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SHORT COMMUNICATION

Tracking transience: a method for dynamic monitoring of biological events in *Arabidopsis thaliana* biosensors

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Abstract

Main conclusion **The activation and level of expression of an endogenous, stress-responsive biosensor (bioreporter) can be visualized in real-time and non-destructively using highly accessible equipment (fluorometer). Biosensor output can be linked to computer-controlled systems to enable feedback-based control of a greenhouse environment.**

Today's agriculture requires an ability to precisely and rapidly assess the physiological stress status of plants in order to optimize crop yield. Here we describe the implementation and utility of a detection system based on a simple fluorometer design for real-time, continuous, and non-destructive monitoring of a genetically engineered biosensor plant. We report the responses to heat stress of *Arabidopsis thaliana* plants expressing a Yellow Fluorescent Protein bioreporter under the control of the *DREB2A* temperature-sensing promoter. Use of this bioreporter provides the ability to identify transient and steady-state behavior of gene activation in response to stress, and serves as an interface for novel experimental protocols. Models identified through such experiments inform the development of computer-based feedback control systems for the greenhouse environment, based on in situ monitoring of mature plants. More broadly, the work here provides a basis for informing biologists and engineers about the kinetics of bioreporter constructs, and also about ways in which other fluorescent protein constructs could be integrated into automated control systems.

Keywords Bioreporter · Fluorescent proteins · Plant stress · DREB2A · Arabidopsis

Introduction

The increase in human population mandates a proportional, if not greater increase in agricultural yields; complicating this goal is the diminishment of natural resources (e.g., water and arable land). Classically, a major approach to addressing these issues has been based on selective breeding or genetic engineering of crops in order to increase their baseline hardiness and/or ability to efficiently utilize resources (Varshney et al. 2011). Concurrent with these approaches have been efforts to develop and apply technologies toward monitoring and understanding the physiological responses of plants to stress (Liew et al. 2008; Chaerle et al. 2009; Wee and Dinneny 2010). This second, more recent approach is based on leveraging the finely tuned and highly sensitive mechanism plants have developed to sense, to respond, and to adapt to changes in their environment.

Appreciation of this internal decision-making process in plants has led to the development of methods to monitor relevant natural physical phenomena, such as, changes in

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the chlorophyll fluorescence spectra (Liew et al. 2008; Chaerle et al. 2009). Another route has been the direct engineering of plants to act as "vital reporters" both of their own health and of internal decision-making processes (so called "biosensors"). By coupling knowledge of the genetic cascades underlying stress responses with reporter proteins [e.g., beta-glucuronidase (GUS), Luciferase (LUC), or fluorescent proteins (FP)], it is possible to visualize genetic events linked to/associated with stress responses (Liew et al. 2008). Indeed, prior research has demonstrated that both endogenous and synthetic promoters can be used as "biomarkers" for a variety of stress conditions, ranging from nutrient deficiencies to pathogen infection, with the appropriate choice of promoter depending on a number of factors, including, ease of interpretation, signal-to-noise ratio, and the timeliness of data acquisition (Liew et al. 2008; Antunes et al. 2011). Thus, biomarkers have the potential of informing the investigator, in real-time, of the magnitude of a wide variety of physiological events.

In particular, the use of FPs has distinct advantages; namely, FP outputs are observable using widely available equipment (e.g., a fluorescent microscope) and require no exogenous additives. As yet, however, there are no implemented demonstrations employing these or other systems for obtaining a rapid, non-destructive monitoring of plants' stress state in real-time. Furthermore, there are no cases where such systems have been linked with grower or environment-control decisions. With the increasing availability of portable meters for measuring fluorescence (Millwood et al. 2003), it is now feasible to transit this technology from the lab to the greenhouses and the fields, thereby utilizing these tools in support of regulating agricultural systems and in enhancing crop yields, an objective of this paper.

For the work reported here, we chose to study a biosensor design based on the well-studied *DREB2A* promoter (*pDREB2A*) controlling a FP reporter (Sakuma et al. 2006a, b; Yoshida et al. 2008). Our choice was motivated primarily by three considerations. First is the fact that much is known about the biology of DREB2A as an activator of stress response pathways (Yoshida et al. 2008; Sakuma et al. 2006a). Secondly, DREB2A can be viewed as a physiological integration point between the heat-stress, ABA/non-ABA drought-stress, and salinity-stress networks (Sakuma et al. 2006b; Todaka et al. 2015). Lastly, our selection of DREB2A was based on earlier reports that this promoter displays a well-defined, discreetly positioned (around temperatures that correspond to significant and unambiguous heat-stress), and strongly induced activation profile (Sakuma et al. 2006b; Hua 2009). Using a promoter that would activate at lower temperatures, we felt would complicate an understanding of our system. Accordingly, for this effort we view *DREB2A* activation as potentially correlated with an overall decision to address non-trivial stressors.

Heat stress was selected as the model stressor because temperature is a significant environmental stressor and because temperature can be reliably and precisely controlled in both the laboratory, and in the greenhouse. Our major objectives were to enable the direct observation of genetic changes in real-time and to enable a plant to effectively "communicate" with a computer, thereby bridging the divide that exists between agricultural control systems and biosensor elements. Using this approach we aimed to resolve a major weak point in how a grower understands and interprets when a plant experiences stress. Namely, we consider an approach for evaluating a plant's stress status before phenotypic manifestations of yield loss have developed. Given that plants are informed and active participants in their environments, we consider how a biosensor-based approach provides insights of internal, biological decision-making processes that allow plants to respond to and even alter their external environment.

Through utilizing commercially available equipment, we advance plant biosensor research by developing a method for continuous FP monitoring that can be linked to a greenhouse feedback control system. Not only have we validated the metering system against classical metrics of fluorescent microscopy and RT-qPCR, but we also demonstrate the applicability of this approach as both a scientific tool and as a control interface. Through this system, it should be possible to enable plants to report stress conditions and to signal the need for environmental adjustments. Such a point is especially salient given that many research groups have either identified or developed promoters that activate in response to known types of stress (Liew et al. 2008; Liu et al. 2011; Hou et al. 2012; Venter and Botha 2010); however, the ability to implement these genetic constructs as enactable early-warning systems that report the signal output to a human or computer monitor, remains to be established. This capacity for communication would help avoid/reduce the consequences of stressors on the plants, which, in an agricultural setting would translate into increased crop yield.

Materials and methods

Construction of reporter cassette

Briefly, the pDREB2A reporter cassette was assembled by cloning the −1.8 kbp region upstream of the DREB2A's start codon into a pGreenII0179 plasmid carrying H2B:YFP and a NOS terminator. Primers used for the isolation of the promoter fragment were AAGGTACCACGGTCG

TCGAAGAAAAGAGA and ATGGATCCCTCCTTCCCA GAAACAACACA, with the restriction sites underlined. The topology of the associated expression cassette is provided in Online Resource 1.

Generation of biosensor plants

Transgenic *Arabidopsis thaliana* (Col-0 background) were generated using the Agrobacterium floral-dip method (Clough and Bent 1998). All seeds were sterilized prior to plating by sequential washes in isopropanol (5 min), bleach containing 0.02 % Tween 20 (10 min), followed by three washes with sterile water. Final transgenic lines were selected by identifying plants that showed both a low level of YFP expression at room temperature and a strong YFP induction profile upon being heat shocked. This screening was accomplished using fluorescent microscopy and manually induced heat shocks, with the screening process occurring up to the T3 generation. The selected lines were used for subsequent experiments (unless otherwise noted).

Heat-stress experiment equipment and design

All heat-shock characterization experiments were conducted using a Precision Thelco Model 2 benchtop oven. The temperature regulator was adjusted to maintain 38 °C whenever the oven was on. Precise measurements were taken using three wireless temperature sensors (Crossbow MTS420 motes) placed in the oven adjacent to the plant samples. 4- to 5-week-old plants (with approximately ten leaves) were grown at 23.5 °C (8 h light, 16 h dark) and used for the heat shock experiments. At the beginning of each experiment a clamp, linked to an optical fiber cable, was gently affixed to a leaf and the whole plant placed into the above-described oven, so that the whole plant was subjected to specific durations of pulsed 38 °C heat, as indicated in the figures. Fluorometer measurements were made from the clamped leaf using either a GFP Meter or GFP III Meter (Opti-Sciences Inc). Both meters had custom firmware upgrades (performed by Opti-Sciences Inc.) that enabled time-course measurements.

qRT-PCR assay

Leaf tissue was collected from plants along a time-course of 0, 0.5, 1, 2, 4, 5, and 24 h time points. RNA was extracted using TRIzol (Life Technologies). cDNA was synthesized from 2 μg total RNA in a 25 μl reaction volume using M-MuLV Reverse Transcriptase (New England Biolabs). The cDNA was diluted two-fold with water before qPCR. Each qPCR reaction consisted of 5 μl of diluted cDNA sample, 10 μl of SsoFast EvaGreen Supermix (Bio-Rad), and 1 μl of each 10 μM primer, to which water was added for a final volume of 20 μl. The Bio-Rad CFX96 System Thermocycler was used for all qPCR experiments. Primers used for H2B:YFP, *DREB2A* (AT5G05410), and *ACTIN1* (AT2G37620, reference gene) appear in Online Resource 2. All qPCR reactions were run in triplicate with a standard curve for each gene, with the relative expression level of H2B:YFP and *DREB2A* normalized to *ACTIN1*. For confirmation, an independent replication of the experiment was run with a new cycle of heat shock, RNA extraction, cDNA synthesis, and qPCR quantification being performed. The resulting trends from the two independent experiments are highly similar; Fig. 1 represents only one of the experiments.

Miniature greenhouse experiments

A small indoor test greenhouse was constructed with a simple temperature control system capable of maintaining temperatures between room temperature (25 °C) and 41 °C, a range that includes all temperatures used in our characterization experiments. The actuation specifications are described in the supplementary materials as Online Resource 3. The greenhouse had two distinct operating modes corresponding to different experimental protocols:

1. For precise characterization experiments the greenhouse could be programed to track staircase-shaped temperature profiles irrespective of the measured fluorescence measurement (open-loop).
2. For system integration experiments the greenhouse operated in "closed-loop" mode, computing the appropriate temperature based on the fluorescence measurements alone for the purpose of achieving and maintaining a target fluorescence measurement.

In the latter case, experiments proceeded as follows: after a 4 h period at room temperature during which a baseline fluorescence level was determined, a target fluorescence level was specified as a multiplicative factor of the baseline. The greenhouse was then switched to the closed-loop mode and left undisturbed for 36–48 h, after which time data were downloaded for analysis in MATLAB.

Results

Validation of a real-time, high-resolution, and non-destructive monitoring method

In order to validate the Opti-science General Fluorescence Probe fluorometer (GFP meter) for measuring the levels of fluorescent protein (FP) expressed by heat-stressed "biosensor" plants, we first compared the GFP meter

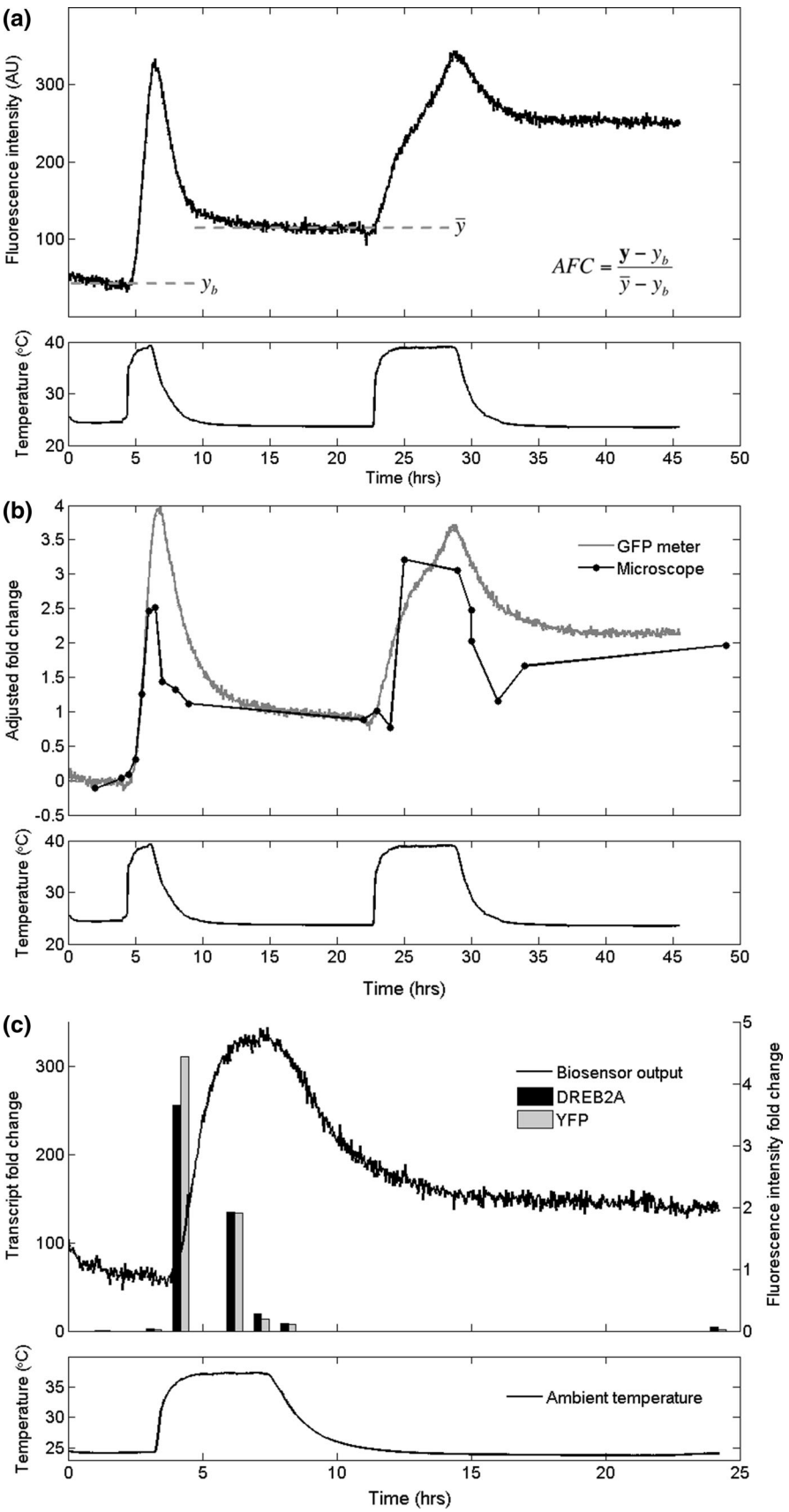


Fig. 1 Comparing meter results to classical techniques. **a** Illustration of the normalization process with marked variables and "adjusted fold change (AFC)" normalization equation. Variables are y_b averaged baseline, *y-bar* averaged baseline after first heat shock, *y* data point being averaged. The "*Y*" axis units are in arbitrary units of fluorescence, as recorded by the meter. **b** Plant response to heat stress as measured by meter and microscope. Meter and microscope data are from one plant each, with normalization described in **a**. **c** Biosensor fluorescence compared to transcript levels (via qRT-PCR). All qRT-PCR time points are from different individuals in the "crop" that were heat shocked with a single-metered ("biosensor") plant. Note how the relative mRNA abundance approximately correlates with the slope of the raising fluorescence trace. "Fluorescence intensity fold change" refers to normalizing the fluorescence against the average initial (unstressed) basal levels of fluorescence recorded by the meter

readings with standard fluorescence microscopy measurements. Because quantitative comparisons of measurements came from different hardware, each with its own calibration function, it was necessary to establish a normalized baseline for comparison. Based on the fact (for the GFP meter) or assumption (for the microscope) of a biased linear calibration, a direct comparison requires two-point normalization to correct for scale and to offset differences. Since quantitative comparisons were performed exclusively for double-pulse experiments, we established a normalization formula for shift/scale correction using two points (Fig. 1a). The calculated R^2 value of 0.85 found between the normalized traces (Fig. 1b) confirmed that there is a strong correspondence between the outputs of these two methodologies, which is in agreement with previously reported results from Opti-Science comparing the meter readings against spectroscopic measurements (Millwood et al. 2003).

Additionally, the validation experiments demonstrated the distinct advantages of using the GFP meter-based approach over a fluorescence microscope, including (1) a reduction in manpower needed to prepare the tissue for monitoring, and (2) a simplification of the data collection protocol. The second property is significant, because the ability to program the meter to make nearly continuous measurements allows for an unprecedented temporal resolution of the FP kinetics, especially when compared to measurements classically obtained with the microscope. Furthermore, the fluorescence microscope setup required repeated re-sampling of the plant (taking a new leaf) for each new measurement, whereas the meter-based approach utilized one leaf that remained attached to the plant for the entire time-course of the experiment.

Next, in order to demonstrate how well the fluorescence kinetics of the meter's observations corresponded with the underlying molecular events of our reporter construct, we used qRT-PCR to compare mRNA abundance of the reporter construct at different time points during the meter's measurements (Fig. 1c). As a comparison point against the endogenous stress-response, we also measured the mRNA levels of the native *DREB2A* gene. Not only did the reporter transgene expression mirror the endogenous gene, but the overall pattern in fluorescence protein accumulation paralleled trends in the mRNA levels. Mathematically, the rate of protein accumulation has long been seen as related to mRNA concentration (Alon 2013).

The observed shift in baseline after each heat stress was expected partly as a result of stable protein accumulation. However, the reason for the transitory "spiking-and-rapid-decay" behavior observed is not clearly understood. This latter phenomenon could be unreported behavior revealed by our fine-level observation of FP-dynamics in planta, or represent a dynamic associated with the H2B-YFP reporter protein. Additional considerations of this matter are included in the "Discussion." Because this behavior was not a focus of the project, no further investigation was made on these observations.

Obtaining high-resolution monitoring of promoter activity over varying conditions

Building on the ability of the GFP meter to measure changes in fluorescence in a real-time and non-destructive manner, we next sought to demonstrate how the biosensor design could be applied to monitoring the effects of dynamic environments. With this approach, it is possible to monitor events that would otherwise be extremely cumbersome to track by other methods, such as fluctuating temperature. Moreover, because of the high temporal resolution of the meter and non-invasive protocol of the whole experiment, it is possible to observe closely spaced, subtle responses of a single, uninjured plant.

The low effort and ability to automate the entire experiment provided the opportunity to study in situ promoter activities in novel ways, as described in the methods section. These results are illustrated in Fig. 2, in which the temperature was either cycled or incrementally raised in a staircase fashion. Fig. 2a experiment demonstrates a practical, real-world situation regarding how plants respond to fluctuating temperature; Fig. 2b highlights the utility of the method as a basis for further scientific research, such as identifying the activation parameters of a promoter. Specifically, Fig. 2b confirms that *pDREB2A*'s induction temperature is approximately 34 °C [in agreement with other reports (Hua 2009)]. Moreover, we suggest that the significantly pronounced response at 37 °C could be either from intrinsic or history-dependent properties.

In both experiments in Fig. 2, the manpower efforts necessary to gain very clear data were trivial once the experimental apparatus was assembled. Based on this advantage, we also decided to study how the proportional response varied across the generations of our transgenic line. The results of this work are illustrated in Fig. 3, which shows that the overall (normalized) behavior of the biosensor line remains highly similar among plants and generations.

Closing the control loop: establishing a computer-plant interface "handshake" and reciprocal feedback

An important objective of this work was to demonstrate a method for interfacing a computer with a biological signal, thereby enabling an infrastructure that could be used to optimize plant health (and yield) during times of environmental stress. Such a system is conceptually illustrated in

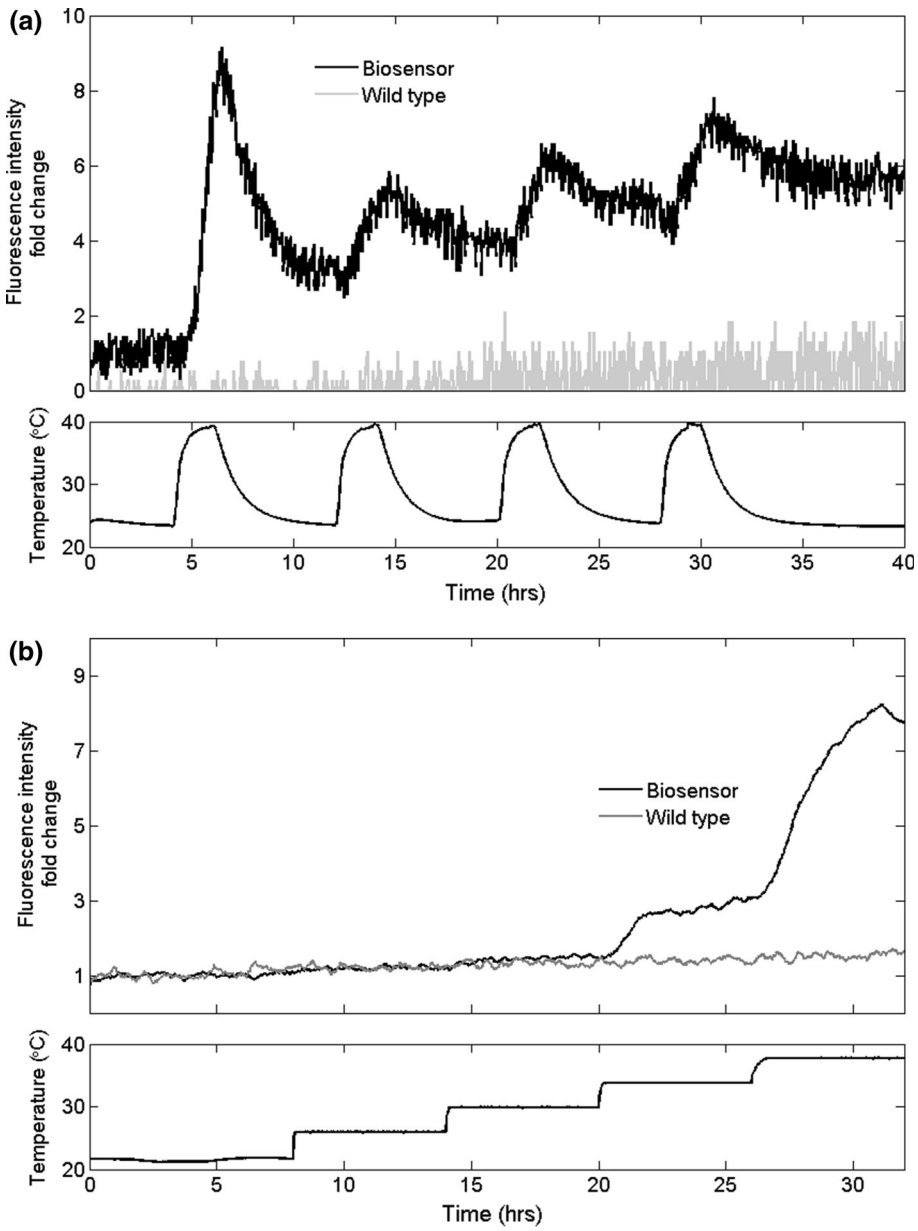


Fig. 2 Dynamic observation of "biosensor" output relative to temperature. **a** Pulsed on/off temperature oscillations, studying dynamic promoter activation/deactivation through FP-proxy; **b** staircase increase in the ambient temperature, with the goal of identifying the threshold temperature that induces *pDREB2A* expression. "Fluorescence intensity fold change" refers to normalizing the fluorescence against the average initial (unstressed) basal levels of fluorescence recorded by the meter

Fig. 4a, in which we view the biosensor plant facilitating a control system based on two feedback loops: an outer loop based on reading the biosensor for determining the environmental parameters, and an inner environmental control loop that realizes and maintains these parameters (by means of mechanical actuators and sensors for abiotic factors like temperature or moisture).

Toward the objective of demonstrating how FP-based biosensors can inform environmental control system decisions, we assembled a miniature greenhouse (details of which are discussed in Online Resource 3) that could be interfaced with our GFP meter, and hence, to the plant. To test the setup, we chose to task the greenhouse with maintaining a defined fluorescence level in the plant, which echoes a previous demonstration in a yeast system (Milias-Argeitis et al. 2011).

Using insights gained from the previous open-loop characterization of the biosensor dynamics, and considerations from control theory (see "Discussion"), we postulated the effectiveness of the relay controller shown in Fig. 4a, which is located inside the block labeled C_F in Fig. 4b. Qualitative simulations are shown in Fig. 4c, with the results of a representative closed-loop experiment using an *Arabidopsis* biosensor shown in Fig. 4d. Taken together these results not only support our postulate that our underlying system can be modeled as a hysteretic (history-dependent) loop, but also

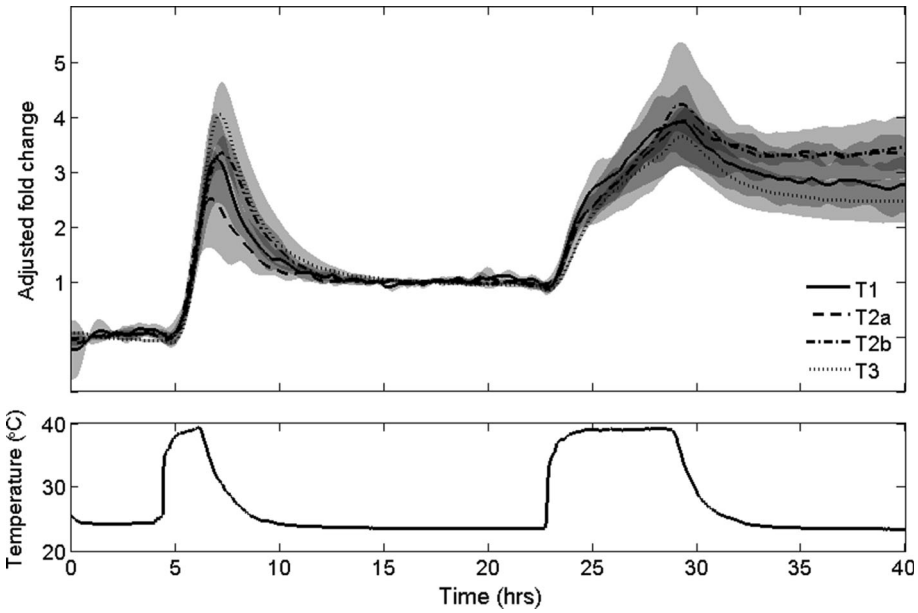


Fig. 3 (Normalized) Heat-shock response by different generations of the reporter line. *Each line* represents the average of four individual plants, with the *shaded area* representing one standard deviation. "*T#*" refers to the generation being tested, with "*a*/*b*" referring to the testing of two *different lines* from the same generation. All data are normalized as previously shown in Fig. 1a

that integrating the biosensor-feedback loop into the control system is relatively straightforward.

Discussion

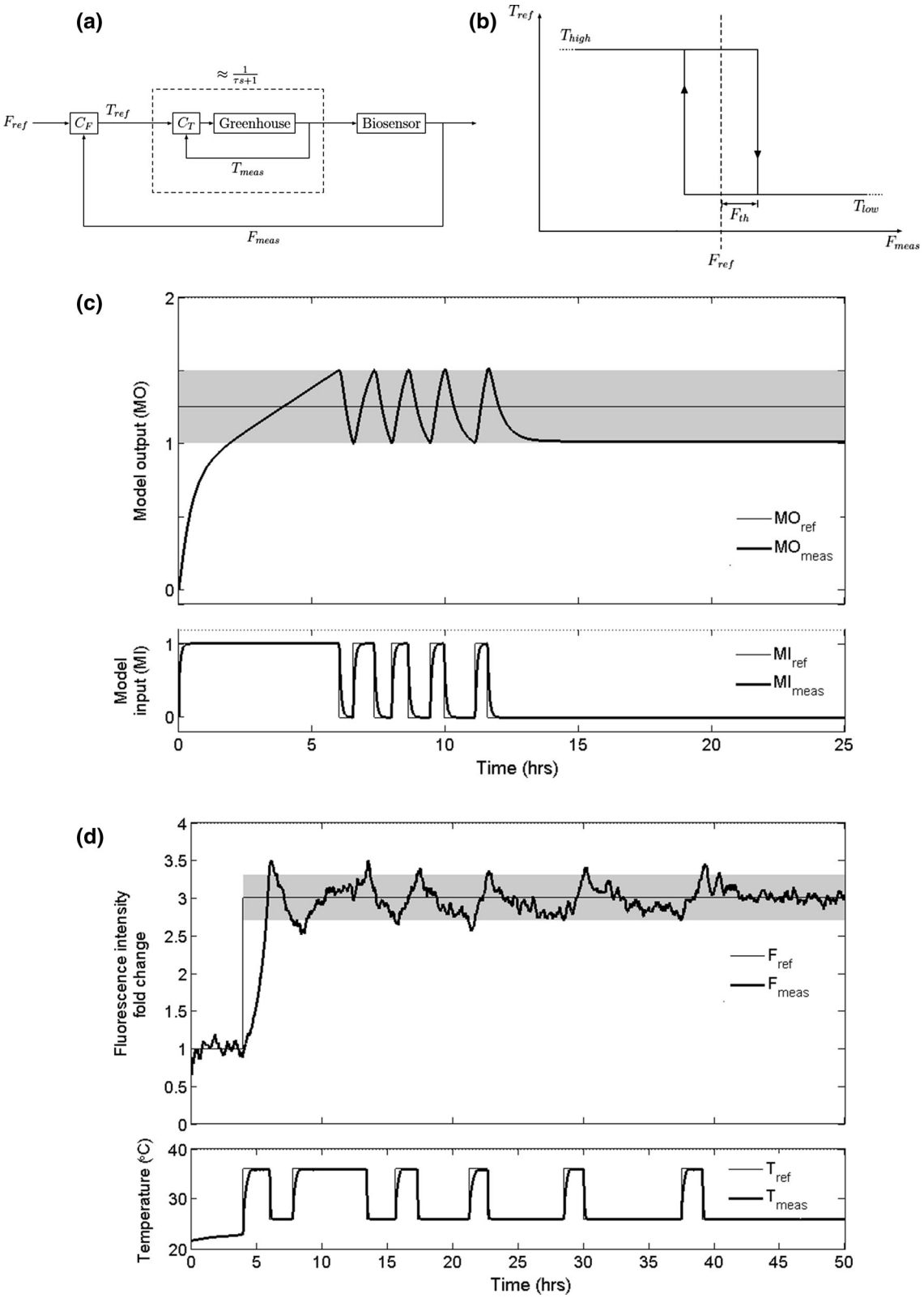
Since the first suggestions of utilizing fluorescent proteins in plants to track biological processes, this approach has seen increasing prominence. Today, there are examples of identified or synthetically designed genetic components that can be linked to GFP for rapidly converting details of plant health into a fluorescence output (Liew et al. 2008; Liu et al. 2011; Hou et al. 2012; Venter and Botha 2010). Complementary to this work are the continuing efforts directed at increasing the automation of crop maintenance, as well as improving the overall efficiency of agriculture (Edan et al. 2009).

Yet, the linkage between the biology and engineering aspects of "intelligent automation" within the cultivation process have remained disjointed; little work has been done on linking GFP-based "biosensor" systems with the technical and theoretical components of environmental control systems.

On one level, our experiments highlight the simple utility of using the GFP meter for real-time monitoring to observe and to understand biological dynamic behavior. On another level, we also demonstrate proof-of-concept work, successfully bridging the divide between plants and environmental control systems. This higher-level linkage is important because in order to address the issue of resource limitations now facing agriculture, it will be necessary to optimize yield in relation to energy input/expenditure. Integrating plants into control system architecture enables such optimization, because this allows biological events to be observed on the same time scale as mechanical actuations, as discussed subsequently. Thus, it becomes possible to correlate plant physiology/biology, yield, and resource allocation directly within the context of agricultural production systems.

Biosensors can be utilized as tools for understanding dynamic biological behavior

The metering methodology outlined in this paper is a resilient, non-destructive approach for acquiring high-resolution, long time-scale data from a single plant. Our work therefore could have utility in addressing a new generation of inquiries about the genetic responses of plants, which includes questions regarding the behavioral consistency observed across individual plants, the expression "dynamics" (i.e., kinetics, behavior, and trends) of promoters/genes under different or changing conditions, and observations related to plants' "(long-term) memory" of past stress. The ability to amass significant amounts of data with minimal effort makes the meter/FP system particularly well suited to such objectives. Many of these potential gains have been hinted at through the various experiments demonstrated in the Results section. For example, as seen in the characterization experiments of Fig. 1c, and in the distribution plot of Fig. 3, there is a highly consistent response trend among the individual crop members and the biosensor plants. This raises questions such as (1) what is it about the nature of the promoter that enables such highly synchronized dynamic behavior across individuals, and, (2)


(a)
$\approx \frac{1}{\tau s+1}$
F_{ref}
C_F
T_{ref}
C_T
Greenhouse
Biosensor
T_{meas}
F_{meas}
(b)
T_{ref}
T_{high}
T_{low}
F_{th}
F_{meas}
F_{ref}
(c)
Model output (MO)
MO_{ref}
MO_{meas}
Model input (MI)
MI_{ref}
MI_{meas}
Time (hrs)
(d)
Fluorescence intensity fold change
F_{ref}
F_{meas}
Temperature (°C)
T_{ref}
T_{meas}
Time (hrs)

◀**Fig. 4** Greenhouse-biosensor feedback control experiment. **a** Block diagram representation of the entire closed-loop system. *Dashed line* indicates component described by the provided equation. Appropriate abbreviations are C_F fluorescence controller, C_T temperature controller, *biosensor* the engineered transgenic plant, T_{ref} target temperature determined by C_F, T_{meas} measured temperature, F_{meas} measured fluorescence. **b** Hysteresis curve defining block C_F's response parameters. Additional abbreviations are F_{ref} target/reference fluorescence level, F_{th} threshold of fluorescence levels, T_{high} temperature set-point corresponding to when heaters are active, T_{low} temperature set-point corresponding to when heaters are inactive. **c** Simulated run of control system program using an empirically derived model of temperature's effect on "biosensor" output. **d** Results from a representative closed-loop experiment demonstrating the ability to achieve feedback control. "Fluorescence intensity fold change" refers to normalizing the fluorescence against the average initial (unstressed) basal levels of fluorescence recorded by the meter

why has evolution selected for such tight regulation of *DREB2A* across many individuals? For the latter of these biological questions, we suggest that the reason is related to the *DREB2A* gene's position as a "signal integrator" of at least three stress response networks (heat, drought, and salinity), and also to its function as an activator of downstream networks (Sakuma et al. 2006a, b; Todaka et al. 2015). An argument for such stringency is that because stressors are extremely destructive, and that because any adequate/systemic response may require significant energy allocation away from growth, that the range of response-profiles conferring optimal survival may therefore be very limited. Thus, population homogeneity might have arisen as a result of only a few types of behaviors being optimal for an individual's survival.

These results demonstrate a unique advantage of being able to track individual plants for the full length of an experiment. By following several plants within a "population" it is possible to directly (and in a facile manner) question how tight a population's response synchronization is for any particular stimulus. Given recent work establishing that single-cell populations can exhibit divergent and stochastic behavior under the same conditions (Altschuler and Wu 2010), exploring if or how much such behavior is seen at the level of individual, multicellular organisms, such as higher plants, would be of great interest. Additionally, meter time-coursing of many individuals can help avoid (or, at least, alleviate) some of the inherent assumptions of synchronicity among population members that often implicitly underlie qPCR or Western blotting time-course experiments.

On a more systems biology/control theory level, there may be value in implementing some natural "signal integrator" promoters as biosensor/biomarker components. This reasoning is based on the likelihood that such promoters could help to identify whether stressor conditions within the context of a dynamic environment are triggering larger response networks, in addition to particular subsets of the transcriptomes (as reported by using a very stress-type specific promoter).

DREB2A promoter functions as a mathematical "integrator"

At the most abstract level, the meter/FP combination we characterized echoes Olson and Tabor's (2014) concept of an oscilloscope for biological systems, whereby the meter is able to readout dynamic changes at the level of gene activity (as given via FPs). High-density data that can be used to visualize the detailed effects of fluctuating environments, transient and persistent, lend itself to establishing gross input/output models, rather than deriving models from first principals (a process that would require a more detailed quantitative understanding of the underlying molecular interactions than we currently possess). Again, using *pDREB2A* activity as an example, it is clear from all the collected sensor outputs, and in light of an understanding of common control system representations, that the biosensor system we are measuring approximately "integrates" (in the mathematical sense) the input over time. When the temperature input is a step, and the temperature is rapidly increased to a new steady state, the sensor output in steady state is roughly a ramp (an upward linear trend after a transient). When the input is a pulse, and the temperature is increased for a period of time and then returned to room temperature, the output in steady state is a step.

Interpreting these signatures from the perspective of control theory (Åström and Murray 2008) suggests that any representation of the dynamic behavior of this system should include a mathematical integrator, which has the important implication that the system is easy to control in the iterative sensing/computation/actuation paradigm, a topic touched on at the end of this section. Furthermore, the "step response" of fluorescence to a temperature pulse suggests that our sensor system might have memory-like properties. Specifically, that the final (steady-state) level of each step is related to the duration of the applied pulse. However, because the work here was focused on application of the sensor system to environmental regulation, no exploration was conducted regarding the mechanism of this proposed memory. We do, however, encourage further identification and exploration of other systems whose steady-state levels afford insight into the organism's history.

Finally, in considering the possibility of mining such data for new insights of gene dynamics, it is also important to consider some of the caveats of our current implementation. The most important and obvious point in this regard is that the meter ultimately measures the level of the FP reporter. Thus, the type of data retrieved can be highly

influenced by factors affecting the reporter's stability. In this context, we should note that because we were using a YFP fused to the full length H2B histone protein (originally chosen to take advantage of the nuclear localization for improving the signal-to-noise ratio) as the reporter output, there are some questions as to the behavior we observed. Because there are no previous detailed reports on the H2B:YFP fusion protein's in vivo dynamics, and also because histone content is dynamically regulated at the protein level (Singh et al. 2009), some of the observed behaviors of the reporter protein—namely the transient "spike-decay-stabilize" seen in the figures—may relate to the particular fusion construct that we utilized. Additionally, some comment is necessary about the remarkable stability of the fluorescence level over long periods of time. Because GFP [the base protein core for YFP (Tsien 1998)] is noted to have a general half life around 24 h (Corish and Tyler-Smith 1999), we are prompted to consider whether the flat line of fluorescence intensity is due to an equilibrium between H2B:YFP production and decay, or the result of potential chromatin sequestration of protein (Boisnard-Lorig et al. 2001).

While the stable H2B:YFP construct was sufficient for tracing the activation and activity of *pDREB2A* for feedback control purposes, we suggest that other FP-constructs should also be considered in the future. First, this includes FPs tagged with degron signals (often referred to as "destabilized FPs"), which turnover more rapidly and have been reported to be able to track mRNA levels more dynamically (Li et al. 1998; Hackett et al. 2006). Second, we suggest that consideration of the classical FP-fusions to the gene products of the promoters of interests should allow for factoring in the effect of post-translational regulation on the "effective output level" of a gene, and a real-time relative quantification of protein. Lastly, we encourage the utilization of rapid-maturation FPs (Pédelacq et al. 2006) or variants of, especially because maturation time has been one factor considered an issue in utilizing FPs for tracking purposes (Mazo-Vargas et al. 2014).

Overall, we propose that the ability to obtain and analyze such quantitative real-time data, as we have here described, will enable new understandings of the nature of biological processes, while also improving our ability to model genetic processes in plants. Furthermore, the ability to track and characterize leads to the ability to predict and to control. It is this capacity to predict and to control that ultimately enables enhancement of agricultural output.

Biosensors are functional components that complete and enhance the control loop

In considering the merits of biosensors and their appeal for augmenting control systems it is important to consider the differing roles that electromechanical and biological sensors currently play in protected agricultural settings. Electromechanical sensors are very fast, inexpensive, and quite accurate with proper calibration. However, the environmental states that they quantify are de facto external to the growing plants, and hence are generally non-linear correlates of the plant stress state. In contrast, biosensor systems are relatively expensive and slow to measure, but because they are fundamental and internal to the growing plants, when properly chosen they directly relate to the stress state of the organism. As such, the best approach to maintain the optimum environment for healthy plants is to combine sufficient biosensor-based measurements to establish environmental set-points, with electromechanical devices to maintain the required environment.

Within the development of electromechanical control systems is the sensing–computation–actuation paradigm. In this paradigm a factor of interest that needs to be controlled is measured (sensing), a decision then is made regarding the appropriate action (computation), and finally the decision is enacted upon the system (actuation). The middle step, i.e., computation requires (1) a representation of the system's expected behavior, and (2) a specification of the system's goal, in order to define what action is considered "appropriate." These two requirements are often derived from first-principles analysis or from detailed simulation, and presume an understanding of the system to be controlled.

This entire sequence (sensing/computation/actuation) is repeated as necessary until the goal is achieved, with success only possible when iteration proceeds fast enough. If sensing is too slow, the system may change so much between measurements that recovery is impossible. It is for this reason that many classical stress-quantification techniques are not fully adequate for the task of enabling efficient and effective corrective action within the relevant time spans of certain crop stressors (e.g., temperature, drought, or light stress). Furthermore, slow measurements of rapidly changing systems may also obscure dynamic properties of the system that could be critical when building a suitable representation of the expected behavior for the purposes of the computation step.

Our sensor system was designed to reduce measurement delays, for the purposes of understanding the dynamic behavior of the *pDREB2A* promoter in response to heat stress, and applying that understanding to build an environment control system to regulate the activation of the *pDREB2A* promoter. Specifically, we demonstrate a process in which the biosensor is queried at the same rate as the electromechanical climate sensors, creating a seamless and automatic interface between biosensor and electromechanical sensor which allows the climate control system to act at similar time scales to the plant's internal stress response dynamics.

Overall, our work demonstrates that the activation level of an endogenous stress-responsive promoter, here based on a H2B:YFP construct tracing the activity of *pDREB2A*, can be visualized in real-time and non-destructively using highly accessible equipment. The ability to obtain high-resolution data allows for identification of transient and steady-state behavior that is not readily observable by conventional means. Furthermore, we have interfaced the biosensor output with a computer-control system to achieve in situ monitoring of a mature plant, enabling feedback-based control of a greenhouse environment. The work presented here can serve as a bridging point that links the many successfully designed biosensor constructs with automated climate control systems, in addition to enabling a broad spectrum of new experimental protocols based on real-time monitoring, and the reuse of the same plant at different stages of its growth cycle.

Author contributions statement All authors conceived and intellectually contributed to the design of the experiments. GH conducted and processed all of the data retrieved from metering experiments. CM and KJ produced all biological measurements and prepared plants. GH, CM, KJ, and LF wrote the manuscript. All authors agreed on the results.

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