

Review

Pleiotropy, a feature or a bug? Toward co-ordinating plant growth, development, and environmental responses through engineering plant hormone signaling

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The advent of gene editing technologies such as CRISPR has simplified co-ordinating trait development. However, identifying candidate genes remains a challenge due to complex gene networks and pathways. These networks exhibit pleiotropy, complicating the determination of specific gene and pathway functions. In this review, we explore how systems biology and single-cell sequencing technologies can aid in identifying candidate genes for co-ordinating specifics of plant growth and development within specific temporal and tissue contexts. Exploring sequence–function space of these candidate genes and pathway modules with synthetic biology allows us to test hypotheses and define genotype–phenotype relationships through reductionist approaches. Collectively, these techniques hold the potential to advance breeding and genetic engineering strategies while also addressing genetic diversity issues critical for adaptation and trait development.

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Introduction

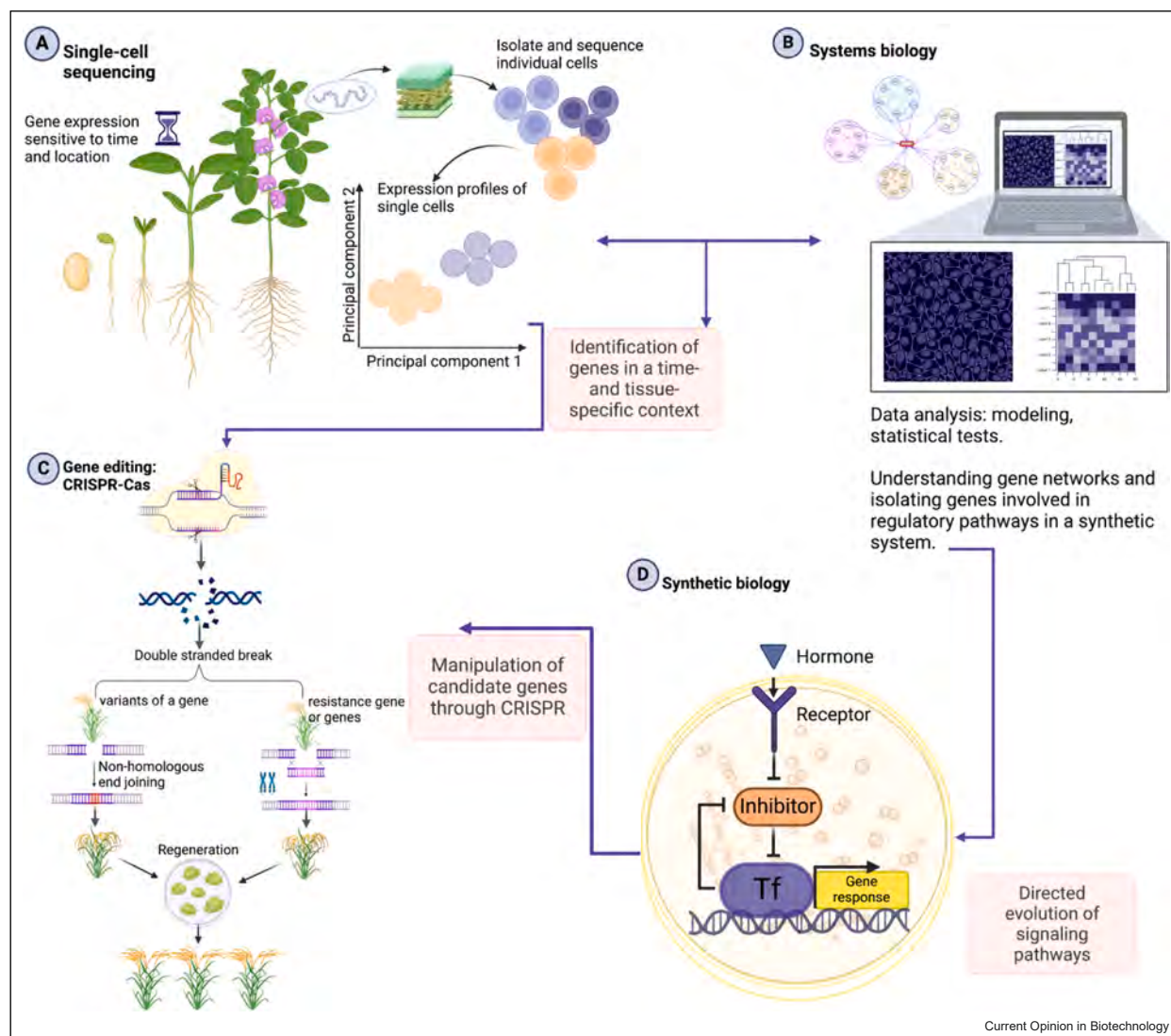
The compounding challenges of minimizing climate change and sustaining global public and environmental health are becoming more urgent as the world’s population is estimated to grow to 10 billion by 2050 [1]. These challenges necessitate a myriad of technological

solutions to sustainably expand agricultural productivity and reduce wastes [2]. Developing our ability to rationally co-ordinate plant growth, development, and environmental responses through genetic engineering is pivotal. It ensures the security of the expanding portfolio of vital plant-based goods, including food and feed, fiber, pharmaceuticals, and fuels. Although substantial progress has been made in gene editing technologies facilitating genome manipulation with remarkable ease, there remains considerable ground to be covered in identifying candidate genes and variants that yield desirable improvements in plant phenotypes. Plant hormones and their gene regulatory networks co-ordinate nearly every aspect of plant development, growth, and environmental responses. Therefore, we posit that hormone signaling networks are potentially prime targets for rationally engineering plant phenotypes. Nonetheless, regulatory networks are rather complex. Plant hormone signaling pathways possess numerous negative feedback loops, as well as high interconnectivity between signaling pathways designed to rapidly adjust gene expression in space and time in response to developmental and environmental signals. This complexity poses challenges in identifying (1) the specific genes within a pathway that are the main drivers of a desired trait; (2) genes that can be targeted for manipulation without impacting other essential metabolic functions; and (3) natural or engineered gene variants that could quickly enhance or diminish a specific function to improve the desired trait. Here, we review recent literature, which views and exploits plant hormone response pathways as tuning knobs of plant phenotypes. We highlight the challenges and recent successes in altering plant phenotypes through targeted manipulation of plant hormone signaling pathways (Figure 1). Finally, we look forward to how new technologies and approaches are advancing spatiotemporal understanding and engineering of plant development, growth, and environmental responses via hormonal signaling.

Tuning knobs in plant development

Hormones and their genetic orchestrations act as the primary conductors, directing and harmonizing transcriptional responses and metabolic changes. They contribute to plants’ ability to perceive and respond to various endogenous cues and environmental stimuli.

Figure 1



Integration of single-cell, systems, and synthetic biology with emerging scarless gene editing technologies, showcasing their potential to improve trait development in crops. **(a)** Understanding of time- and tissue-specific developmental processes are rapidly advancing through the application of single-cell techniques. **(b)** Advances in computational methods are necessary for reducing the network of genes and variants identified in single-cell sequencing to identify hypothetical causal mechanisms of phenotypic change. These hypotheses can then be fed into gene editing approaches **(c)** or further interrogated via synthetic systems **(d)**, where minimal and high-throughput methods can explore the new sequence–function of candidate genes and variants. This figure was created with BioRender.com.

Historically, significant emphasis has been placed on understanding the structural aspects at both the phenotypic and molecular levels and environmental responses facilitated by hormone signaling transduction. Previous work, particularly genetic screens, has often identified mutations that exhibit pleiotropic effects, perhaps due to their ease of identification and dominant nature [3–7]. While these discoveries have helped us to define these signaling pathways. This often leads to the narrow conclusion that targeting plant hormone signaling for trait improvement is perhaps misguided as it may

lead to off-target, negative, or unintended traits, that is, ‘a bug in the code’. However, we prefer to view pleiotropy of plant hormone signaling not as a bug, but a feature — several plant phenotypes could be improved through advancing our understanding of and ability to engineer these tuning knobs for plant development. Particularly as gene families have expanded, there is the possibility for reduced pleiotropy. Several studies quantifying genome-wide pleiotropy have revealed that most genes and single nucleotide polymorphisms (SNPs) are not highly pleiotropic, affecting only

between one and six phenotypic traits [8]. Additionally, complex relationships between gene expression domains and protein accumulation and function, in both time and space, can now be parsed with genetic engineering to affect only specific phenotypic traits [5,9].

To capitalize on this feature of plant hormone signaling pathways, it is imperative to understand how cells, their transcriptional state, and gene regulatory networks function throughout development, growth, and in response to environmental stimuli. Emerging single-cell technologies hold potential in describing cell populations, subpopulations, and their differentiation in a time- and space-dependent manner. Single-cell technologies can provide insightful information regarding cellular heterogeneity transcription factor binding and chromatin accessibility [10], proteome content [11], and specific hormonal orchestrations in cell development [12]. This unprecedented resolution is highly promising as we strive to understand plant responses throughout development. Particularly, as we attempt to manipulate gene expression to enhance plant adaptability to stress conditions — such as drought, flooding, pests, and pathogens — while also maintaining crop productivity.

Examples showcasing the potential of single-cell approaches for connecting molecular orchestrations to whole-plant phenotypes in a tissue- and time-specific manner have been demonstrated. For instance, transcriptional changes in *Arabidopsis* during early lateral root development exemplify the potential application of single-cell technology at a very specific stage of development and in individual cells [13]. Research has illustrated how abscisic acid (ABA) mediates salt stress responses in a spatiotemporal manner. It has also shown that the concerted function between hormonal pathways drives the complete temporal architecture of stress responses [14,15]. The co-ordination of gene expression during lateral root development has recently been defined by single-cell sequencing and cell-type-specific repression of critical transcription factors and chromatin modulators was used to confirm the effects of these candidate genes [13]. Moreover, the control of seed size and oil content in *Arabidopsis* has been linked to the extended expression of *WRI1* during specific times in seed development [13]. Tissue- and time-specific regulation of rice crown root development has been elucidated through the antagonistic relationship between cytokinin and auxin [16]. Additionally, recent research has highlighted that single-cell analysis can capture context-specific brassinosteroid regulatory genes that are important in the development of *Arabidopsis* roots [12]. Therefore, all these examples provide future directions wherein the expression of genes and the accumulation of their products at single-cell resolution can aid in identification of candidate genes.

Coupled with systems and synthetic biology, this approach will allow us to define cell-type-specific gene networks and pinpoint candidate genes that can influence developmental outcomes in a more precise and targeted manner. Specifically, genes involved in or regulated by hormonal signaling pathways — such as auxin, ethylene, cytokinin, ABA, jasmonic acid, salicylic acid, brassinosteroid, gibberellin, strigolactone, and karrikin, which serve as pivotal players in all stages of plant life — are likely to allow us to tune valuable plant phenotypes. Many genes involved in hormone biosynthesis, transport, and signal transduction also belong to expansive gene families that regulate their signal transduction [17–19]. These gene networks may provide flexible tuning knobs, featuring both positive and negative regulators, with relatively well-defined sequence–function relationships. Additionally, potentially tissue-specific expression domains will allow for the identification of regulators specific to certain times and places in relation to a desired phenotype.

The utilization of key regulators and their cross-talk holds potential for crop improvement

Growth and development involve a sequence of morphological, physiological, and anatomical changes. These changes encompass all stages of a plant's life cycle — embryogenesis, vegetative stage, reproduction, and senescence. Throughout these developmental stages, cascades of signaling events occur, crucial for driving growth processes, with hormones playing a central role.

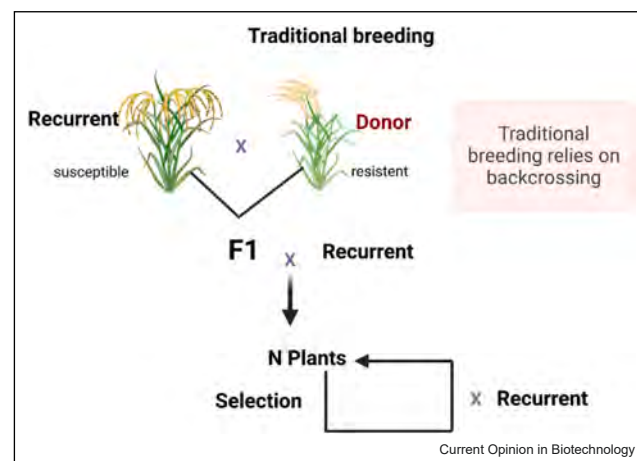
Hormonal signaling pathways follow a structured logic, wherein each hormone typically interacts with a corresponding receptor. In the presence of the hormone, the receptor regulates inhibitor levels, while in its absence, inhibitors bind to transcription factors, thereby repressing downstream gene expression [18,20–25]. Thus, the inhibition or formation of complexes between transcription factors and inhibitors plays a pivotal role in controlling hormone-mediated gene expression and orchestrating the downstream response within the pathway (Figure 1d). Nevertheless, there exist numerous feedback, alongside interplay and cross-talk among signaling pathways [26]. These complexities are exemplified by interactions, such as those between auxin ARF10/ARF16-IAA8-TIR1/AFB and abscisic acid ABI3 modules, which collectively control seed germination [27]. Also, the cytokinin response factor CRF4 is intricately involved in the regulation of YUC genes, a family of flavin monooxygenase proteins responsible for auxin biosynthesis, consequently influencing auxin levels [28]. Furthermore, the interplay of gibberellin-regulated DELLA and auxin-regulated ARF-Aux/IAA interactions regulates both cambial activity in poplar and also fruit initiation in tomato [28,29].

One of the most successful stories about the use of hormone regulators in crop improvement resulted from genetic variation in gibberellin signaling. Genetic variants in *DELLA*, negative regulators of gibberellin signaling, facilitated the creation of semi-dwarf wheat varieties by Borlaug during the 'green revolution', through increasing accumulation of DELLAs [30] reviewed in Refs. [31–34]. Consequently, this led to reduced gibberellin signaling, significantly contributing to the increase in agricultural production. This success story demonstrates how key regulators and their cross-talk can be utilized for crop development. It also highlights pleiotropy as a beneficial feature harnessed to produce both lodging resistance and increased partitioning of nutrients into grains as opposed to stems in wheat varieties. While there are numerous examples that could be cited regarding hormones, their cross-talk, and their potential for engineering crop phenotypes in recent research, *DELLA* represents a narrative that is particularly noteworthy. This story mirrors the imperative we face to double agricultural productivity to feed the approximately 10 billion inhabitants by 2050. A similar scenario faced during the 'green revolution'. However, the crucial difference now lies in our responsibility to do so sustainably, especially as natural resources become increasingly scarce. Additionally, there are still regions that have not benefited from mechanization technologies developed during the Green Revolution. The genetic redundancy and diversity within the gene families regulating plant hormone signaling provide both a robustness and adaptability in fine-tuning of plant phenotypes [35].

Improvements in agricultural production have been driven through classical breeding techniques that target improving desired plant traits (Figure 2). These techniques involve the selection of improved varieties from large breeding populations with traits, such as improved yield, harvestability, or adaptation to different biotic and abiotic factors [36–41]. While traditional breeding practices and genomics-informed methodologies, such as genome-wide association studies (GWAS), quantitative trait loci mapping, and genomic selection, have played crucial roles in advancing agricultural productivity [42,43], they have also contributed to narrowing genetic diversity within crop species [41,44–46]. This narrowing is a consequence of prolonged domestication processes and laborious variety development, often requiring extensive backcrossing efforts to integrate desired traits into high-yielding cultivars (Figure 2). Moreover, these techniques have limitations in identifying key regulators within the context of pleiotropy that could enhance trait diversity under different conditions [34]. Primarily, they lack integration of spatial and temporal specificity in plant development, which is influenced by environmental inputs that contribute to the underlying molecular mechanisms.

Combining the aforementioned approaches with expression-based techniques such as expression GWAS (eGWAS)

Figure 2



Illustrates the introgression of a desired trait through traditional breeding. This figure was created with [BioRender.com](https://www.biorender.com).

and transcriptome-wide association studies (TWAS) can enhance statistical and resolution power. This integration enables the identification of candidate genes and small-effect loci, thereby offering a more robust approach to understanding pleiotropy within and beyond signaling pathways [32,43,47–51]. Additionally, integration of pleiotropy models into multitrait GWAS studies may also increase power in detecting associations [49]. With the rapid advances in genome editing, we are now able to introduce novel genetic variations to adjust the intricacies of ongoing processes, such as meristem differentiation and the formation of organs resulting from it (Figure 1). Moreover, fine-tuning the hormonal signaling networks driving these processes could potentially allow for the rational engineering of the next generation of crops [32,47]. This is particularly encouraging considering the aforementioned productivity challenges in agriculture. In the near future, the combined use of technological advances and deeper understanding of how plant hormone signaling pathways drive crop productivity and responses to stressors will allow us to make progress on a global scale.

Exploring genetic variation in crop improvement: leveraging synthetic and systems biology approaches

Systems and synthetic biology play crucial roles in understanding and leveraging genetic variation within complex networks. In systems biology, we simulate and predict the interactions of multifaceted networks (Figure 1b). Meanwhile, synthetic biology empowers the deconstruction of these gene networks, enabling the rational design and bottom-up construction of entire regulatory pathways (Figure 1d). This approach allows for the fine-tuning of pathways and the exploration of new sequence–function spaces within a pathway. It potentially opens new regions

of phenotypic space and offers innovative strategies for genetic engineering and biotechnology. This process is vital for reprogramming plant development through a predictive biological framework, allowing for the anticipation of potential outcomes in a high-throughput manner [52].

To rationally design plant morphology, it is imperative to understand growth and development in a spatiotemporal context, as well as the underlying genetic variation. This will help identify time-specific interactions and expression patterns, allowing fine-tuning of their resulting phenotypes (Figure 1a). The integration of single-cell resolution transcriptome, metabolome, and proteome data will provide an unprecedented ability to predict how changes in molecular function in a gene or its variants will affect cell, tissue, and whole-plant phenotypes [53,54].

While single-cell RNA sequencing offers high spatiotemporal resolution transcriptomics data, its limitation lies in focusing solely on gene expression profiles. This overlooks crucial details, such as the mismatch between mRNA and protein abundance as well as chromatin accessibility [10–12,55,56]. To address this gap, the incorporation of cutting-edge single-cell methodologies for proteomics and assays for transposase accessible chromatin sequencing become indispensable [57,58]. These techniques play a pivotal role in elucidating the regulation and functionality of transcriptomes, protein expression levels, and the epigenetic mechanisms regulating tissue-specific biological functions [12,13,57,58].

In the context of crop improvement, integration of these different types of single-cell resolution data along with increased time resolution will shed light on how important traits develop over time. This will provide valuable information for breeding programs aimed at enhancing crop productivity, resilience, and adaptation to changing environmental conditions. For instance, membrane microdomains and protein partitioning at various subcellular levels play crucial roles in growth, development, and stress responses, including hormone biosynthesis, cellular signaling, and disease resistance [59–61]. These processes highlight the need for enhanced spatiotemporal resolution in single-cell and subcellular proteomics technologies. This would greatly benefit crop improvement efforts by providing insights into the molecular mechanisms at the cellular level underlying important traits [56]. The specific information about gene expression at precise cellular locations and times could also facilitate the dissection of pleiotropy.

Certainly, single-cell technologies, despite their advancements, encounter challenges, particularly in the context of plants, owing to the inherent complexity of plant cells and their surrounding polysaccharide-rich

walls [62]. Additionally, single-cell technologies are not yet sensitive enough to detect low abundance molecules. This results in a high number of undetectable molecules that nonetheless may be playing a significant role in signaling and cellular responses [63]. Efforts are underway to overcome these challenges and improve the application of single-cell technologies in crop improvement [53,64]. Critical to this effort will be integration of comparative evolutionary approaches to understand how genetic variation relates to spatiotemporal changes in biomolecule accumulation. Genetic variation serves as the cornerstone of evolutionary processes, with mutations acting as the fundamental source of this variation. Historically, the generation of variant traits in crop plants was through introgression via labor-intensive and time-consuming mutagenesis-based breeding. This essentially required screening of large mutagenized populations, presenting significant challenges in terms of time, space, and reproducibility [65–67]. Synthetic biology, leveraging heterologous systems, can rapidly provide a molecular understanding of protein function and signaling pathways. This facilitates the selection of protein variants with desired functions through screening, massively parallel assays, or directed evolution [68–72].

Transgenic-based breeding comprises a potent source of external variation, addressing the issue of reduced genetic diversity found in modern crops. However, it encounters significant public opposition, and the regulation of genetically modified organisms involves lengthy approval processes [67]. CRISPR-Cas gene editing and base editing, in contrast, can significantly reduce breeding time to as little as 5 years, compared to the 10 years often required for mutagenesis- and transgenic-based breeding and also reduce concerns of transgenics (Figure 1c) [67,73,74]. Notable applications of CRISPR-Cas in crop improvement include recent engineering feats, such as Pairwise's enhancement of mustard greens. This has resulted in greens with improved nutrient content compared with regular lettuce salad while eliminating the pungent taste typically associated with mustard [75]. Additionally, scientists at Sanatech Seed have introduced the first Sicilian Rouge tomatoes in Japan, engineered to contain higher levels of γ -aminobutyric acid [76]. While these technologies represent the future, the identification of candidate alleles and genes, as well as the final step involving the regeneration of whole plants [77] remains a bottleneck.

Moving forward, there is a pressing need for a coalition of various techniques to address the limitations in identifying genes and their variants that should be targeted to generate diversity in specific traits. Comparative evolution coupled with expression analysis of hormonal pathways could prove to be a valuable asset alongside the current cutting-edge technologies [47,78,79]. Furthermore, the synthetic biology toolbox adds another

dimension to this approach, as it allows for the testing of different variants with much ease and in a high-throughput manner. This knowledge can then be transferred to more complex organisms such as plants. Additionally, synthetic systems have proven to provide great insights in hormonal pathways functionality that can later on be translated into prediction of a diverse array of phenotypes [68–70].

Conclusions

The integration of synthetic and systems biology, particularly at the single-cell level, and advanced genome editing technologies facilitates the acceleration and enhancement of breeding programs. These advancements enable the identification of genes responsible for specific traits in a time and context-specific manner, thereby reducing pleiotropic effects. Moreover, they facilitate the discovery of untapped trait diversity in crops and offer the capacity to expand beyond existing genetic diversity. By leveraging the combination of these technologies alongside conventional approaches, we can achieve the rational design of traits and develop the next generation of improved crops crucial for meeting the demands of our growing population.

CRedit authorship contribution statement

D.F.N. conceptualized, planned, designed figures, and wrote the manuscript; R.C.W. conceptualized, planned, supervised the work, and wrote some of the manuscript. All authors revised and approved the final manuscript.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare no conflict of interest.

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