to improve crop nutrient use efficiencies, measure environmental pollution as a result of over-fertilization and help analyze nutrient dynamics in plants. An ideal diagnostic test should reflect the actual fraction of a nutrient that is available to a plant ("bioavailable"), be inexpensive, technically accessible, simple, rapid, portable, and provide both temporal and spatial resolution in both plant tissues and soil. Many current diagnostic tests for crop nutrients do not fulfill these requirements (Preverill *et al.*, 2001).

Standard nutrient diagnostic tests involve older methods of chemical extraction (e.g., hot acid), multi-step chemical conversions, separation and finally detection (e.g., spectrophotometry) (Preverill *et al.*, 2001). These tests can suffer from limitations including the following: (i) high costs that limit sampling, which is especially problematic for highly mobile nutrients that change over space and time (e.g., nitrate); (ii) they report the total but not bioavailable nutrient pool (e.g., immobile nutrients such as phosphate); (iii) they are inadequately sensitive (e.g., molybdenum); (iv) they require large sample amounts; (v) the results can be difficult to calibrate and may not predict crop yield; and (vi) they do not report the *in vivo* spatial localization of nutrients in plant tissues (Preverill *et al.*, 2001).

Anthocyanins, the blue-red pigments of plants (Fig. 1a), may serve as an example to help solve some of the limitations of current plant nutrient diagnostics. In the early twentieth century, maize geneticists used subtle visible changes in the color, intensity, spatial and temporal patterns of anthocyanins to understand how the environment (e.g., phosphate limitation) affects these metabolites and to screen for genetic mutants that affect pigmentation (Lindstrom, 1923). Here lies the historical lesson: by converting the invisible into the visible, and making it measurable, large numbers of plants can be screened cost-effectively and with excellent spatial and temporal resolution.

A technology that is capable of turning invisible crop nutrients into visible outputs is the whole-cell biosensor: in simplest terms, a whole-cell biosensor detects a stimulus inside or outside a cell and causes a visible reporter to change in expression (D'Souza, 2001). There are many types of biosensors (D'Souza, 2001), but plant biologists are most likely able to work with whole-cell biosensors based on their knowledge of biology and available equipment. In this review, the biosensors discussed are primarily limited to transgenic whole-cell biosensors.

The most common whole-cell, plant-based biosensor consists of a transgenic plant that expresses a truncated native promoter that responds to a stimulus, fused to a reporter gene (Sadanandom and Napier, 2010) (Fig. 1b). The main design variable in a promoter biosensor is the choice of the reporter gene, typically *uidA* (*gus* encoding glucuronidase) (Jefferson *et al.*, 1987), firefly luciferase (*luc*, analogous to *lux* from the bioluminescent bacterium, *Vibrio harveyi*), green fluorescent protein (*gfp*) or fluorescent derivatives (D'Souza, 2001;

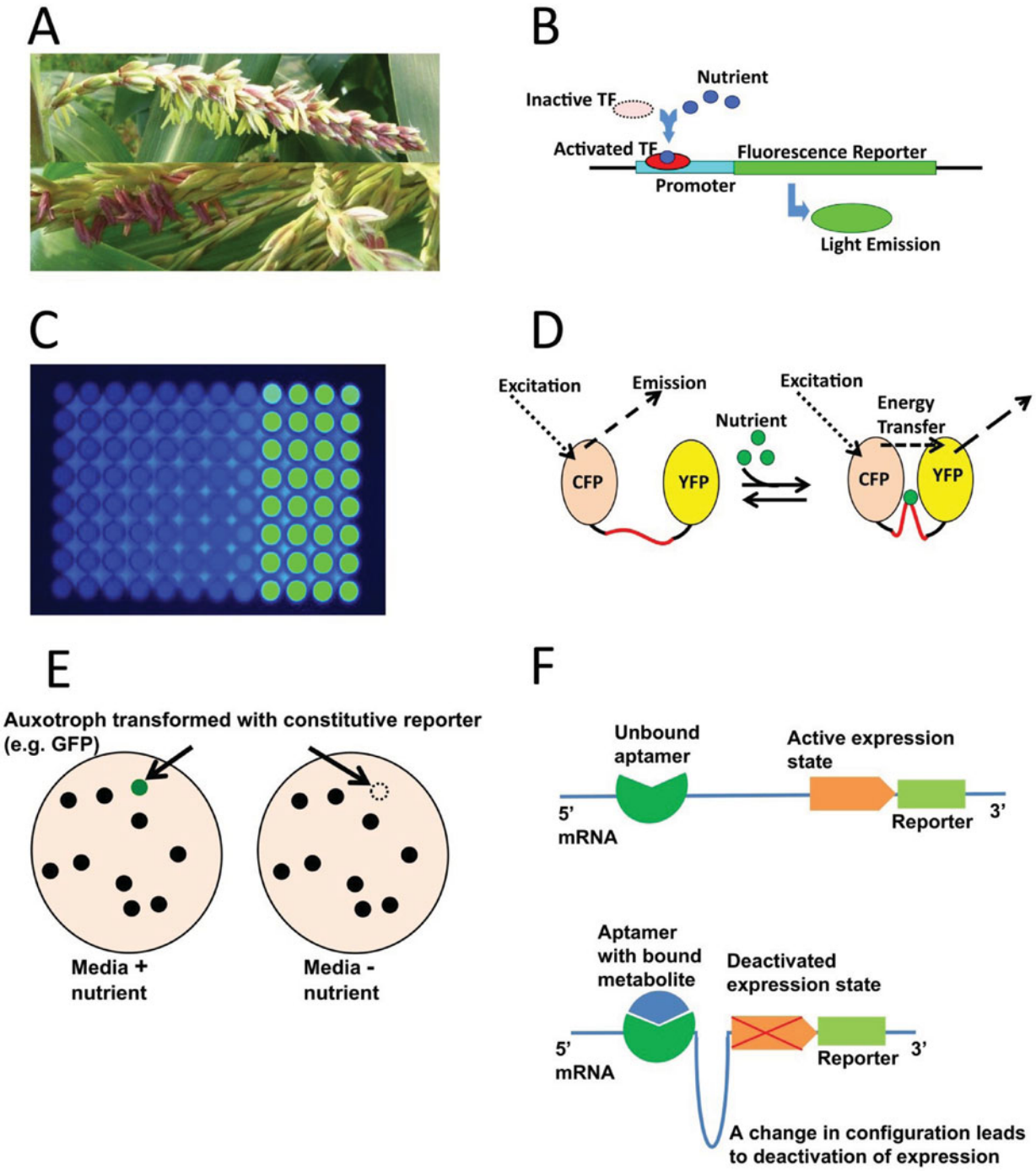

FIG. 1. Summary of principles underlying transgenic whole-cell biosensors. (A) Example of anthocyanin pigmentation on maize anthers. (B) General design of a promoter-reporter biosensor. (C) Example of a *lux*-output microbial biosensor exposed to increasing concentrations of its target analyte in a 96-well plate, imaged with a photon detection CCD camera. (D) General design of a FRET biosensor. (E) A potential auxotroph biosensor. (F) General design of a riboswitch biosensor.

Sadanandom and Napier, 2010) (Table 1). Depending on the reporter used, biosensor activity can be measured either visually using the naked eye (i.e., for GUS), with a photon-capture camera (i.e., for firefly luciferase or lux) (Chinnusamy *et al.*, 2002), or with a micro/macroscope equipped with appropriate excitation and emission filters (i.e., for GFP and its derivatives). For semi-quantitative analysis, tissue extracts must be made, and the samples read using single chamber detectors (a spectrophoto meter for GUS and GFP, a luminometer for luciferase) or related 96-well plate readers (example shown in Fig. 1c).

TABLE 1
Features of potential transgenic whole-cell biosensor reporters

Reporter	Application	Advantages	Disadvantages
GUS/LacZ	•Tissue-level resolution for microscopy •Reporter to quantify extracts	•Can use inexpensive light microscope or direct viewing •Excellent contrast for plant microscopy	•Moderate tissue resolution due to substrate diffusion •Requires reagent •Moderate sensitivity and linearity •Stable/poor temporal resolution •Involves laborious enzymatic rate assay to quantify extracts
GFP and variants	•Microscopy at cellular and subcellular resolution	•No diffusion •No reagents required •Multiple fluorophores •Compatible for translational fusions •Inexpensive UV detection for on/off applications	•Moderate sensitivity and linearity •Stable/poor temporal resolution •Requires fluorescence microscope for good resolution
Luciferase/LUX	•Quantitative reporter for environmental responses at multiple stimulus ranges •Soil or tissue extract measurements	•High sensitivity, low background •Linear across several orders of magnitude •Excellent temporal resolution •Lux requires no exogenous reagents	•Luciferase requires reagents •Requires luminometer or CCD detector •Poor fine scale resolution due to photon scatter •Instability of luciferase in tissue extracts
Anthocyanins Carotenoids Chlorophyll	•Microscopy at cellular resolution	•Can use inexpensive light microscope or direct viewing	•No reagents •Requires specific genetic background •Requires sophisticated genetic engineering •Not useful for quantifying extracts

In addition to promoter-based biosensors, nuclear-encoded FRET (Forster Resonance Energy Transfer)-based biosensors, first developed by the lab of Roger Tsien (Miyawaki *et al.*, 1997), have recently been engineered in plants (Okumoto *et al.*, 2008). In a FRET biosensor, two different fluorophores (e.g., GFP variants) are linked by a polypeptide that has specific ligand-binding ability; upon ligand binding, there is a dramatic conformational change of the whole structure, mediated by flanking hinge adaptor peptides, bringing the two fluorophores together in a more optimal configuration to permit energy transfer from the excited fluorophore to an acceptor fluorophore (Fig. 1d). The excited acceptor fluorophore subsequently re-emits the fluorescence energy at a longer wavelength, whereas florescence emission of the donor fluorophore is quenched—either or both signals can be measured. The central ligand-binding peptide is often derived from the bacterial maltose binding protein (MBP) family—a wide-range of ligand sensors normally found in the periplasmic space of bacteria (periplasmic binding proteins, PBPs). PBPs can be readily mutagenized to alter target ligand specificity (Okumoto *et al.*, 2008). If the entire FRET biosensor (fluorescent indicator protein, FLIP) is expressed constitutively, the rapidity of the ligand-induced conformational change permits real-time reporting of the ligand. FRET-based screening requires fluorescence and confocal microscopy.

A significant disadvantage of plant-based biosensors is that a transgene must be introduced and optimized in every genotype of interest; this can be time consuming and may limit field studies. Complementary to plant-encoded biosensors are microbial biosensor cells that can be used to measure analytes by co-inoculation with either plant or soil extracts, for example in a 96-well fluorescence/luminescence plate reader (D'Souza, 2001) (Fig. 1c). Microbial biosensors can also be applied onto the surface of intact or freeze-thawed plant tissues; such surface tissue cracks and/or freeze-drying cause leakage of endogenous metabolites that can then be sensed by the microbial cells and visualized using the appropriate imaging device.

Transgenic bacterial and yeast biosensors are attractive tools for plant biology for a number of reasons. First, microbial cells are easy to transform and screen in large numbers, permitting complex engineering and fine-tuning. Second, because this technology does not involve the construction of transgenic plant lines, these biosensors can be used to assay large numbers of diverse plant genotypes. Third and finally, the diversity of Earth's micro-environments has resulted in microbes that have adapted to sense and catabolize large numbers of substrates, which has created an expansive library of potential biosensors (Falkowski *et al.*, 2008).

In addition to promoter and FRET-based biosensors, there are two additional types of biosensors available in microbes: first, there is a large collection of auxotrophs—mutants that cannot grow unless a particular nutrient is added exogenously (Lengeler *et al.*, 1999). By transforming an auxotroph with a constitutive reporter, reporter output (e.g., lux) becomes a proxy for induced growth, creating a highly sensitive biosensor (Fig. 1e). For example, a bacterial auxotroph for glutamine has been used to engineer a lux-based biosensor to quantify glutamine in corn extracts (Tessaro *et al.*, 2012). A disadvantage of such biosensors is that any stimulus that alters general growth can potentially confound the output results, so proper testing and controls must be applied (Tessaro *et al.*, 2012).

A second class of biosensors, primarily available in bacteria, is the riboswitch. In a riboswitch, a small chemical ligand binds typically to a target nucleic acid polymer (aptamer), such as in the 5'-Untranslated Region (UTR) of mRNA, resulting in a change in its conformation; this may result in premature transcriptional termination or masking of the Shine-Dalgarno (SD) sequence required for ribosome binding (Fig. 1f) (Henkin, 2008). Though riboswitches have begun to be identified in plants (e.g., in which the conformation of the 3'-UTR RNA is altered, leading to altered splicing) (Bocobza and Aharoni, 2008), for now most discovered riboswitches exist in bacteria.

Whether plant or microbial, a user may wish for a nutrient biosensor to possess several features (summarized in Table 2). In particular, a biosensor must be specific to the target nutrient amongst a diverse pool of plant and soil compounds. Furthermore, it is ideal if the biosensor output is linear (or mathematically transformable into a linear output) across a wide concentration range. An ideal biosensor is sensitive (high signal:noise ratio) and perhaps tunable to different concentration ranges. A specific challenge when using any biosensor for plant and soil extract measurements is that the signal output must be robust (consistent against background noise) in the presence of varying inorganic and organic compounds or even competing microflora that are present in different soil types, plant species and tissues. Other criteria for the design of optimal biosensors, that may or may not be necessary, include tuneability (ability to adjust the sensitivity of the biosensor to measure different concentration ranges of the target analyte), scalability (e.g., ability to assay thousands of samples in parallel if needed), low cost (including labour) and appropriate biological resolution (e.g., tissue or subcellular) depending on the application (Table 2). However, there are tradeoffs between these design principles. For example, enhancement of one design feature may result in increased cost. Furthermore, improving one design feature may have a negative effect on another criterion (e.g., improving linearity across multiple orders of magnitude can reduce sensitivity).

TABLE 2
Biosensor engineering design principles

Criterion	Explanation
Specificity	Selectivity for the primary target analyte over potentially interfering compounds
Sensitivity	High signal output, low background
Linearity	Linear reporter expression across multiple orders of magnitude or ability to mathematically transform into a linear output
Tunable	Sensitivity can be adjusted to different concentration ranges of the target chemical
Robustness	Performs well under different stress conditions (e.g., resistant to changes in pH, salts, temperature, etc.)
Biological resolution	Organism, tissue, cell or subcellular level signal, depending on application
Scalable	Practical to assay thousands of samples, depending on application
Cost/Labor	Minimum reagent cost, preparation time, minimal number of steps and personnel expertise

Here, we review transgenic plant and microbial whole-cell biosensors that have been used specifically to detect macronutrients and micronutrients in plant tissues and soils. As such examples are limited, we also extensively review candidate biosensors that have the potential to detect plant nutrients with the objective of catalyzing the research and development of these technologies. We conclude by discussing current limitations and future improvements needed, as well as the long-term outlook of nutrient biosensors for plant and soil research.

II. BIOSENSORS FOR PRIMARY PLANT MACRONUTRIENTS

A. Inorganic Nitrogen (N)

1. *Nitrate (NO_3^-)*

Potential plant-based biosensors are available for nitrate in both monocots and dicots. In dicots, including *Arabidopsis thaliana*, a 43-bp pseudo-palindromic nitrogen response element (NRE) was identified in the promoter of a gene encoding nitrite reductase (*NiR*) as well as in the 3' region of the nitrate

reductase (NIA1) gene; both elements were shown to be necessary and sufficient for nitrate-responsive gene transcription (Konishi and Yanagisawa, 2010, 2011). NRE-like elements were also identified in monocots including rice, maize and sorghum; in particular an 8 bp element was conserved in both monocot and dicot nitrate-responsive genes (Konishi and Yanagisawa, 2010; Liseron-monfils *et al.*, 2013). Nitrate-inducible biosensors have been engineered that consist of 4 copies of the NRE fused to either *gus* or *luc* (Konishi and Yanagisawa, 2011, 2013). In the promoter region of the *Arabidopsis NIA1* gene, three non-NRE motifs (myb, ALFIN1, E-box) have also been discovered which were observed to be necessary and sufficient for nitrate-responsive transcription of fused GUS reporter constructs (Wang *et al.*, 2010).

With respect to bacterial biosensors, biosensors for nitrate based on the *narG* promoter (e.g., *narG*:*GFP*) have been constructed in *Enterobacter cloacae* and *Escherichia coli* based on the two-component NarX/L and NarQ/P systems (De Angelis *et al.*, 2005). Using the biosensor, the authors showed that nitrate levels on soil-grown roots were higher when nitrate had been added to the soil. The biosensor holds potential for quantifying nitrate in plant extracts.

2. Ammonium (NH_4^+)

Potential microbial biosensors for ammonium have been designed using promoters that are activated by the NtrC transcription factor that is induced during ammonia limitation (Atkinson *et al.*, 2002). The responses of NtrC-binding promoters *glnA, glnK* and *nac* were all examined by fusing each to lacZ. It was found that the *glnA* promoter was immediately activated following the same limitation while *glnK* and *nac* were only moderately activated. A strain of *E. coli* containing multiple recombinant promoter constructs each fused to different fluorescent proteins might detect a wide concentration range of ammonia in plant extracts, though specificity may be a problem as these promoters respond to amino acids and potentially other nitrogenous compounds (Atkinson *et al.*, 2002).

Another potential strategy for a whole-cell bacterial biosensor of ammonium is the use of auxotrophic strains that have a requirement for exogenous nitrogen. *Ammoniphilus oxalaticus* and *A. oxalivorans* have been identified as being ammonium-dependent, requiring $\geq$0.07 M ammonium (Zaitsev *et al.*, 1998). Growth of these species was shown to be ammonium specific and not induced by sodium (Na^+) or potassium (K^+). These bacterial species were isolated from the rhizosphere of sorrel, signifying their potential as a bioindicator of ammonium in plant root zones. These bacteria could be turned into a visual assay by introducing a constitutive plasmid expressing GFP or LUX, though the strains have only a limited response to ammonium (Zaitsev *et al.*, 1998).

The bacterium, *Nitrosomonas europaea,* has also been explored in terms of its capabilities as an ammonium biosensor (Nguyen *et al.*, 2011). This lithotrophic ammonium-oxidizing bacterium was transformed with a plasmid containing constitutively expressed *luxAB*. Bioluminescence was correlated with ammonium oxidation activity. Using this methodology the authors were able to quantify bioavailable ammonium from rice paddy soil, and demonstrated that ammonium availability increased within days after the addition of urea to soil. This biosensor was shown to be specific for ammonium, and it did not respond to urea or any of the 20 amino acids at 100 μM (Nguyen *et al.*, 2011). A biosensor that measures oxygen consumption by *N. europaea* has also been used to quantify ammonium in the range of 0–200 μM (Bollmann and Revsbech, 2005).

3. Urea [$CO(NH_2)_2$]

With respect to potential plant-based biosensors for urea, a gene encoding an H^+/urea co-transporter (*AtDur3*) has been identified in *Arabidopsis* which is heavily induced after application of urea to the roots of nitrogen-starved plants (Kojima *et al.*, 2007). A transgenic line expressing an *AtDUR3*-promoter:*GFP* construct has been engineered which allows for visualization of the root-transporter protein with single cell resolution including root hairs. Though the fusion construct was not quantified, expression analysis of the native gene showed that transcript accumulation was reduced by nitrate or ammonium, but increased following addition of urea (Kojima *et al.*, 2007).

4. Total inorganic nitrogen (ammonium + nitrate + nitrite)

To determine the effects of dissolved nitrogen on phytoplankton, a promoter biosensor (*glnA*:*luxAB*) for total inorganic nitrogen (ammonium, nitrate, nitrite) was integrated into the cyanobacteria *Synechococcus* strain GSL, and used to quantify bioavailable nitrogen along a lake depth profile (Gillor *et al.*, 2003).

B. Phosphorus (P)

Bioavailable phosphate (PO_4^{-}) is often the limiting nutrient for legumes, and in tropical soils in general (Mengel and Ernest, 2001). Plant-based phosphate responsive promoters have been shown to hold promise as phosphate biosensors. A GUS fusion to the *Arabidopsis AtIPS1* promoter (construct *AtIPS1:GUS*), a member of the phosphate-responsive *TPSI1/Mt4* gene family, was activated under phosphate starvation conditions in seedling leaves, roots and phloem (Martín *et al.*, 2000). Expression of *AtIPS1* was also shown to be reversible. The expression of *AtIPS1* was claimed to be specific to phosphate starvation, as the gene was not induced by limiting potassium or nitrogen, though auxin and cytokinin were shown to modulate expression of *AtIPS1* under phosphate limiting conditions (Martín *et al.*, 2000). The *AtIPS1:GUS* fusion was used to localize the specific cell types affected by phosphate starvation in *pho1* mutants defective in xylem loading of phosphorus (Martín *et al.*, 2000). The *AtPS1:GUS* biosensor facilitated a successful screen of 25,000 Arabidopsis plants to identify mutants which were affected in their capacity to adapt to phosphate-limiting conditions (Rubio *et al.*, 2001). The authors were able to use this method to discover

that a MYB domain is common to several phosphate-starvation response proteins (Rubio *et al.*, 2001). The mutant screen also resulted in the identification of a phosphate transporter traffic facilitator, *PHF1* (Gonzalez *et al.*, 2005). An invaluable tool for plant breeders would be to use such nutrient biosensors as a screen for improved nutrient uptake or utilization (e.g., plants that switch on *IPS1:GUS* at lower concentrations of phosphorus).

In addition to plant-based biosensors for phosphate, good examples exist of bacterial phosphate biosensors. Random insertions of a transposon (Tn) carrying a reporter gene were used to capture phosphate-responsive promoters in the rhizosphere-colonizing bacteria, *Pseudomonas putida* (Strain WC358, *TnlacZ*) (de Weger *et al.*, 1994) and *P. fluorescens* (Strain DF57-P2, *Tn5luxAB*) (Kragelund *et al.*, 1997). In the former example, biosensor bacteria were inoculated and recovered from soil, and were used to report bioavailable levels of phosphate in bulk sand or soil from the rhizospheres of potato, tomato and radish (de Weger *et al.*, 1994). In the latter example, a photon-capture camera was used to image activation of the phosphate-lux biosensors at zones of phosphate starvation on barley roots directly (Kragelund *et al.*, 1997). Reporter fusions of downstream promoter targets of the two-component *PhoB/PhoR* extracellular phosphate sensing system were used to measure phosphate reduction in *E.coli* and rhizosphere colonizer *P. fluorescens* DF57 (*phoA:lux*) (Dollard and Billard, 2003). The *phoA* biosensor was also used to measure extracellular freshwater phosphate bioavailability in the blue-green bacterium *Synechococcus* sp. strain PCC7942 (Gillor *et al.*, 2002).

In *Bacillus subtilis*, the PhoP/PhoR two-component system is responsible for sensing extracellular phosphate. The regulon is transcribed from the *P1* and *P2* promoters that hold promise as phosphate biosensors, as these promoters switch on when extracellular phosphate is below 0.1 mM (Prágai and Harwood, 2002). The PhoP/PhoR system results in the activation of downstream promoters. In a promoter trapping strategy for these downstream promoters, the pMUTIN system (*spoVG:lacZ)* was used to randomly integrate β-galactosidase next to promoters that were activated during phosphate starvation (Prágai and Harwood, 2002). Nine genes were identified that were activated by phosphate starvation (*yhaX, yhbH, ykoL, yttP, yheK, ykzA, ysnF, yvgO* and *csbD*); these could serve as phosphate biosensors. A similar enhancer trapping strategy could be used in plants to create biosensors for plant nutrients.

Finally, a major assimilate, transport and catalytic form of phosphate is orthophosphate (P_i, PO_4^{3-}) (Maathuis, 2009). Multiple affinity FRET biosensors for intracellular orthophosphate ($H_2PO_4^-$, $HP0_4^{2-}$) have become available (FLIP-P_i) based on the *Synechococcus* phosphate-binding protein (P_iBP) (Gu *et al.*, 2006).

C. Potassium (K^+)

The transcription of *HKT1*, a K^+ transporter gene, has been found to increase in the roots of both wheat and barley under potassium limitation (Wang *et al.*, 1998). The promoter of this gene could be fused to a reporter such as *GFP* or *GUS* in order to make a plant-based potassium biosensor. This gene has the potential to become a valuable biosensor because of the high speed at which transcription is induced: up-regulation occurred after just 4 hours of K^+ limitation in roots (Wang *et al.*, 1998).

Microarray profiling of RNA isolated from potassium-limited *Arabidopsis* roots showed that *AtHAK5,* a gene involved in K^+ uptake, was the only gene that was significantly over-expressed after 48 h of potassium starvation (Gierth *et al.*, 2005). Up-regulation of *AtHAK5* upon starvation was verified by fusing the promoter region of this gene to both GUS and GFP (*AtHAK5:GUS-GFP*), a strategy which could be adapted into a simple biosensor assay for low K^+ in plant roots. Transcription of a high-affinity potassium transporter in *Arabidopsis*, *AtKUP3*, has also been shown to be up-regulated by K^+ starvation (Kim *et al.*, 2013). Fusing the promoter sequence of *AtKUP3* to a reporter might form the basis of a potassium biosensor in plant roots.

With respect to microbial biosensors, an *E. coli* gene, *kup,* was found to be homologous to *AtKUP3* from *Arabidopsis* plants, based on sequence homology analysis and complementation, and could therefore serve as the basis of a whole-cell bacterial K^+ biosensor. Like the plant *AtKUP3*, *kup* is also highly involved in the transport of K^+ (Schleyer and Bakker, 1993).

Bacterial promoter biosensors (e.g., *kdpD:lacZ*) (Rothenbücher *et al.*, 2006) that respond to the two-component KdpDE system, responsible for regulation of K^+ transport (Walderhaug *et al.*, 1992), have been used to detect changes in extracellular K^+, but do not appear to have been applied to plant biology research. However, the Kdp transporter system has been studied in *Rhizobium leguminosarum* (Prell *et al.*, 2012), indicating its potential as the basis of a test for the quantification of K^+ status in the plant rhizosphere.

III. BIOSENSORS FOR SECONDARY PLANT MACRONUTRIENTS

A. Calcium (Ca^{2+})

The use of FRET biosensors for Ca^{2+} has been reported in plant research. The FRET Ca^{2+} biosensor is a fusion of four peptides: calmodulin (CaM), M13 (domain of a myosin light chain kinase), enhanced cyan fluorescent protein (ECFP) and modified yellow fluorescent protein (YFP, known as Venus) (Miyawaki *et al.*, 1997) (Fig. 1d). Binding of Ca^{2+} to CaM induces its interaction with the target peptide M13. The tips of CaM and M13 are fused to ECFP and YFP (Venus), respectively; Ca^{2+} binding causes these two fluorescent domains to interact resulting in an altered ratio of CFP to YFP fluorescence (FRET response). Since the construct changes in color upon Ca^{2+} binding, the biosensor has been called Cameleon (Miyawaki *et al.*, 1997). Readers are encouraged to refer to a detailed methodology paper

that describes the use of Cameleon in plant research, including detection of Ca^{2+} in roots (Swanson and Gilroy, 2012).

In one application, the Cameleon biosensor was expressed constitutively or using the guard cell specific *pGC1* promoter in *Arabidopsis*, in order to measure calcium dynamics in guard cells (Allen *et al.*, 1999; Yang *et al.*, 2008). Though Ca^{2+} spikes in guard cells are typically associated with stomata closing following induction by abscisic acid (ABA), the authors discovered ABA-independent spontaneous spikes in Ca^{2+}; the spikes in paired guard cells of the same stomate were typically not synchronized (Yang *et al.*, 2008).

More recently, the Cameleon system (construct YC3.6) has been used to understand the role of Ca^{2+} signaling during pollen tube-ovule interactions in *Arabidopsis* (Iwano *et al.*, 2012). The authors fused YC3.6 to a constitutive actin promoter (resulting in construct *pAct:YC3.60*) to monitor Ca^{2+} in pollen tubes, though a chromosome insertion also caused expression in synergid cells of ovules allowing a second tissue to be monitored. The researchers also fused YC3.6 to a synergid-expressed promoter (*DD2*) resulting in construct *pDD2:YC3:60*. Using this dual tissue biosensor system, the researchers were able to detect reproducible Ca^{2+} oscillation patterns in synergid cells as the pollen tube approached the ovule; the oscillation peaked as the pollen tube ruptured to release sperm cells. Precisely timed Ca^{2+} spikes were also observed at the tips of pollen tubes as they approached the ovules (Iwano *et al.*, 2012).

There is potential to engineer FRET reporters for Ca^{2+} based on calmodulin in other plant species. Calmodulin includes four EF-hand motifs which give the protein its Ca^{2+}-binding capabilities (Chin and Means, 2000). Other proteins containing this domain have been identified in various species of plants, and in some cases, Ca^{2+} may induce a physical conformational change, which is a requirement for FRET. An example of such a protein is MtCaMP1 from *Medicago truncatula* which contains two EF-hand motifs; this protein was expressed in *Arabidopsis* (Wang *et al.*, 2013). Other proteins that contain the EF-hand motif and therefore show potential as FRET-based biosensors include calcium-dependent protein kinases (CDPK) and calcineurin B-like proteins (CBL) (Grabarek, 2006). Protein kinase Cα (PKCα), a member of the CDPK family, was successfully tagged with a high-fluorescence variant of GFP and expressed in baby hamster kidney cells (Almholt *et al.*, 1999). It was discovered that this protein would migrate to the cytoplasmic membrane in quantities that roughly corresponded to levels of intercellular Ca^{2+}. A different but sensitive FRET Ca^{2+} biosensor (*TN-XXL*) based on the troponin C-binding protein has also been reported (Mank *et al.*, 2008).

Recently, it has been demonstrated that multiple FRET-based biosensors could quantify levels of different stimuli at the same subcellular location without spectral overlap (Su *et al.*, 2013). Previously, it was necessary to perform extensive image calibration in order to ensure that FRET signals did not interfere with one another. The researchers used this new technology to observe the activity of Src kinase and Ca^{2+} simultaneously in HeLa cells, a human carcinoma cell line. It should be possible to apply the same principle to plant cell research in which simultaneous visualization of multiple nutrients would prove invaluable.

With respect to candidate promoter-based biosensors for Ca^{2+}, recently bioinformatics was used to identify four Ca^{2+}-regulated promoter motifs in *Arabidopsis* (Whalley *et al.*, 2011). The authors fused each identified motif sequence to a minimal constitutive CaMV promoter along with luciferase, then expressed these constructs in *Arabidopsis*. As already noted, luciferase is an ideal reporter because of its low background and wide linear range compared to fluorescent-based assays such as FRET (Table 1).

A few possibilities might exist to create whole-cell microbial biosensors to detect Ca^{2+} in plant extracts. For example, the presence of extracellular Ca^{2+} has been shown to promote biofilm growth in the microbe *Pseudomonas putida* (Martínez-Gil *et al.*, 2012). The authors found that increasing extracellular Ca^{2+} caused adhesion protein LapF to multimerize leading to increased biofilm formation. This result suggests the possibility of a visual assay for Ca^{2+} present in plant extracts, though no data in terms of sensitivity was shown. Microbial biofilm formation can be quantified using an OD_{600} assay involving crystal violet staining (Mulcahy and Lewenza, 2011). The Ca^{2+} repressible *ciaH:luc* reporter belonging to the human dental pathogen, *Streptococcus mutans* (He *et al.*, 2008), may be another good candidate for a bacterial Ca^{2+} biosensor for use with plant extracts. Another potential microbial whole-cell biosensor for Ca^{2+} exists in various *Yersinia* strains. Detection of Ca^{2+} by extracellular YopN proteins inhibits transcription of the *yop* genes including *yopE* (Forsberg *et al.*, 1991). A *yopE:gfp* fusion reporter ($pYopE_{138}$:GFP) was shown to be repressed by Ca^{2+} (Freund *et al.*, 2008).

B. Magnesium (Mg^{2+})

No plant-based biosensors for Mg^{2+} were found in the literature, thus this section will focus on bacterial biosensors for possible use with plant or soil extracts. Bacteria such as *E. coli* might serve as effective whole-cell biosensors for Mg^{2+}, because of their ability to equilibrate the intracellular concentration of this ion with extracellular concentrations (i.e., in *E.coli*, from 4×10^{-6} M to 2×10^{-2} M) (Hurwitz and Rosano, 1967). Bacterial promoter biosensors that respond to the two-component *PhoP/PhoQ* system (e.g., *pcgF:lacZ*; *pmrC:lacZ*, *mgrB:yfp*, *mgtB:DsRed*, *retS:lux* in *E.coli, Salmonella enterica or Pseudomonas aeruginosa*) were shown to inversely respond to extracytoplasmic levels of Mg^{2+} (Chamnongpol *et al.*, 2003; Miyashiro and Goulian, 2008; Mulcahy and Lewenza, 2011; Vescovi *et al.*, 1996; Zhang *et al.*, 2009). This sensory system has the potential to be used to measure Mg^{2+} in the rhizosphere, as it has been discovered in soil-inhabiting *Klebsiella pneumonia* (Perez *et al.*, 2009). An innovative potential reporter for Mg^{2+}, using the PhoP/PhoQ system, was recently engineered in *E. coli* (Zhang *et al.*, 2009): by fusing a PhoP/PhoQ responsive

promoter (*Pmgt*) to a lysis gene from bacteriophage lambda, cells were programmed to lyse when extracellular Mg^{2+} was limiting. Lysis was quantified by measuring an engineered GFP that was released upon lysis (Zhang *et al.*, 2009). Additional research must first be undertaken to quantify the sensitivity and specificity of this system before it may be adapted as a biosensor for plant extracts.

A modified version of the *lac* promoter (*lar*) from *E. coli* may also have potential use as a biosensor, as this promoter is activated by Mg^{2+} at low concentrations, but repressed at higher concentrations; limited data suggests that the promoter is less reactive to other divalent ions namely Fe^{2+} and Ca^{2+} (Kandhavelu *et al.*, 2012).

Another potential bacterial biosensor for Mg^{2+} is based on the Mg^{2+} riboswitch (Fig. 1f) (*mgtA*:*lacZ*) from *Salmonella enterica* (Cromie *et al.*, 2006). In this system, cytoplasmic Mg^{2+} acts as a repressor of the 5'UTR M-box riboswitch (Cromie *et al.*, 2006; Dann *et al.*, 2007).

C. Sulfur (S)

Sulfate (SO_4^-) is often limiting in tropical soils (Mengel and Ernest, 2001). With respect to candidate plant-based biosensors for sulfur, the promoter of the *Arabidopsis* sulfate transporter (*SULTR1;1*) has been used to detect sulfur deficiency in *Arabidopsis* roots, but it is repressed by the products of sulfate assimilation, cysteine and glutathione (Maruyama-Nakashita *et al.*, 2005). Luciferase fusions demonstrated that a 16-bp sulfur response element (SURE) within this promoter is necessary and sufficient for activation under sulfur deficiency, though the element contains a putative auxin response factor (ARF) binding site (Maruyama-Nakashita *et al.*, 2005). SURE-like sequences were also observed in the promoter of the *Arabidopsis Nitrilase 3* (*NIT3*) gene involved in auxin biosynthesis; a GUS fusion to this promoter (3kb-*NIT3*:*GUS*) was shown to be strongly inducible under sulfur starvation in both roots and leaves (Kutz *et al.*, 2002). Similarly, a transgenic *Arabidopsis* line named Naoko Ohkama Beta (NOB) has been developed which expresses GFP under the control of a promoter (β_{SR}) that is activated in response to sulfur deficiency (Hirai *et al.*, 1994; Kasajima *et al.*, 2006). Finally, a luciferase fusion to the promoter of the *Arabidopsis PRH43* (*APR2*) gene, a marker for sulfur assimilation, is also up-regulated by sulfur starvation (Hugouvieux *et al.*, 2009). All of these reporter fusions have potential as plant-based biosensors for sulfate.

There are several bacterial promoters that could be useful in the design of a whole-cell biosensor for the detection of sulfate from plant extracts. In particular, promoters of the *ssu* pathway (sulfonate sulfur utilization), responsible for sulfur scavenging, have been shown to be up-regulated under sulfur starvation in *E.coli* (*ssuE'-lacZ*) (van der Ploeg *et al.*, 1999). However, the *ssu* promoter was shown to be responsive to both inorganic and organic sulfur containing compounds (van der Ploeg *et al.*, 1999). Promising sulfur-responsive promoters in this pathway include *atsA* (Murooka *et al.*, 1990) and *tau* (van der Ploeg *et al.*, 1999). In a parallel pathway responsible for scavenging of sulfur from organosulfonates, a *ytmI:lacZ* fusion in *Bacillus subtilis*, was strongly activated by SO_4^{2-} but also sensitive to the sulfur-containing amino acids cysteine and methionine (Choi *et al.*, 2006; Erwin *et al.*, 2005). In the root-colonizing bacteria, *Pseudomonas putida*, an *sfnE:lacZ* reporter was shown to be activated by low sulfate (Kouzuma *et al.*, 2008) and thus this system has potential to report rhizosphere sulfate. The *sfn* genes are involved in scavenging sulfur from volatile organosulfur compounds (Kouzuma *et al.*, 2008). Finally, in *B. subtilis*, the *cysH:lacZ* fusion (cysteine biosynthetic pathway) was activated by sulfur limitation, but repressed by cysteine and methionine (Mansilla *et al.*, 2000).

IV. BIOSENSORS FOR PLANT MICRONUTRIENTS

A. Boron (B)

Boron plays a structural role in plant cell walls and boron soil deficiencies have been reported in 80 countries (Shorrocks, 1997). The promoter of a boron channel *NIP6;1:GUS* is up-regulated in *Arabidopsis* shoot vascular tissues following root boron uptake (Tanaka *et al.*, 2008), and hence, has potential as a plant-based biosensor. A root-acting gene similar to *NIP6;1*, *NIP5;1,* has also been examined in its response to boron (Tanaka *et al.*, 2011). A *NIP5;1* promoter-*5′UTR:GUS* construct was down-regulated in roots as concentrations of boron were increased. The authors were able to determine that a specific region of the *NIP5;1* 5′ UTR was responsible for this boron-dependent regulation, and that this sequence was conserved in rice, maize and grape, indicating the potential to engineer this biosensor in diverse plants. However, the specificity of the fusion construct was not tested.

In rice, a promoter fusion of the boron transporter *OsBOR1* (*OsBOR1:GUS*) was up-regulated in roots under low boron (Nakagawa *et al.*, 2007). Interestingly, in *Arabidopsis*, an *AtBOR1:GFP* translational fusion was localized to the root plasma membrane under low boron, but re-localized to the vacuole under high boron where it was degraded (Takano *et al.*, 2005), demonstrating a novel type of biosensor based on post-translational protein stability.

In barley (*Hordeum vulgare* L.), several miRNAs were identified as being up- or down-regulated in leaf and root tissue in response to boron (Ozhuner *et al.*, 2013). The mRNA targets of several of these miRNAs were also identified, including the transcripts of proteins such as Squamosa promoter-binding-like protein (SLP) and serine/threonine protein kinase. Fusion of these miRNA target sites to reporters such as GFP or luciferase represents an additional biosensor strategy for plants, if the constructs are shown to respond specifically to boron.

On a cautionary note, the specificity of any plant-based biosensor for boron should be analyzed for responsiveness to aluminum which is chemically similar to boron and has been shown to interfere with boron-dependent processes in plants (Jiang *et al.*, 2009).

In terms of microbe-based boron biosensors, in the yeast *Saccaromyces cerevisiae*, a boron efflux pump promoter (*ATR1:GFP*) was up-regulated in response to boron (Kaya *et al.*, 2009) and hence is a candidate biosensor. A bacterium which has been identified as auxotrophic for boron may also serve as a whole-cell biosensor: *Bacillus boroniphilus* requires exogenous boron for growth (Ahmed *et al.*, 2007). To construct a whole-cell biosensor, this strain of *Bacillus* could therefore be transformed with a reporter gene such as *lux* under the control of a constitutive promoter.

B. Chloride (Cl^-)

FRET-based Cl^- biosensors have been developed and tested in mammalian cells. Fluorescence from a YFP mutant, YFP-H148Q, which permits solvent access to the chromophore, was shown to be inhibited by Cl^- ions; the biosensor has been used as a Cl^- bioindicator in mammalian cells though it is pH sensitive (Jayaraman *et al.*, 2000). Improved FRET-based Cl^- biosensors have also been engineered that incorporate various YFP mutations, resulting in the CFP-YFP Clomeleon (Kuner and Augustine, 2000) and the Improved Cl^- Sensor (Markova *et al.*, 2008). Clomeleon has also been applied to plant research to examine changes in chloride influx in *Arabidopsis* roots in the presence of different cations and under salt stress (NaCl) conditions (Lorenzen *et al.*, 2004). In mammalian cells, a YFP variant has also been anchored to the cytoplasmic-facing plasma membrane (Watts *et al.*, 2012). If this design could be replicated in plant tissues, it could provide a method to measure localized chloride concentrations on plant membranes such as root-hair cells.

With respect to candidate bacterial Cl^- biosensors, a regulatory gene (*gadR*) responsible for chloride-induced gene expression, has been isolated from the genome of *Lactococcus lactis*, a species that is widely used in dairy production (Sanders *et al.*, 1997). Upon exposure to Cl^-, the GadR regulatory protein activates the *gadCB* operon from the P_{gad} promoter. In a cassette containing *gadR*, P_{gad} and the start codon region of *gadC* were fused to *lacZ;* the expression level of *lacZ* was correlated with NaCl concentrations ranging from 50 to 750 mM. In a related, innovative biosensor, P_{gad} was also fused to two lysis genes, *lytPR* and *acmA*; upon Cl^- exposure, cells lysed and released PepX (X-prolyl dipeptidyl aminopeptidase), a cytoplasmic enzyme which was then quantified (Sanders *et al.*, 1997). Another candidate bacterial Cl^- biosensor was derived from the halophyte, *Halobacillus halophilus,* isolated from a German coastal salt marsh, which is dependent on Cl^- for growth; in *H. halophilus,* the genes *LuxS* and *FliC* are both transcriptionally induced by Cl^- (Roebler and Müller, 2002; Sewald *et al.*, 2007), and thus represent good candidates for bacterial Cl^- biosensors.

C. Cobalt (Co^{2+})

The physiological relevance of Co^{2+} is poorly understood (Pilon-Smits *et al.*, 2009), and we could not find good candidates for a plant-based biosensor for this element. However, the rhizobial bacterium *Sinorhizobium meliloti*, that colonizes alfalfa nodules, shows potential as a whole-cell biosensor of cobalt for use in the plant rhizosphere (Cheng *et al.*, 2011). Specifically, auxotrophic mutants of the *cbtJKL* operon (strains RmP833, RmFL3108, RmP889), responsible for cobalt transport in *S. meliloti*, require cobalt for microbial growth (Cheng *et al.*, 2011), and could be transformed with a constitutive reporter plasmid in order to engineer a biosensor. The auxotrophs do not respond to Zn^{2+}, Ni^{2+} or Fe^{3+}. LacZ and GFP transcriptional fusions to *cbt* promoters, as well as the promoter of the cobalt transporter *cobT,* were shown to be repressed by $CoCl_2$ and to a lesser extent by vitamin B12, which has a cobalt center. Additionally, in this study, a riboswitch was described that was highly sensitive to cobalt, which caused attenuation of the *cbtJ* transcript. It may be possible to incorporate this riboswitch sequence into reporter genes to create sensitive biosensors.

With respect to other microbial biosensors for Co^{2+}, the promoter of the metal binding protein NmtR from *Mycobacterium tuberculosis* (*nmtR:lacZ*) was shown to be induced by Co^{2+} and Zn^{2+} in *Mycobacterium* but only by Co^{2+} in a strain of the cyanobacterium, *Synechococcus* PCC 7942 (Cavet *et al.*, 2002), demonstrating the utility of introducing biosensors into different hosts to alter either the ligand specificity or signal:noise ratio. The *coa*:*lux* promoter of *Synechocystis* sp. PCC 6803 was shown to be highly inducible and specific to Co^{2+} (compared to Cd, Ni, As, Cu and Cr) except for activation by Zn (Peca *et al.*, 2008). In *Ralstonia eutropha* bacteria, the *cnrYXH:luxCDABE* biosensor was strongly induced by Co^{2+} but also by Ni^{2+} (Tibazarwa *et al.*, 2001) and has potential as a biosensor. A study in *E. coli* revealed several genes that were both up- and down-regulated by cobalt limitation (Fantino *et al.*, 2010). Among up-regulated genes are those that are responsible for the efflux of cobalt such as *rcnA*, thus reporter fusions to *rcnA* might serve as cobalt biosensors. Similarly, in *Rhodococcus rhodochrous,* a known plant colonizing microbe (Finnerty, 1992), the level of the nitrile hydratase gene transcript is cobalt-dependent but may be affected by nitrogenous compounds (Pogorelova *et al.*, 1996). This strain is worth investigating as the basis of a cobalt biosensor.

There are several candidate genes in yeast that are regulated by cobalt availability. One such gene is *Cot1* which confers cobalt tolerance to yeast and has been shown to be up-regulated after addition of cobalt ions but only up to two-fold (Conklin *et al.*, 1992). The gene is also sensitive to rhodium ions, but not to any other divalent ions. Transcriptional or translational reporter fusions to *Cot1* could form the basis of whole-cell cobalt biosensors.

D. Copper (Cu^{2+})

Cu^{2+} is an essential cofactor for ethylene hormone perception in plants (Bleecker, 2000). A plant-based biosensor for Cu^{2+} has been developed by transforming *Arabidopsis* with a yeast Cu^{2+}-inducible system (Granger and Cyr, 2001). This system is composed of the following two components: *AceI*, which encodes a metalloresponsive transcription factor, and a

metalloregulatory element (MRE) enhancer fused to a minimal 35S promoter (35S-90) that is activated by ACE1 in the presence of Cu^{2+}. The authors fused the MRE-35S-90 promoter to *gfp*, which allowed them to visualize a range of $CuSO_4$ concentrations from 0-50 μM in root tissue. The fluorescent effects could also be observed in above-ground tissues to a lesser degree. The specificity of this biosensor was not reported. In terms of future plant Cu^{2+} biosensors, 77 plant promoters have been identified that are up-regulated by Cu^{2+} in *Arabidopsis*, but not by other metals (Zhao *et al.*, 2009). In rice, 882 genes were identified as responsive to Cu^{2+} treatment (Lin *et al.*, 2013) that might serve as the basis of future cereal biosensors.

The alga, *Chlamydomonas reinhardtii,* increases production of cytochrome c_6 (Cyc6) in response to Cu^{2+} deficiency (Bohner and Böger, 1978). Two Cu^{2+}-responsive elements (CuREs) were identified in the promoter of the *Cyc6* gene (Quinn and Merchant, 1995), which could be used as the basis for whole-cell biosensors. The CuREs were shown to be selective for Cu^{2+} compared to silver or mercury. Upon Cu^{2+} deprivation, the CuREs were sufficient to confer Cu^{2+}-dependent expression to an arylsulfatase-encoding reporter gene (*Ars2*).

Cu^{2+} has been found to quench far-red light emission from the fluorescent protein, HcRed, isolated from the reef coral *Heteractis crispa*, making it a possible candidate for transformation and *in planta* detection of Cu^{2+} (Rahimi *et al.*, 2007).

In terms of microbe-based Cu^{2+} biosensors, candidate designs may be based on one of the two-component Cu^{2+} regulators present in different bacterial species (e.g., *CusS/CusR*, *PcoS/PcoR*, *CopS/CopR*). For example, a whole-cell bacterial biosensor for Cu^{2+} has been constructed in *E. coli* by fusing the promoter of *CusC* to red fluorescent protein (*pCusC:rfp*) (Ravikumar *et al.*, 2012). In response to Cu^{2+}, the two component *CopR/CopS* system (Mills *et al.*, 1993) was shown to induce a promoter from a Cu^{2+} resistance operon (*pCOP38:lacZ*) by 100-fold in *Pseudomonas syringae* pv. Tomato; the induction was highly specific to Cu^{2+} (Mellano and Cooksey, 1988). In *Achromobacter* sp. AO22, a heavy metal tolerant soil bacterium, a plasmid encoding CopR, which activates a *cop* box promoter fused to *lacZ,* was shown to be induced by Cu^{2+}, but not by Zn, Pb, Cd, Ag or Hg (Ng *et al.*, 2012). The construct was also successfully expressed in *E. coli*. Another research group demonstrated that promoters that mediate Cu^{2+} transport, *copRZA:lux* and *copB* from *Lactococcus lactis,* were highly induced and specific to Cu^{2+} (Magnani *et al.*, 2008). In parallel studies (Ivask *et al.*, 2009; Riether *et al.*, 2001), strains of *E. coli* and *Pseudomonas fluorescens* were transformed with both *copA:lux* and its Cu^{2+} activated regulator *cueR*. The *copA:lux* fusion was shown to be specific to Cu^{2+} and not Hg, Cd, Zn or Pb, with only moderate induction by Ag. A dual luciferase version of the *copA* biosensor has also been engineered in both yeast and *E. coli* with a high degree of robustness and a wide dynamic range (Roda *et al.*, 2011). Specifically, green luciferase (PpyWT) was placed under the control of the Cu^{2+} inducible *copA* promoter, while red luciferase (PpyRE8) was placed under the control of a promoter induced by anhydrotetracyclin (ATc). The latter reporter was used as an internal control to buffer against other chemicals in the media that might affect cell viability. Additionally, these biosensor cells were embedded in a solid polymeric media matrix that allowed storage for up to one month. The biosensor was shown to be sensitive to a wide concentration range of Cu^{2+} from 10^{-8} to 10^{-3} M (Roda *et al.*, 2011).

The *ComR/ComC* system in *E. coli* is another two-component regulatory pathway that has been adapted to form a copper biosensor; in this system, *lux* was fused to the *ComC* promoter (*pComC:lux*), shown to be repressed by ComR in the presence of Cu^{2+} (Mermod *et al.*, 2012). ComR was shown to be specific to Cu^{2+} but not to Co^{2+}, Cd^{2+} and Ni^{2+} (Mermod *et al.*, 2012).

There are additional candidate Cu^{2+} biosensors of interest. By random *Tn5luxAB* insertions in *Pseudomonas fluorescens*, another Cu^{2+}-reporter strain (DF57-Cu15 luxAB) was discovered and successfully tested on soils as well as on barley straw (Tom-Petersen *et al.*, 2001). Finally, in yeast, another candidate copper-inducible biosensor is the *cup1:lacZ* system (Lehmann *et al.*, 2000).

E. Iron (Fe)

In terms of a candidate plant-based iron biosensor, a good candidate may be a transcriptional reporter fusion of the iron-regulated transporter IRT1 in *Arabidopsis* since the transcript is up-regulated in iron-deficient roots (Connolly *et al.*, 2002; Kerkeb *et al.*, 2008). However, *IRT1* promoter fragments have not apparently been characterized, and may be regulated by zinc and possibly cadmium (Connolly *et al.*, 2002; Kerkeb *et al.*, 2008). The *Arabidopsis* paralog *IRT2* is also up-regulated in root tissue by iron starvation (Vert *et al.*, 2001). The promoter-GUS fusion of this gene was induced under iron deficiency in roots, especially in the root epidermis and root hairs, though the specificity of this promoter to iron was not reported (Vert *et al.*, 2001). The transcript of LeIRT1, the tomato ortholog of IRT1, was also up-regulated in roots upon iron deficiency (Eckhardt *et al.*, 2001), suggesting IRT1-based biosensors could be built in different plant species.

AtNRAMP3 is an additional *Arabidopsis* gene responsible for metal transport which could form the basis of an iron biosensor (Thomine *et al.*, 2003). An AtNRAMP3 promoter:GUS fusion was up-regulated in the vascular bundles of roots, stems and leaves upon iron deficiency, though the sensitivity or specificity of this response was not analyzed (Thomine *et al.*, 2003).

In terms of microbial biosensors for iron, genes from three species of *Pseudomonas* (*P. aeruginosa, P. fluorescens* and *P. syringae*) may be of interest. Siderophores are molecules that chelate iron. In *P. aeruginosa* (strain 7NSK2) and *P. fluorescens*, the siderophore, pyoverdin, was shown to be quantifiable by absorbance at 400 nm (Gupta *et al.*, 2008; Leclère *et al.*, 2009). Addition of Fe^{3+} was shown to alter the absorbance of pyoverdin from 400 nm to 450 nm, and this response was highly specific to Fe^{3+} even compared to Fe^{2+}. The studies showed that Fe^{3+} decreased pyoverdin production, which could be measured using

this simple assay, making the test accessible to labs equipped with a simple spectrophotometer. However, certain salts, especially $CaCl_2$, were shown to interfere with the linearity of the assay based on their tendency to limit iron bioavailability (Leclère *et al.*, 2009). In *P. syringae,* a promoter fusion was constructed with the gene encoding the pyoverdin siderophore (*Ppvd:GFP*) (Joyner and Lindow, 2000; Loper and Lindow, 1994). The biosensor was used to test iron bioavailability in both the rhizosphere and leaf surface of bean plants without freeze-thawing, and the results showed that there was significant variation in iron availability to *P. aeruginosa* on plant tissue surfaces (Joyner and Lindow, 2000). The *P. syringae* transcriptome has since been studied in detail in terms of differential gene regulation in response to iron availability (Bronstein *et al.*, 2008). In total, 386 such genes were identified in *P. syringae* with microarray analysis, and many were found to associate with the regulators Fur, PvdS, HrpL or RpoD, indicating the potential for additional biosensors to be designed in this bacterium. Subsequently, because the bacterial epiphyte *P. fluorescens* (strain A506) produces an antibiotic against a blight pathogen in response to iron, a derivative biosensor (*Ppvd:inaZ*) was introduced onto the surface of pear and apple flowers to monitor iron bioavailability and hence the potential distribution of antibiotic accumulation (Temple *et al.*, 2004). The results showed that the flowers of these plants were iron-limited for this microbe.

Another major class of bacterial iron biosensors are promoter constructs bound by iron-activated repressors (Fur/DtxR/IRR/RirA/IdeR), including *irp1:lacZ* and *irp6:lacZ* (*Corynebacterium diptheriae*) (Qian *et al.*, 2002), *cir:lacZ* (*E.coli*) (Griggs and Konisky, 1989) and *mrgC:lacZ* (*B.subtilis*) (Chen *et al.*, 1993). As iron causes degradation of iron response regulator (IRR) proteins rapidly such as in Rhizobia (Qi *et al.*, 1999), a GFP-translation fusion to these repressors might be ideal candidates for a real-time biosensor, including in legumes.

Finally, a positive feedback microbial biosensor for iron has been engineered using an innovative synthetic biology approach (Cuero *et al.*, 2012). In *E. coli*, the authors fused an iron-responsive, aptamer-containing riboswitch (ribosomal *rpoS*) to a gene (*tonB*) which encodes a voltage-gated ion channel that transports iron and other metals. The TonB protein was translationally fused to a YFP reporter. The purpose of this design was to improve the strain sensitivity and signal to iron by coordinating iron uptake with increased gene expression.

F. Manganese (Mn)

Mn is an important catalytic center of the photosynthetic machinery and an important enzymatic co-factor in plants (Yamaguchi *et al.*, 2002). We could not find good candidates for a plant-based biosensor for Mn. GFP fusions to Mn transporters have been described in *Stylosanthes hamate (*ShMTP1), a tropical legume, and in *Arabidopsis* (AtMTP11) (Delhaize *et al.*, 2003, 2007), though these constructs did not appear to be Mn-responsive.

Several Mn biosensors exist in bacteria. In *B.subtilis*, the *mntH:lacZ* (NRAMP metal ion transporter promoter) and *mntA:lacZ* (Mn ABC transporter) fusions were shown to respond to Mn, but not to other metals; these promoters are controlled by the Mn homeostasis regulator *MntR* (Que and Helmann, 2000). Similarly, in the legume nodulator, *Sinorhizobium meililoti* (Strain 1021, mutant pMan), *mntA:gfp* was shown to be repressed by Mn (Platero *et al.*, 2004) while *sitA:gusA* (Mn ABC transporter) was strongly inhibited by Mn in the presence or absence of iron and was used to visualize GUS expression in nodules (Chao *et al.*, 2004). In *Rhizobium leguminosarum*, the *sitABCD* operon (Mn ABC transporter) was shown to be Mn-responsive, repressed by Mur, a regulator of Mn uptake (Díaz-Mireles *et al.*, 2005). In cyanobacteria, *Synechocystis* sp PCC6803, a *mntCAB:luxAB* biosensor (ABC transporter) was used in a mutagenesis experiment to discover the two-component regulators ManS/ManR (Ogawa *et al.*, 2002); the *mntC* promoter is specific to Mn but not to other metals (Yamaguchi *et al.*, 2002).

A fungal biosensor for Mn was constructed by fusing the *Mnp* (manganese peroxidase) promoter from *Phanerochaete chrysosporium* (a white-rot fungus capable of degrading lignin) to the reporter *ura1* (orotidylate decarboxylase [ODase]) (Godfrey *et al.*, 1994). The reporter was induced by extracellular Mn, though extracellular nitrogen has also been known to affect the *Mnp* promoter (Godfrey *et al.*, 1994). A more practical reporter might be helpful.

G. Nickel (Ni^{2+})

In an approach that could be used for other nutrients, *Arabidopsis* microarrays were used to bioprospect for promoters that were activated by Ni^{2+}, but not other metals (Cu, Cd, Zn), resulting in transgenic *Arabidopsis* plants containing a Ni^{2+}-responsive promoter-based biosensor (*AHB1*:*GUS*) (Krizek *et al.*, 2003). As a periplasmic Ni^{2+} binding protein has been identified (NikA) (De Pina *et al.*, 1995), it should also be possible to engineer a FRET-based Ni^{2+} biosensor in plants.

Numerous bacterial biosensors also exist for Ni^{2+}. For example, a *cnrYXH: luxCDABE* reporter in *Ralstonia eutropha* strain AE2515 has been used as a highly sensitive biosensor of Ni^{2+} in soils, though it is also activated by cobalt, but not other metals (Tibazarwa *et al.*, 2001). Measurements of soil Ni^{2+} using the *Ralstonia* biosensor correctly predicted Ni^{2+} accumulation in corn grain, leaves and potato tubers (Tibazarwa *et al.*, 2001). The *Ralstonia* biosensor also demonstrated that Ni^{2+} bioavailability to *Alyssium* plants is maximal at pH 5.1 to 6.0 under different soil texture types (Everhart *et al.*, 2006). In *Mycobacterium smegmatis*, *nmtA:lacZ* has been shown to be activated by Ni^{2+} as well as cobalt, but not by other metals; promoter activation was shown to be dependent on the regulator NmtA (Cavet *et al.*, 2002). In *E.coli*, NikR was shown to negatively regulate the *nikABCDE:lacZ* operon (Ni^{2+} ABC transporter) under high intracellular Ni^{2+} (Chivers and Sauer, 2000; De Pina *et al.*, 1995). Finally, a very promising Ni^{2+} biosensor was

constructed in the blue-green alga, *Synechocystis* sp. PCC6803, in which a *nrsBACD:luxAB* fusion was shown to be both specific and sensitive for Ni^{2+} (Peca *et al.*, 2008).

As an additional note, Ni^{2+} availability increases beneficial recycling of hydrogen released by *Rhizobium* nitrogenase in nodules (Ureta *et al.*, 2005). As bacterial biosensors can only detect Ni^{2+} bioavailability in free-living bacteria, it has been suggested that a biosensor that measures Ni^{2+} bioavailability within nodules is still needed; accordingly, an assay based on bacteroid hydrogenase protein processing, as detected by an immunoblot, has been suggested as an alternative Ni^{2+} bioassay (Ureta *et al.*, 2005).

In *E. coli*, it has been noted that Ni^{2+} addition will promote biofilm formation, encoded by the *csg* operons (Perrin *et al.*, 2009). Different *csg* promoter fusions (*csgB:gfp, csgA:gus, csgD:gus*) were induced by Ni^{2+}, though the specificity of these constructs was not examined.

H. Molybdenum (Mo)

Mo is an important co-factor (MoCo) in plant nitrate assimilation (nitrate reductase), ABA biosynthesis (aldehyde oxidase), detoxification of excessive sulfite (peroxisomal sulfite oxidase), purine metabolism (xanthine dehydrogenase), and essential for most nitrogenases required for bacterial nitrogen fixation in nodules (Kisker *et al.*, 1997; Schwarz and Mendel, 2006). Mo is limiting in acidic soils and is taken up by plants as molybdate (MoO_4^{2-}) (Baxter *et al.*, 2008). In *Arabidopsis*, if improved, the promoter of the Mo transporter MOT1 could potentially be used as a plant-based biosensor, because MOT1 mRNA levels were shown to increase in shoot tissues when Mo was added to roots (Tomatsu *et al.*, 2007). Fusions of the *Mot1* promoter to *gus* and *gfp* have both been reported (Baxter *et al.*, 2008; Tomatsu *et al.*, 2007). Similarly, by using random mutagenesis, a *Chlamydomonas reinhardtii* strain (strain DB6) was generated which was deficient in MOT1 activity and unable to grow on nitrate media without supplementation of molybdate (Li *et al.*, 2009). This auxotroph could be transformed with a constitutive reporter gene and used as a sensor for bioavailable Mo.

Externally applied Mo is known to increase the level of frost tolerance in various plants as it is a cofactor required by some abscisic acid (ABA) biosynthetic enzymes (Sun *et al.*, 2009). CBF14 is a transcription factor in wheat that has been shown to be significantly up-regulated by Mo in spring wheat even in the absence of low-temperature stress, although the induction was much more pronounced in cold temperatures (Al-Issawi *et al.*, 2013). The promoter of the *Cbf14* gene, if fused to a reporter, could form the basis of a biosensor to assay Mo status in spring wheat, an extremely valuable crop plant. In general, promoters of genes encoding MoCo biosynthesis enzymes or enzymes requiring MoCo, have potential as Mo biosensors in various plants, but more research is required in this area.

A key class of candidate bacterial biosensors for Mo are promoters regulated by ModE, a central sensor of intracellular Mo (Grunden *et al.*, 1996); this includes *modA:lacZ* in *E.coli* that is activated by Mo limitation (Rech *et al.*, 1995). The Mod sensor has also been identified in other organisms including known plant colonizers such as *Xanthomonas axonopodis* pv. *citri* (Balan *et al.*, 2006), as well as the nodule-former *Bradyrhizobium japonicum* in which a P_{modA}-*lacZ* transcriptional fusion was used to observe reporter activation upon Mo limitation (Delgado *et al.*, 2006).

Regulatory proteins within *Rhodobacter capsulatus,* a phototrophic purple bacterium, have been reported to be down-regulated in the presence of Mo. One such protein is MopA, a homologue of ModE and a regulator of high affinity molybdate transport and nitrogenase activity (Solomon *et al.*, 2000; Wang *et al.*, 1993). MopA binds to the Mo-box element such as in the promoter of the *anfA* gene; when fused to *lacZ*, variants of Mo-box elements within *mop* promoters have been shown to be activated or repressed by Mo (Müller *et al.*, 2010). Mo-box promoters could serve as effective biosensors, though promoter expression may be affected by nitrogenous compounds.

Finally, a periplasmic Mo-sensing regulatory protein (MorP), has been observed in *Desulfovibrio alaskensis*; the transcript was reported to be up-regulated 168-fold in response to Mo, but the data was not shown (Rivas *et al.*, 2009). The promoter of this gene has potential as a Mo biosensor. The MorP protein was not up-regulated by Fe, W (tungsten) or Zn; however a small induction of expression was observed upon addition of Cu^{2+} to the growth medium.

I. Selenium (Se)

Se has now been implicated as a plant micronutrient and has also been shown to be required for optimum human health (Zhu *et al.*, 2009). In terms of a plant-based Se biosensor, the promoter of the gene encoding Selenium Binding Protein 1 was fused to luciferase (*SBP1:Luc*) and shown to be up-regulated in both *Arabidopsis* roots and shoots in response to Se (+VI) (selenate, SeO_4^{2-}), but not Se (+IV) (selenite, SeO_3^{2-}), which are two soluble forms of Se (Hugouvieux *et al.*, 2009). The promoter construct, however, was also affected by sulfur, cadmium and copper (Hugouvieux *et al.*, 2009). Using this biosensor, Se was also visualized in intact seedlings using a CCD (charge-coupled device) camera.

Most plants in nature do not tolerate high amounts of Se, however there are a few species in the genus *Astragalus,* such as *A. racemosus,* with the ability to hyperaccumulate Se (Davis, 1972). *Astragalus racemosus* was studied for its transcriptional response to selenate and selenite with fluorescent differential display analysis (Hung *et al.*, 2012). Nine Se-responsive genes were identified as being differentially expressed under Se treatment, and tissue localization of the transcripts was also performed. One transcript *(CEJ367)* was highly induced by both selenate and selenite (1920 and 579-fold, respectively), and might be fluorescently tagged to form the basis of a sensitive Se biosensor.

With respect to other candidate plant based biosensors, in broccoli (*Brassica oleracea*), quantitative PCR identified several genes that were up-regulated upon Se addition, especially *APS1* (ATP sulfurylase 1) and *SMT* (selenocysteine Se-methyltransferase) which both participate in the Se uptake pathway (Ramos *et al.*, 2011). It has been demonstrated that SMT can be quantified with immunoblot analysis (Sors *et al.*, 2009).

Selenocysteine (Sec) is the twenty-first amino acid in some species, including bacteria, and its translational insertion requires reprogramming of the stop codon UGA, in part due to an mRNA secondary structure called the Sec insertion sequence (SECIS); UGA readthrough is enhanced by Se (Sandman, 2003). In *E.coli,* read-through of a UGA-SECIS:*lacZ* fusion was strongly up-regulated by selenite (Sandman, 2003), demonstrating a novel type of biosensor. SECIS elements have been found in the model alga *Chlamydomonas reinhardtii* (Novoselov *et al.*, 2002), but we found no reports of SECIS elements in higher plants.

Due to its need to synthesize selenocysteine, the archeon *Methanococcus jannaschii* can only grow in the presence of Se (Rother *et al.*, 2001), making it a candidate for a bacterial auxotrophic biosensor by incorporation of a constitutively expressing reporter. In *Methanococcus voltae* (strain VI), $NiFe^-$ and $NiFeSe^-$ hydrogenase promoters were shown to be strongly repressed or activated by Se respectively. The $NiFe^-$ hydrogenase promoter reporter constructs, *vhc:lacZ*/*vhc:gus*, and *frc:lacZ*/*frc:gus,* were strongly repressed by Se (Noll *et al.*, 1999; Sun and Klein, 2004), making them good Se biosensors. Similarly, a promoterless Tn5 transposon carrying *luxAB* was used to capture the *gutS* promoter in *E.coli* based on its up-regulation by selenite (Guzzo and Dubow, 2000).

J. Zinc (Zn)

Zinc is an important co-factor in a large number of genes in plants, including zinc finger transcription factors (Ciftci-Yilmaz and Mittler, 2008). A plant-based Zn biosensor has been engineered that utilizes FRET for quantification of environmental levels of this ion (Adams *et al.*, 2012). To engineer a FRET response, *PtZNT* coding for a Zn-binding protein was modified such that a fluorescent reporter was fused to each end of the sequence and placed under the control of the *PtZNT* promoter, resulting in the construct *ProPtZNT:DsRed:PtZNT:ECFP* (Adams *et al.*, 2012). This sequence was expressed in *Arabidopsis* and poplar trees (selected for their fast growth rate), both of which were able to differentiate between control (1 μM) and high (10 mM) levels of Zn in leaf tissue, though the robustness of the biosensor was variable (Adams *et al.*, 2012).

In microbes, biosensors based on ZntRA, a Zn efflux system responsible for Zn detoxification, have potential. In this system, ZntR activates transcription of the efflux protein ZntA in the presence of zinc (Pruteanu *et al.*, 2007). Reporter fusions to the *zntA* promoter (*zntA:lacZ* and *zntA:lux*) were shown to be activated by zinc, but also by other metals (Ivask *et al.*, 2009; Pruteanu *et al.*, 2007; Riether *et al.*, 2001), possibly limiting their use as biosensors.

Two biosensors for Zn have been engineered using the ZraSR system in *E.coli.* At higher concentrations of Zn, ZraSR is a two-component membrane associated sensor kinase system that responds to exogenous Zn and causes metal efflux (Ravikumar *et al.*, 2012). First, the promoter of *zra* was fused to GFP, and the resulting construct (*pzraP:gfp,* construct pCRGZ1) was shown to display a very linear relationship between ion concentration and fluorescence (Ravikumar *et al.*, 2012). Second, the promoter of *zra* was used to drive a zinc binding protein (ZBP) that was translationally fused to the outer membrane protein OmpC to facilitate extracellular sensing of Zn (construct CZ1056); though as the fusion protein was found to adsorb Zn, it was not stable (Ravikumar *et al.*, 2012).

In addition to biosensors for Zn based on efflux systems, in *E. coli* and *B. subtilis*, the import systems for Zn are regulated by Zur, which represses transcription of import machinery operons in the presence of Zn. In *B. subtilis*, import machinery gene promoters *yciC:lacZ* and *ycdH:lacZ* are strongly repressed by Zn, but not most other metals (Gaballa and Helmann, 1998), making them good biosensors for Zn.

Czc's are metal tolerance systems in diverse microbes that have potential as biosensors for Zn. In *Pseudomonas putida* X4, both *lacZ* and an enhanced version of *gfp* (*egfp*) were fused to the Zn-responsive *czcR3* promoter (*czcR3*:*lacZ*; *czcR3:egfp*) (Liu *et al.*, 2012). When expressed in *P. putida*, it was shown that the fluorescent output of this sensor strongly correlated to Zn measurements taken using an atomic absorption spectrophotometer (AAS) (Liu *et al.*, 2012). The biosensor was not activated by other divalent cations (Cu^{2+}, Cd^{2+} or Co^{2+}), and was successfully applied to measure Zn in diverse soil types (Liu *et al.*, 2012). This biosensor could be easily applied to detect Zn levels in plant extracts.

In *Synechococcus* PCC7942, the SmtB repressor-dependent *smtA:lacZ* reporter, was shown to be de-repressed by Zn, but not by other metals (Cavet *et al.*, 2002), also making it a good candidate biosensor for Zn. Also in *Synechocystis* (sp. PCC 6803), the *coaT*:*lux* promoter was shown to be activated by Zn, but it was more strongly induced by Co^{2+} (Peca *et al.*, 2008) as already noted earlier. Finally, as an additional note of interest, Zinpyr-1 is a fluorescent dye that has also been used for the quantification of free Zn levels in plant and microbial cells (Sinclair *et al.*, 2007).

V. BIOSENSORS FOR THE TOXIN ALUMINUM (Al^{3+})

Aluminum is not a plant nutrient, but rather a toxin that is particularly problematic in tropical soils (Mengel and Ernest, 2001). With respect to candidate plant-based biosensors for aluminum, glutathione-S-transferase promoter fusions (*pAtGST1:GUS* and *pAtGST11:GUS*) in *Arabidopsis* induce GUS expression in leaves in response to Al^{3+} exposure in the roots, though they are also induced by other heavy metals (Ezaki *et al.*, 2004).

These promoters represent a very useful type of biosensor technology for plant research, because the biosensor signal is transduced from the hidden root to the visible shoot. A more specific Al^{3+} biosensor is the *Arabidopsis* malate transporter promoter (*AtALMT1:GUS*) or other promoters regulated by the zinc finger protein STOP1 (Sawaki *et al.*, 2009) that are not activated by other metals or oxidative stress but are primarily induced by Al^{3+} in roots (Kobayashi *et al.*, 2007; Zhao *et al.*, 2009). A transcriptome analysis of *Arabidopsis* roots in response to Al^{3+} (in addition to other metals) suggests that there are 103 highly up-regulated promoters that are specific to Al^{3+} (Zhao *et al.*, 2009), all of which hold promise as plant-based biosensors. The promoters of plant genes encoding organic acids (e.g., malate) or their corresponding root transporters (*MATE-a* and *MATE-b*), which were up-regulated following Al^{3+} exposure, also hold potential as aluminum biosensors (Eticha *et al.*, 2010; Rangel *et al.*, 2010).

Bacterial promoter-based biosensors for Al^{3+} also exist that are not activated by other heavy metals, including *fliC:luxAB* and *xyl:luxAB* in *E.coli* (Guzzo and Dubow, 1994; Guzzo *et al.*, 1992).

VI. SUMMARY, LIMITATIONS, AND FUTURE IMPROVEMENTS NEEDED

In this article, we have reviewed and suggested possibilities for transgenic plant and microbial biosensors that detect macro- and micro-nutrients critical for plant growth. Several trends were apparent. With respect to the types of whole-cell biosensors that are available, the most common biosensors are based on promoter-reporter fusions (Fig. 1b), including promoters of genes that encode nutrient transporters, followed by sensors based on two-component and other regulatory systems; more rare are biosensors based on auxotrophs (Fig. 1e), FRET-based sensors (Fig. 1d) and riboswitches (Fig. 1f). We found very few examples of nutrient biosensors that were extensively engineered using principles of synthetic biology or directed evolution, suggesting that opportunities exist for improved biosensor designs, for example to improve sensitivity, linear response and activity across a wide concentration range. Improved biosensor designs are much needed, as many potential biosensors reviewed here were limited or under-studied for these attributes (e.g., some biosensors only showed a two-fold increase in response to a nutrient). Also needed are critical analyses of the costs of assaying analytes with these biosensors compared to traditional tests, as we found this information generally lacking in published studies.

The literature review demonstrates that the availability of transgenic whole-cell biosensors varies for plants (e.g., several biosensors exist for Ca^{2+}, K^+, Fe) and microbes (e.g., several biosensors exist for P, Cu^{2+}, Fe, Zn), but in general, there were many more microbial biosensors than plant-based biosensors. For example, we could find no transgenic plant-based biosensors for Mg, NH_4^+, Co or Mn, pointing to avenues for future research. Furthermore, some plant-based biosensors were reported to act only in roots, potentially limiting the technology to plants grown on agar. As a subset of the biosensors originated from species that can hyper-accumulate one or more of these nutrients, or can thrive in environments where the nutrient exists in high concentrations (i.e., microbes in heavy-metal soils), it may be useful to bioprospect such extreme environments to fill gaps. Plant microarray responses to nutrients might help to predict promoter motifs for use as biosensors. Often, we found examples in plants where gene expression in response to soil nutrients was characterized, but promoter-reporter constructs were not built. In general, more research is needed to define specific promoter motifs to be able to engineer promoter-based biosensors rationally. Though many transgenic microbial biosensors exist, we could find few examples where they were used for plant or soil biology applications, suggesting that opportunities exist for plant and microbial biologists to collaborate.

The literature suggests that current transgenic whole-cell biosensors often lack specificity and cross-react to related compounds (e.g., different nitrogen fertilizers, divalent cations), those with different valencies (e.g., Fe^{2+}, Fe^{3+}) or partner ions (e.g., Na^+ vs. Cl^-). A significant problem is that in many studies, the specificity of a potential biosensor was not fully characterized. We found very limited research on how biosensors respond at different pH levels which is important for robust testing of soils/rhizospheres, since pH is known to affect nutrient bioavalability (Mengel and Ernest, 2001).

Finally, the majority of the nutrient biosensors reviewed here used common reporters (GFP, lux/luciferase, lacZ/GUS), though we also found a few examples where unusual reporters were employed (biofilms, lysis initiation). We could find no examples of nutrient biosensors that used reporters based on natural plant pigments (carotenoids, anthocyanins, chlorophyll) (Antunes *et al.*, 2006) (Table 1). As these plant pigments are visible to humans without detection equipment, they may have applications as biosensor reporters in a field or greenhouse setting.

VII. THE POTENTIAL OF BIOSENSORS FOR PLANT AND SOIL RESEARCH

Transgenic plant and microbial biosensors hold long-term promise for plant and soil research (summarized in Table 3). Plant-encoded biosensors may be most useful when only one or a few plant genotypes (i.e., a model species) will be required, due to the significant time needed to make transgenic plants, whereas microbial biosensors will be more beneficial when large numbers of plant genotypes require sampling. With these constraints in mind, both plant and microbial nutrient biosensors have significant long-term potential for plant biology research.

Transgenic microbial biosensors could be used to accelerate the exploration of plant genetic diversity in order to facilitate crop improvement. For example, nutrient biosensors might aid in screening large plant germplasm collections to identify parents for breeding programs (e.g., selection for genotypes with

TABLE 3
Comparison of current and possible future applications of the major classes of transgenic whole-cell biosensors for plant and soil research

	Plant biosensor		Microbial biosensor			
Application	Promoter	FRET	Promoter	Auxotroph	Riboswitch	FRET
High throughput plant mutagenesis screens	Yes	No	Yes	Yes	Yes	Yes
Plant natural variation genetic screens	No	No	Yes	Yes	Yes	Yes
Plant germplasm selection	No	No	Yes	Yes	Yes	Yes
Plant breeding (progeny phenotyping)	No	No	Yes	Yes	Yes	Yes
Plant flux analysis	•Yes •Non-destructive •Delayed reporting	•Yes •Non-destructive •Real time	•Yes •Destructive assay	•Yes •Destructive assay	•Yes •Destructive assay	No
Plant cellular or subcellular microscopy	No	Yes	No	No	No	No
In situ soil microscopy	No	No	Yes	Yes	Yes	Yes
High throughput soil extract sampling	No	No	Yes	Yes	Yes	Yes

higher nitrogen uptake). The germplasm collections of rice and wheat alone consist of 107,000 and 150,000 accessions, respectively (CIMMYT 2013; IRRI 2009). Microbial nutrient biosensors could be used to discover genotypes that are more efficient at taking up or assimilating soil nutrients to improve fertilizer usage. For example, the leaves of various local plant species in Sub-Saharan Africa (e.g., *Tithonia diversifolia*) are known to hyper-accumulate significant concentrations of organic nitrogen; these plants can be used as mulches to reduce the synthetic fertilizer requirements of subsistence farmers (Kimetu *et al.*, 2004). This observation leads us to wonder if there are other rich sources of invisible macro- and micro-nutrients that exist in the tissues of the >300,000 other land plants that could be discovered inexpensively using microbial biosensors. Additional microbial biosensors could be used to screen for decreased uptake of aluminum in the soil, a problem in irrigated and tropical soils (Mengel and Ernest, 2001), rather than having to wait for signs of visible damage or reduced growth. Using 96-well plates, extracts from these crop accessions could also be explored for micronutrient content to aid crop biofortification efforts (e.g., Ca^{2+}, Fe, Se, Zn) (Uncu *et al.*, 2013). Microbial biosensors might help breeders to quantify nutrients in 10^4-10^6 progeny, for example to facilitate selection for improved nutrient uptake or scavenging across multiple geographic locations and growing seasons. Nutrient biosensors could also be used as tools in mutagenesis screens to help discover the overlying network of regulatory genes involved in plant nutrient uptake and transport. Finally, microbial biosensors for use with soil extracts may help researchers to characterize plant genotype-soil interactions at the field level, providing unprecedented spatial and temporal (seasonal) resolution of nutrient fluxes. In turn, this could help researchers better understand crop yield variability at the field level. Such an application would capitalize on a key advantage of microbial biosensors compared to analytical chemistry: microbes measure only the bioavailable and not bulk fraction of soil nutrients.

In terms of transgenic plant-based nutrient biosensors, they have significant potential to help researchers who use model systems such as *Arabidopsis* to undertake basic research. For example, plant-based biosensors could be used to help researchers

understand the spatial-temporal dynamics of plant nutrient uptake and transport including at the cellular and subcellular levels, including in source and sink organs. Looking into the future, plant-based biosensors that communicate nutrient availability below ground to a visible output above-ground, may help researchers understand the effects of soil ecological variation (e.g., rhizosphere microbes) and biophysical variation (e.g., soil pH, moisture texture and nutrients) on plant metabolism. For such applications, the nutrient sensor would be in the root, but the transducer would be a mobile signal transmitted to a linearly responsive reporter expressed in the shoot. Perhaps to allow biosensors to have a competitive advantage over conventional analytical chemistry techniques, older reporters that require destructive sampling, such as *lacZ* or *gus*, could be swapped with *lux/luc* or *gfp,* to permit non-invasive detection.

We caution that none of these technologies will substitute for full ionomic profiles of plants (Wiechert *et al.*, 2007); rather their primary use will be in situations where only one or a few minerals require quantification and/or localization.

VIII. CONCLUSIONS

Plant scientists are being called upon to optimize crop yields for biofuels and industrial materials as well as to feed the expected three billion additional people by the end of the century, while more efficiently utilizing fertilizers and adapting to degrading soils (Karp and Shield, 2008; Rothstein, 2007). By turning invisible nutrients into visible signals, akin to anthocyanins, transgenic whole-cell biosensors have tremendous potential to improve the tissue/cellular resolution of nutrient detection in plants and soils, and may also reduce the cost and labor required. In the past, the focal point of plant biosensor research has been the detection of environmental pollutants, but as we have demonstrated, biosensors designed against soil-derived nutrients may have a greater long-term impact on plant biology research. Transgenic plant and microbial-encoded biosensors promise to permit the exploration of much larger numbers of plant samples (e.g., different developmental stages, diverse species, germplasm accessions or breeding populations) than has ever been realistically envisioned to help improve our understanding of plant-soil nutrient interactions, and they may facilitate crop improvement.

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