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Gene regulatory circuitry of plant–environment interactions: scaling from cells to the field

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Abstract

Plant growth and development is the product of layers of sensing and regulation that are modulated by multifactorial environmental cues. Innovations in genomics currently allow gene regulatory control to be quantified at multiple scales and high resolution in defined cell populations and even in individual cells or nuclei in plants. The application of these 'omic technologies in highly controlled, as well as field environments is revolutionizing the recognition of factors critical to spatial and temporal responses to single or multiple environmental cues. Within and pan-species comparisons illuminate deeply conserved circuitry and targets of selection. This knowledge can benefit the breeding and engineering of crops with greater resilience to climate variability and the ability to augment nutrition through plant–microbial interactions.

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Keywords

Gene regulation, Transcription, Networks, Cell-population, Single cell, Stress, Environment sensing.

Abbreviations

3C, Chromatin conformation capture; ATAC, Assay for transposase-accessible chromatin; DAP, DNA affinity purification; ChIP, Chromatin immunoprecipitation; CRE, cis-regulatory element; FACS, Fluorescence-activated cell sorting; FANS, Fluorescence-activated nuclei sorting; GRO, Global run-on; HRPE, Hypoxia response promoter element; INTACT, Isolation of nuclei tagged in specific cell types; m6A, *N*6-methyladenosine-modified mRNA; PARE, Parallel analysis of RNA ends; scRNA, seq- Single cell RNA sequencing; TF, Transcription factor; TRAP, Translating ribosome affinity purification; uORF, Upstream open reading frame.

Introduction

Plants are multisensory integrators of the cues of their ever dynamic environment; viability, growth and ultimately fecundity are the product of the integration of these inputs. Besides the perception of light, adjustments to temperature, humidity, water and nutrient availability, plants sense and respond to a plethora of microbial interactions in cultivated and natural environments. All of these inputs are modulated spatially and temporally within the shoot canopy, root system and rhizosphere community. A suboptimal environment may have rapid or prolonged consequences: short term temperature extremes can promote pests or pathogens, whereas nutrient deficiencies or water limitations may increase mutualistic interactions with soil microbes. To limit crop yield loss in Earth's increasingly unpredictable cultivated environments, it is of value to understand the genetic mechanisms whereby plants limit the negative impacts of stress resiliency on growth, whether through developmental reprogramming, metabolic adjustments or promotion of mutualistic trans-organism interactions [1].

Single stress studies in model systems have yielded invaluable mechanistic insight into environmentally activated signal transduction and responses, yet more complex experimental designs are required to replicate a plant's native ecophysiological context. While such investigations are more challenging to execute and interpret, they are instrumental in translating basic research to the field where complex environments are the norm. The implementation of evolving 'omic technologies primarily in studies of *Arabidopsis thaliana* has fostered the discovery of transcriptional and post-transcriptional regulators and regulatory circuits that activate and modulate protective strategies. Environmentally regulated gene networks are increasingly investigated with advanced 'omics methods that leverage greater spatial and temporal resolutions, cross-species comparisons and studies designed in native agro-environments (Table 1). Regulatory networks underlying resilience have also been refined in relation to selection pressures that influence fitness and yield. Here, we consider recent progress and present challenges in defining the gene

Table 1

Representative studies of multi-scale high-resolution analyses of responses to the environment.

'Omics method	Species	Tissue and *Condition*	Major Findings	Refs.
Multi-scale				
ATAC-seq, RNA-seq, ChIP−seq, MethylC-seq, Hi-C, HiChIP	Maize	Leaf *field*	Epigenomic signatures used to predict functional short and long-range cis-regulatory elements	[55]
RNA-seq Proteomics Phosphoproteomics	Maize	23 tissues *control*	Lack of correlation between mRNA and steady-state protein levels; integration of multiscale data improves confidence in gene regulatory networks	[56]
RNA-seq Metabolomics	Maize	Leaf *individual plants. field*	Leveraging of plant-to-plant variation in at two 'omic scales used to associate genes with abiotic and biotic responses	[57]
Hi-C ATAC-seq RNA-seq	Rice, *indica* and *japonica* ssp.	Seedling *heat*	Chromatin accessibility changes and gene expression during heat stress are correlated with chromatin structure; chromatin structural changes are more pronounced in heat-resilient *indica* variety	[58]
ATAC-seq RNA-seq	Rice	Leaf *heat shock, water deficit, circadian, field*	Integrated temporal accessible chromatin, CREs and mRNA data to infer environmentally modulated TF-target gene relationships	[59,60]
INTACT-ATAC-seq Nuclear RNA-seq RNA-seq TRAP-seq Ribo-seq	Rice, *Medicago*, tomato, *Solanum pennellii*	Root tip *submergence*	Conservation of motif use to regulate deeply conserved stress response genes	[17]
Chromatin accessibility Histone ChIP	Potato	Tuber *cold stress*	Cold stress increases chromatin accessibility in gene bodies; induces bivalent H3K4me3-H3K27me3 modification associated with up-regulation,	[61]
RNA-seq TRAP-seq mRNA half-life	Arabidopsis	Seedling *plate, pathogen*	Mutant and multi-scale analyses identify highly unstable immune response mRNAs	[62]
RNA-seq Ribo-seq	Arabidopsis	Leaf *Flg22 elicitor*	5' leader and uORF sequences provide temporal translational control in response to elicitor; used to engineer disease resistance in rice without yield penalty	[63,64]
DAP-seq RNA-seq Histone ChIP Proteomics Phosphoproteomics	Arabidopsis	Seedling *jasmonic acid*	Cross-talk between JA and other hormone signaling pathways prioritizes JA responses; Identification of novel TFs of JA responses downstream of master TF MYC2	[3]
INTACT-ATAC-seq Histone-, RNAPII- and TF-ChIP-seq Nuclear RNA-seq RNA-seq TRAP-seq	Arabidopsis	Whole seedlings *temporal hypoxia*	Identified concordance from transcriptional activation through the translation of core hypoxia-responsive genes; temporal discordance between transcription and mRNA accumulation of heat-responsive and other genes	[8]

Table 1 (*continued*)

'Omics method	Species	Tissue and *Condition*	Major Findings	Refs.
Cell Population				
sc-ATAC-seq sc-RNA-seq	Rice	Roots; dissected and protoplasts *control*	Inference of putative regulators that drive ground tissue differentiation; transcriptome conservation between rice and Arabidopsis varies between cell types	[65]
ATAC-seq TRAP-seq	Tomato	Root cell populations *plate, pot, field*	Cell-population enriched gene transcripts; comparative analyses between cell type and species illuminate similarities and distinctions associated	[28]
RNA-seq TRAP-seq	*Medicago*	Root cell populations *Rhizobial symbiosis*	Translational upregulation of mRNA decay machinery in epidermis and cortex promotes nodulation; association of lncRNA with ribosomes sequesters miRNA to enhance nodulation	[66]
RNA-seq proteomics	Arabidopsis	Root *15 cell types, 6 developmental zones*	Alternative splicing patterns and lincRNAs vary across cell types and developmental zones	[67]
RNA-seq TRAP-seq	Arabidopsis	Root cell populations *pathogenic and mutualistic fungi*	Pathogenic fungi inhibit suberization of the endodermis; antimicrobial secondary metabolite biosynthesis varies based on fungal species in a cell type-specific manner	[9]
Nuclear RNA-seq	Arabidopsis	Leaves and guard cells *Water deficit*	Guard cells respond to water deficit more rapidly than whole leaf nuclear transcriptomes; carbohydrate metabolism changes are specific to guard cells during water deficit	van Weringh et al., *bioRxiv* https://doi.org/10.1101/2021.04.15.43999
Single-cell/nuclei				
FANS-ATAC-seq	Maize, B73 and Mo17 inbred lines	Seedling, roots, tassel and ear primordia, axillary buds *greenhouse*	Accessible chromatin of multiple tissues is used to associate TFs and active CREs of genes as loci for genetic variants influencing phenotype	[27]

CRE, *cis*-regulatory element, JA, jasmonic acid.

regulatory circuitries that integrate environmental cues to enable appropriate modulation of development for ultimate fitness. We focus on analyses of gene regulation from the tissue to cell-type and single-cell levels and their implementation to advance crop resilience.

Methodologies

Environmental regulation of gene activity can occur at numerous steps in the gene expression continuum, from histone modifications and chromatin accessibility and transcription to alternative splicing, regulation of mRNA turnover and translation, to post-translational processes (Figure 1). Multi-omic methods for genome-scale and gene-targeted study of gene regulation continue to become more sensitive and targeted (reviewed by [2]). Highly integrated multi-omic analyses are largely limited to Arabidopsis, for example, the time series analysis of transcription factor (TF)-chromatin binding, transcriptome and phosphoproteome [3]. To date, most analyses of stress responses have been at the organ level and focus on the polyadenylated mRNA transcriptome. Because the homogenization of organs required for mRNA extraction indiscriminately masks mRNA dynamics in specific cells, methods developed in Arabidopsis to evaluate developmentally defined populations of cells are increasingly deployed in crops to evaluate environmental regulation of gene activity in specific populations of cells. These include fluorescence-activated cell or nuclei sorting (FACS and FANS, respectively), laser capture microdissection, the

isolation of tagged nuclei in specific cell types (INTACT) and translating ribosome affinity purification (TRAP) (reviewed by [4]). Single cell RNA sequencing (scRNA-seq) using microfluidic devices has also been accomplished in plants (reviewed by [5] and [6]). INTACT and TRAP are complementary approaches that rely on cell population-specific promoters and are advantageous in the study of environmental responses because the purification of tagged nuclei and ribosomes can be carried out on tissues that have been rapidly frozen to preserve signatures of transient stress responses. INTACT facilitates evaluation of epigenetic regulation (DNA methylation, histone modifications, chromatin accessibility) by use of the assay for transposase-accessible chromatin (ATAC)-seq, as well as nuclear RNAs, whereas TRAP provides a readout of the transcript isoforms associated with ribosomes. Finally, individual plant nuclei can be used for both nuclear RNA and ATAC-seq [7]. The feasibility of capture of environmental responses by scRNA-seq has yet to be demonstrated.

Multi-scale data integration

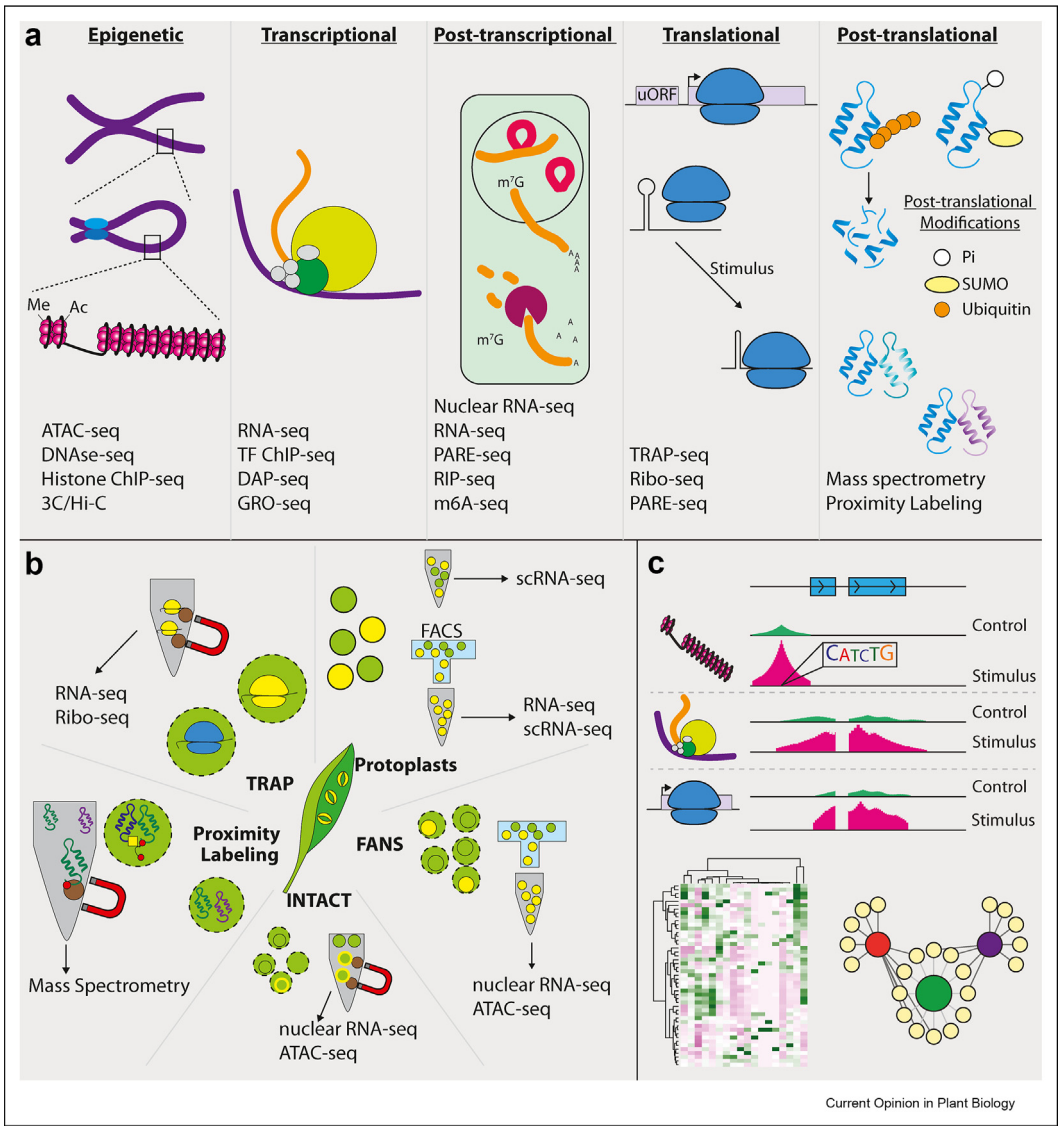
Integration of different data types provides a high-resolution perspective on biological processes and stress responses, revealing detailed knowledge of gene regulatory networks for the identification of critical factors that drive phenotypes of interest. Data integration across regulatory levels, cell types and different species enable the discovery of genes pertinent to traits of interest with high accuracy.

The importance of resolving gene expression across regulatory levels is exemplified by the disparity between transcriptomes and translatomes in response to environmental stimuli [8,9], including in response to time of day. Polysome levels are low at dawn and peak during the day [10]. The translatome is also modulated by time of day, with a greater proportion of the genome regulated at the polysome association level than the total transcript level by the clock in Arabidopsis. Translatome-specific diurnally cycling genes serve critical roles in protein synthesis, mitochondrial function, photosynthesis, and the cell cycle [10,11]. An examination of circadian control of the heat stress response translatome observed that only 70% of cycling heat-responsive transcripts were also responsive at the translatome level, whereas half the heat-responsive translatome was not responsive at the total transcript level [11]. Interestingly, induction of most heat-up-regulated genes occurred when heat stress occurred outside of the gene's peak expression, suggesting that heat stress raises the nadir of heat-responsive genes but not their peak [11]. This not only emphasizes how the time of day can influence experimental outcomes but also may be important for the improvement of heat resilience in terms of climate change as heatwaves become increasingly common [12] and non-peak temperatures become progressively warmer [13,14].

TFs that drive stress resilience are of particular importance for crop improvement because they regulate gene networks and thus have a large impact on phenotypic outcomes. Transcript abundance studies such as RNA-seq can identify putative TFs of interest. However, because TFs can be regulated post-translationally by covalent modification and proteasome-mediated turnover, TF mRNA abundance patterns do not necessarily correlate with their activity [15,16]. Furthermore, predicting the impact of TFs requires knowledge of downstream targets. Reynoso et al. (2019) addressed this question in the context of the rapid response to submergence in root tips, exploiting the variation in flooding resilience across rice, *Medicago truncatula*, tomato and *Solanum pennellii* to examine conservation of submergence responses [17]. By integrating chromatin accessibility determined by INTACT-ATAC-seq with differential regulation of nuclear and polyadenylated transcriptomes and the translatome, they found that upstream regions of evolutionarily conserved submergence-upregulated genes not only become more accessible during submergence but the open regions are also enriched in specific TF motifs (WRKY, bHLH, MYB and HRPE [hypoxia response promoter element] associated with class VII ERFs). Genes with more copies of a TF binding site had more pronounced submergence upregulation, narrowing down possible critical regulators of the submergence response to TFs that bind one of these elements. Despite species-specific bias for the four different enriched TF motifs in submergence-regulated genes, a deeply conserved submergence response network was evident across dicots and monocots [17]. Manipulation of this conserved network is potentially a significant step towards improving flooding tolerance in crops, as modifications could be effective in diverse species. On the other hand, understanding the differences in networks between flooding tolerant and intolerant species can assist in improving flooding resilience in the most sensitive crops.

Pan-species analyses provide valuable insights, and conservation of genomic responses has been a powerful approach towards identifying key conserved response regulators [17,18]. Sequencing has enabled studies to include more diverse plants outside of established model systems, facilitating the study of stress responses in species that occupy niche marginal environments [19–21] or exploring conservation in basal lineages. Recent work in arbuscular mycorrhizal fungi-colonized plants in *Marchantia polymorpha* and several angiosperms demonstrated the transfer of lipids as a carbon source from the host plant to the fungal symbiont was an ancient adaptation and that the TFs and core machinery that control this nutrient exchange is conserved across

Figure 1



Multi-scale approaches to study mechanisms of environmental regulation of gene expression. (**a**) Gene regulation occurs at many levels. Many genome-scale methods facilitate the evaluation of gene regulation from epigenetic through post-transcriptional levels. (**b**) The suite of microgenomic methods used by plant biologists to gain cell-population, cell- and sub-cellular resolution from chromatin to protein complexes [54]. (**c**) Conditionally regulated TF-gene networks can be inferred by integration of accessible chromatin exposing TF binding sites of gene transcripts monitored by nuclear RNA-seq, total RNA-seq or translated mRNA RNA-seq (TRAP or Ribo-seq). Network pipelines may require TFs to be co-expressed. 3C, chromatin conformation capture; ChIP, chromatin immunoprecipitation; DAP, DNA affinity purification; GRO, global run-on; m6A, *N*6-methyladenosine-modified mRNA; PARE, parallel analysis of RNA ends; RIP, RNA immunoprecipitation.

land plants [22]. Such analyses not only provide insight into the evolutionary history of plant interactions with their environment but also reveal high-value manipulation targets for the improvement of many crops.

Cell-type and cell-population resolution

Transcriptomics on total tissue samples obscures cell type-specific responses [23,24]. Environmentally regulated responses at the cell population level have been studied by capturing cells that express GFP regulated by cell- and region-specific promoters by fluorescence-activated cell or TRAP as elegantly demonstrated over ten years ago [23,24]. Subsequent development of INTACT to profile cell population-specific chromatin states, and more recently, scRNA-seq expands our ability to probe stress responses with the necessary resolution to identify, model, and test control circuits. These approaches reveal cell population-specific responses, as well as interactions between cell populations, such as the discovery that vascular perception of low phosphate

status promotes root hair development in the epidermis in Arabidopsis using scRNA-seq [25]. Cell population specificity can be layered with multi-scale data integration to achieve a high-resolution perspective on gene function, as has been carried out to identify photoperiodic regulators of flowering time using INTACT-ATAC-seq and nuclear RNA-seq in phloem companion and epidermal cells [26]. In a recent *tour de force*, the application of single-nucleus ATAC-seq to maize (*Zea mays* L.) tissues enabled the recognition of cell-specific accessible *cis*-regulatory elements as sites of nucleotide variation associated with phenotypic diversity [27].

Cell-population level analyses have recently been adapted to crops. Kajala et al. [28] (2021) used TRAP-seq and INTACT-ATAC-seq to profile translatomes and chromatin accessibility in cell populations in tomato roots of plate-, pot- and field-grown plants. They also performed a pan-species analysis of meristematic cell populations in tomato, Arabidopsis and rice in plate-grown seedlings. While many processes were found to be conserved across species, key development regulators were also shown to have functionally diverged, such as the expanded role of the homeobox gene *KNAT1* to control xylem development in tomatoes in contrast to its role in regulating shoot apical meristem architecture in Arabidopsis. Interestingly, conservation of the translatome varied amongst different cell populations, with the meristem translatome, being well-conserved across the three species whereas the endodermis, vasculature, and meristematic cortex translatomes had diverged significantly, suggesting that proliferating cells in the root meristem are more developmentally constrained, while the other tissues examined exhibit greater developmental and evolutionary plasticity. This confirms that translation of knowledge between species is not straightforward and emphasizes the value of cross-species analyses and expansion of high-resolution studies to more diverse species [28].

Field studies

Transcriptomic studies are highly sensitive to environmental conditions. While laboratory studies allow precise control of environmental conditions, it is difficult to replicate all of the variables found in the field, which can result in significantly different results between outdoor and controlled environment studies, such as the disparities shown in cell population-specific translatomes among plate-, pot- and field-grown tomatoes [28]. Field studies have the advantage of representing the complex environment in which crops are grown without the need to make assumptions about or replicate the many external variables that determine growth. Some of these variables are challenging to replicate, such as the microbiome, as intensive agricultural cultivation selects for certain microbial taxa not only in the rhizosphere but also in field soil compared with surrounding native soils [29–31]. These domesticated microbial populations can negatively impact plant growth compared with native microbiota or sterile growth substrates [29,31], highlighting the complexity of agricultural fields compared to controlled laboratories that begin with ideal growth conditions.

Field studies can integrate plant stress responses with microbial community profiles or phenotypic outcomes of agronomically relevant traits. In a 17-week experiment, Varoquaux et al. [32] examined transcriptomic changes in response to pre- or post-flowering drought in field-grown sorghum (*Sorghum bicolor* L.). Interestingly, most drought-responsive genes were unique to either pre- or post-flowering drought and were more temporally dynamic pre-flowering than post-flowering, suggesting developmental plasticity of drought responses. Genes associated with arbuscular mycorrhizal symbiosis, however, were consistently downregulated by drought. Whereas arbuscular mycorrhizal symbiosis improves drought resilience and yield outcomes in other cereals [33,34], the energy requirements to maintain the symbiosis can be too high to sustain during drought and thus must be minimized. A complementary microbiome analysis found that drought impacts root and rhizosphere microbial populations overall, enriching for monoderm over the diderm bacteria found on well-watered sorghum roots [35]. Investigating both transcriptomics and microbiome populations in the field at such high temporal resolution is an approach ripe for network analysis to identify regulators that drive drought resilience and microbial community composition.

An important question is whether yields can be maintained under drought, which typically slows vegetative growth and can delay reproductive development and fecundity. This is best explored in the field under conditions used for agricultural production. A leaf transcriptome study of 120 varieties of rice grown in a wet paddy with intermittent drought performed a multivariate analysis with the >15,000 transcripts detected by 3′-end sequencing to recognize genes and processes under selection by drought [36]. Drought was shown to limit selection on the transcript abundance of photosynthetic genes but promote selection for early flowering, necessary for fecundity under drought. Water deficit stress also selected for higher expression of a MADS-box TF, known to promote early flowering, marking this TF as a drought-escape gene. This exploration of the use of transcriptomes to evaluate adaptive evolution identifies genes that may be targeted for yield stability.

Field studies incorporating cell-type or cell-population level analyses are limited. Such experiments are inherently more challenging because of environmental unpredictability and scale, as well as difficulties in sample collection, such as the delay between sample

collection and transport to the laboratory for processing (e.g. protoplasting for scRNA-seq), which can perturb molecular signatures. Methods for analysis of targeted cells that permit rapid preservation of tissue without extensive dissection or equipment, such as TRAP and INTACT, are thus well-suited for cell population-resolution field studies. Using TRAP-seq to compare cell type translatomes across field-, pot- and plate-grown tomato plants, Kajala et al. [28] demonstrated that while a core set of cell population enriched genes are enriched across conditions, most enriched genes were only enriched in specific conditions. These results demonstrate the feasibility of cell population-level analyses in the field and point to the promise of such work to identify regulatory networks that can be exploited to improve agronomically relevant traits.

Multifactoral approaches

As plants must coordinate growth and development with available resources and limitations imposed by stress, there is cross-talk between different stimuli. Nutrient deficiencies, for example, have been shown to impact the uptake of or responses to other nutrients [37–39], which is expected given nutrient transporter promiscuity [40] or nutrient-dependent changes in root suberization [41] that could have broad implications on nutrient acquisition. The ratio of nitrogen (N) to phosphorus rather than absolute abundance has been known to be critical for optimal plant growth [42], but recent work on Arabidopsis and rice has shown that direct cross-talk between nitrate and phosphate signaling underlies this phenomenon with the nitrate transceptor NRT1 gating phosphate starvation responses at high nitrate concentrations [43,44]. Such signaling integration equilibrates the acquisition and utilization of limiting nutrients to maximize growth.

Nutrient acquisition is not only balanced with the abundance of other nutrients but also the availability of water. Nearly 20% of the maize transcriptome is uniquely differentially regulated in response to combined N and water deficit in a greenhouse setting [45]. The interaction between nutrients and water is critical not only because both are necessary for plant fitness but also because water is the solvent for mineral nutrients. Water availability can thus impact both the nutrient amount and concentration. Swift et al. (2019) varied the absolute amount of N and water provided to rice plants under wet paddy field cultivation, with a design that isolates the effects of each stress individually, as well as probing for synergy and the impact of N concentration [46]. Most N-responsive genes were impacted by either N amount or N concentration but not both, and more genes responded to the interaction between N and water availability than either limitation alone. Interestingly, grain yield, biomass accumulation and water use efficiency were most closely correlated with genes responding to N concentration or synergistically to N and water rather than to water or N availability alone [46], making these subsets of genes prime targets for improving crop performance.

Conclusions

As technologies to probe gene activity advance, defining gene regulatory networks at high-resolution in complex environments will provide the resources necessary to understand plant by environment interactions in native and agroecological contexts. Multi-scale and cell population-resolved approaches can refine the prediction of multisensory integration of environmental information. They identify key targets that mediate responses to stimuli as well as their mode of regulation, including specific regulatory features, such as chromatin structure and *cis*-regulatory element presence and accessibility, and variations in RNA sequence determinants, such as uORFs. Such depth not only improves our understanding of gene regulatory networks but also empowers the manipulation of these networks for crop improvement by use of CRISPR/Cas9 editing and dCAS9 transcriptional control systems (reviewed by [47]).

Timing is a key aspect of gene regulation. As discussed earlier, gene activity can be heavily controlled both diurnally and/or by the circadian clock and important regulatory events can be obfuscated based on sampling time. However, the timing of regulatory events at different levels relative to one another is also critical. Methods that enable the capture of the nuclei of the few cells responding to microbes or other stimuli are also needed. Single-cell multi-omics that profiles transcriptomes and chromatin states from the same cells coupled with trajectory inference may prove instrumental in disentangling the temporal dynamics of different modes of regulation.

While cell population-specific genomics methods are effective for environmental response analyses, there remain challenges for single-cell assessment of environmental responses. Spatial transcriptomics that provides precise localization of mRNAs within cells of tissues has not been adopted in plants [48,49], but provides significantly more information about the distribution of transcripts across tissues and will be instrumental in studying stress responses, for example, the distribution of transcripts near or distal from sites of infection or herbivory. In addition, the accuracy and sensitivity of nucleic acid detection have outpaced that of protein quantification; single-cell proteomics thus remains in its infancy (reviewed in [50]) and the spatial resolution at which we understand proteome-wide post-translational regulation is limited compared to processes up to translation. Finally, single-cell approaches will also highlight the inherent stochasticity of gene expression

[51]. While an analytical challenge, stochasticity can influence phenotypic outcomes [52,53], and integration of this layer of gene regulation with non-stochastic processes will provide a more comprehensive understanding of environmental responses.

Author contributions

G.Z.A. and J.B.-S. wrote the paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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