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Review Article

Applications of cell- and tissue-specific 'omics to improve plant productivity

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The individual tissues and cell types of plants each have characteristic properties that contribute to the function of the plant as a whole. These are reflected by unique patterns of gene expression, protein and metabolite content, which enable cell-type-specific patterns of growth, development and physiology. Gene regulatory networks act within the cell types to govern the production and activity of these components. For the broader organism to grow and reproduce successfully, cell-type-specific activity must also function within the context of surrounding cell types, which is achieved by coordination of signalling pathways. We can investigate how gene regulatory networks are constructed and function using integrative 'omics technologies. Historically such experiments in plant biological research have been performed at the bulk tissue level, to organ resolution at best. In this review, we describe recent advances in cell- and tissue-specific 'omics technologies that allow investigation at much improved resolution. We discuss the advantages of these approaches for fundamental and translational plant biology, illustrated through the examples of specialised metabolism in medicinal plants and seed germination. We also discuss the challenges that must be overcome for such approaches to be adopted widely by the community.

Introduction

Plants have been used continuously in a wide array of foods, medicines, textiles and construction materials since their domestication ~10 000 B.C. [1,2]. However, it was only after the nineteenth century that major scientific breakthroughs were made in plant breeding, driving significant advances in productivity. The development and application of 'omics technologies in recent years, such as high-throughput sequencing, proteomics and metabolomics, have enhanced research into plant biology tremendously [3–7]. As a result, we have gained a better understanding of the genetics and complex biological processes that underpin plant productivity. These discoveries have been translated to real-world agriculture through improved breeding practices and biotechnology solutions that have increased yield [8–10].

Plant tissues consist of many different cell types that each have specific functions. These functions are driven by distinct biochemistry, physiology and gene expression programmes. The concerted activity of cell types determines the properties of plant tissues and organs but their correct function requires careful coordination and integration of signalling networks [3,5,11–15]. 'Omics technologies have been applied extensively to determine the mechanisms controlling the accumulation of proteins and metabolites within organ and tissue types [4,6,16,17]. Recent advances in single-cell resolution 'omics methods, such as single-cell RNA-seq (scRNA-seq), have further advanced our ability to understand spatiotemporal responses to biological stimuli and compartmentalisation of functions [11–15]. These methods are not yet mature or widely adopted but widespread enthusiasm exists amongst the plant science community. In this respect, the Plant Cell Atlas (PCA) framework has been initiated to overcome the knowledge gaps and technical challenges that stand in the way [18,19].

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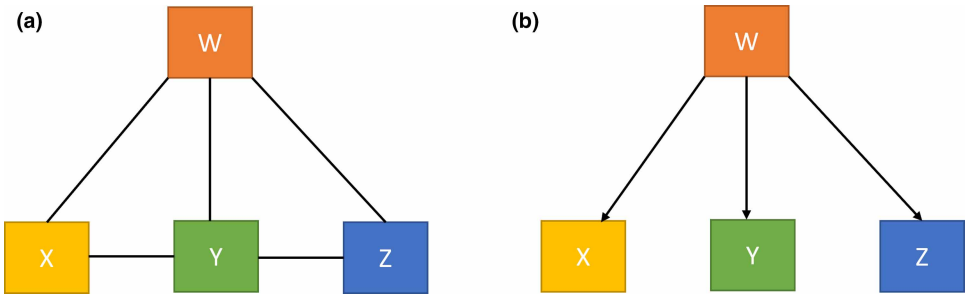

Figure 1. Simplified models depicting gene networks used in biological research.

Coloured boxes represent genes or transcription factors (nodes) and black lines/arrows represent the presence of a connection between nodes (edges). (**a**) In a gene co-expression network, genes W, X, Y and Z have similar patterns of expression, and are therefore connected. However, this type of network does not meaningfully reflect how these genes interact. (**b**) In a gene regulatory network, edges have directionality and depict causal relationships between nodes. For example, W, X, Y and Z are co-expressed and correlated. However, X, Y and Z do not interact with each other even though they are regulated by W.

Cellular function is closely linked with spatial location [20]. Consequently, we need to understand both expression of gene products at the single-cell level and the spatial context of cells. In other words, the knowledge of where and how closely individual cells are located relative to one another in native tissues is required to understand the intercellular communication that occurs upon biological stimulation. However, the isolation of single cells for single-cell analysis methods, such scRNA-seq, typically requires tissue dissociation and so loses this spatial information. Spatial transcriptomics overcomes this issue by physically localising gene expression in distinct areas of tissues [21]. The method can be considered analogous to classic RNA *in situ* methods but assays complete transcriptomes rather than individual transcripts. This approach can be combined with scRNA-seq to survey the expression of gene products of distinct cellular subpopulations within intact plant tissues [20].

Individual, gene-by-gene analysis is common in plant science research, but does not provide the holistic, systems level view of biological processes needed to understand the organism as a whole [22,23]. This realisation has given rise to the concept of gene networks as shown in Figure 1 [22,23]. The underlying principle of this powerful approach is that a biological system can be represented as a network graph of interacting genes or gene products [24]. Gene co-expression network analysis has been applied extensively to plant tissues to identify genes with co-ordinated expression patterns during development or exposure to environmental cues (Figure 1a) [25–27]. Similarly, gene regulatory network analysis has been employed to decipher the hierarchical relationship between transcription factors, their target genes and associated signalling pathways (Figure 1b) [23,28]. Extension of these approaches to the analysis of cell types at single-cell resolution has begun and will provide a clearer, deeper understanding of the regulatory programmes that underpin cellular processes [29–31].

In this review, we provide an overview of how 'omics technologies have been applied to study plant tissues, illustrating this through two major examples; specialised metabolism in medicinal plants, and seed germination in cereal crops. We highlight knowledge gaps, provide insight into the types of analyses that could be performed at the single-cell level and the biological insight this would enable, and discuss how network theory and spatial transcriptomics could be leveraged. We also discuss the challenges that must be overcome along the way.

Glandular trichomes in cannabis and the production of cannabinoids

Phenotypic plasticity is a unique and defining property of plants. Plants are immobile and therefore must adapt to the range of dynamic local environmental conditions, rather than move to escape them. Challenges they face include wide daily temperature variation, water and nutrient availability, pathogen and animal attack [32–36]. Alterations of metabolism are an important component of adaptation mechanisms [37]. Accordingly, plants produce a plethora of specialised (also known as secondary) metabolites via complex metabolic pathways. These pathways are dynamic and can quickly alter the profile of specialised metabolites thereby enabling plants

to adapt and mediate environmental interactions [37,38]. Many specialised metabolites are produced and accumulate to high levels in particular cells or organs that have unique properties (Figure 2) [39–41].

Cannabis sativa exhibits remarkable specialised metabolite diversity and is currently the focus of much attention from the pharmaceutical industry, breeders and researchers. It is a prolific producer of cannabinoids and terpenes in specialised structures called capitate stalked glandular trichomes (Figure 2a) [25,42]. Over 120 cannabinoids have been identified, amongst which Δ^9-tetrahydrocannabinol and cannabidiol are the most abundant and of primary interest to the community [43,44]. More than 100 terpenes have been reported, but are largely understudied compared with the cannabinoids [42,45]. Proteomics has been successfully used to identify several hundreds of proteins in individual cannabis tissues, including the glandular trichomes, some of which are involved in the biosynthesis of secondary metabolites [46,47]. However, there is remarkable variation in secondary metabolite profiles between cannabis cultivars/strains [48,49]. The mechanisms underlying this might be determined by comparing the glandular trichome-specific proteomes of cannabis cultivars that have different metabolite profiles (termed chemotypes). The integration of such studies with tissue-specific transcriptomics and metabolomics analyses, as well as cultivar-specific therapeutic or recreational user experiences, could reveal the broader regulatory mechanisms of secondary metabolism and identify uncharacterised minor compounds that have unexplored therapeutic or industrial benefits.

The glandular trichome is an excellent model to study cell-specific gene regulation. It is composed of a multicellular stalk and a globose head of secretory cells where metabolites are synthesised and accumulate [25]. Transcriptomics, gene co-expression network analysis and metabolomics have been applied to the cells of glandular trichomes, identifying genes associated with the production of major compounds that are primarily or solely active in these cell types [25,26,45,50]. Glandular trichomes were enriched by mechanical means then comparisons made to different cell types, which allowed trichome-specific processes to be identified [25,26,47]. Gene regulatory network analysis is an ideal way to extend these studies. It would enable the identification of transcription factors and their target genes that could be used in breeding programmes or in biotechnology applications. The function of gene regulatory network components can be examined and manipulated using techniques such as gene editing [51,52]). Functional characterisation through *in vivo* mutational analysis would help validate the relationships between network components and the phenotypes that the networks influence. The effect of transcriptional regulators within networks might also be investigated at single-cell resolution using high-throughput *in vitro* approaches, for example, targeted Perturb-seq (TAP-seq) [53,54]. It is also possible to reconstruct parts of gene regulatory networks in heterologous systems, such as yeast, to identify the essential constituents and core parameters of these networks [55]. This synthetic biology approach has

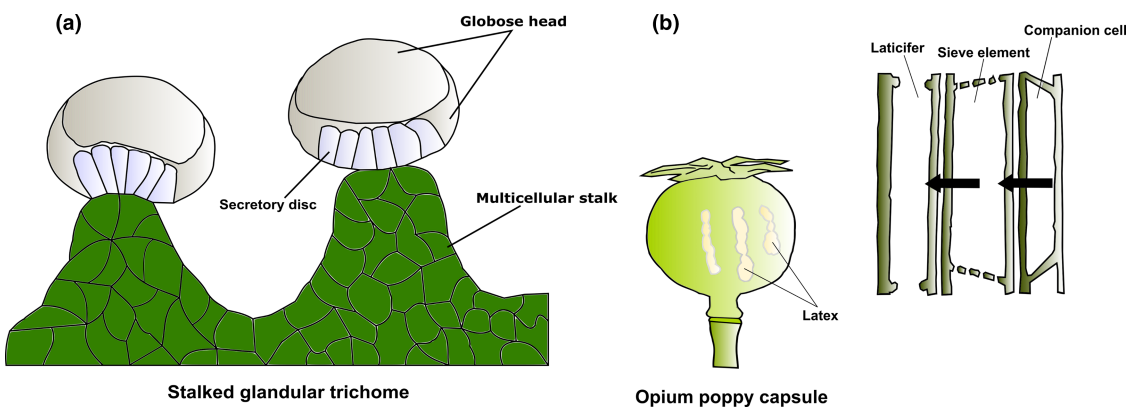

Figure 2. Site of specialised metabolites synthesis, accumulation and storage in *Cannabis sativa* and *Papaver somniferum* (opium poppy).

(**a**) The stalked glandular trichome of cannabis consists of a globose head which protrudes from the epidermal surface via a multicellular stalk. The head region contains several secretory disc cells that produce and store specialised metabolites such as cannabinoids and terpenes. (**b**) The opium poppy capsule wall contains an extensively branched network of companion cells, sieve elements and laticifers where latex exclusively occurs. Latex represents the site of alkaloid accumulation in poppies.

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successfully been applied in yeast to study plant hormone signalling pathways and the same principle could be used for cannabis to recapitulate specialised metabolism pathways in heterologous systems [56,57].

Transcription factors and genes that drive cannabis glandular trichome initiation and development, and consequently the production of specialised metabolites, are attractive targets for industrial producers who wish to increase or manipulate yield. Transcription factors that regulate specialised metabolism have been identified in the glandular trichomes of *Artemisia annua* and *Solanum lycopersicum* [58–60]. A recent study by Qin et al. demonstrated that overexpression of transcription factor *AaMYB17* in *A. annua* increased glandular trichome density by 1.3–1.6 fold. Production of the specialised metabolite artemisinin, which is an anti-malarial compound, also increased by 50% [58]. Defining the gene regulatory network surrounding *AaMYB17* would allow us to better understand how it regulates these biological processes, providing both increased mechanistic understanding and additional candidate genes for crop improvement. Methods that identify transcription factor target genes genome-wide, such as DNA affinity purification sequencing (DAP-Seq) and chromatin immunoprecipitation sequencing (ChIP-seq), could be applied to reveal the suite of regulated genes [61–64]. This information could then be related to the genetic and biochemical regulation of specialised metabolism. Transcription factors associated specifically with glandular trichomes in *C. sativa* have also been identified and similarly promising avenues could be followed for the study of cannabinoid and terpenoid biosynthesis pathways [65].

Tissue-specific localisation of alkaloid biosynthesis and storage in opium poppies

Papaver somniferum (opium poppy) is another important medicinal plant. The various products of its benzylisoquinoline alkaloid (BIA) pathway have therapeutic uses including for pain relief, in cancer treatment and as cough suppressants [66,67]. Synthesis and accumulation of BIAs occurs in three distinct cells types of poppy capsules; the companion cells, sieve elements and laticifers (Figure 2b) [68,69]. Summarised, transcription and translation of the majority of BIA pathway genes take place in the companion cells, then the corresponding functional enzymes (NCS, 6OMT, NMCH, 4′OMT, SalSyn, SalR and SalAT) are exported to sieve elements where alkaloids are synthesised. Finally, the alkaloids are transported to the laticifers for storage [68,69]. This has been determined by localisation of expression of individual enzymes in the pathway, using biochemical methods. This individual gene/enzyme approach does not inform us about the surrounding regulatory context, which could be defined using cell and tissue-specific 'omics studies. Thus far, however, global BIA gene expression has only been investigated at the resolution of organs (leaves, stems, roots, etc.) [70,71]. In future, the isolation of single sieve elements, laticifer cell types and companion cells from a range of poppy cultivars with differing alkaloid contents, and the comparison of cellular gene expression profiles and underlying gene networks among cultivars, would shed more light on the transcriptional regulation differences among opium poppy lineages.

One striking property of opium poppy cultivars is their remarkable variation in alkaloid content and composition. For instance, some plants produce large amounts of morphine and codeine while others synthesise high levels of thebaine or oripavine [72]. This is due to the extensive artificial selection the species has been subjected to which has resulted in genetic variation between cultivars, landraces and lines. Whilst the biochemical properties of many enzymes in the BIA biosynthetic pathway have been defined, comparatively little analysis has been conducted of the effects of natural variation in these enzymes between poppy cultivars nor of the underlying gene regulatory mechanisms and the roles they have in generating this variation [66–71,73]. A recent study by Li et al. [71] determined that the BIA genes were predominantly co-expressed, irrespective of whether they were physically clustered within the genome or not. This finding led to the discovery of uncharacterised genes that may be involved in various stages of the pathway. However, their conclusions were based upon analyses of a single poppy cultivar. The same study demonstrated a link between copy number variation (CNV) of BIA genes and alkaloid production in 10 opium poppy cultivars. This raises the question of how gene CNV can affect the topology of gene networks across genotypes of the same species. Therefore, it would be beneficial to compare cultivar-specific gene networks to have a better understanding of how networks interplay with natural variation underpinning alkaloid production. Comparison of cell-type-specific gene expression profiles and the underlying gene networks among cultivars across developmental stages would allow us to define the regulatory mechanisms controlling BIA variation between different opium poppy lineages.

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Events within individual cells and cell types of germinating cereal seeds

Seed germination is a key step in the life cycle of plants. It is an intricate process that involves a series of morphological, physiological, and biochemical changes that transform the metabolically inactive seed into a highly active seedling [74–76]. Proper timing of germination has a direct influence on crop yield as it ensures that the seedlings have the opportunity to thrive under appropriate environmental conditions [77]. The seed itself is the most valuable product of plants, especially cereal crops, and accounts for more than 60% of food sources around the world [76].

Seed germination has been studied extensively in *Arabidopsis thaliana*, with strong spatial and temporal organisation of growth, development and physiological processes found to exist [78–81]. For example, the decision to break dormancy and proceed with germination is made by small numbers of cells in discreet regions of the radicle, by control of the biosynthesis and perception of the hormones, abscisic acid and gibberellic acid [78]. Specific genes must be activated and repressed at the right times in the various cell types of the seed for this to occur successfully [81]. Similarly, tight spatiotemporal regulation must occur in germinating cereal seeds, but our knowledge of this is extremely limited. A recent study by our laboratory identified co-expressed tissue-specific genes and key TFs at different stages of germination in seeds of barley, an important cereal crop and model for genomics studies of monocot species [76]. We did so by applying laser-capture microdissection (LCM) coupled with RNA-seq. Although LCM can spatially resolve and compartmentalise gene expression, it does so at the bulk tissue level with a minimum resolution of tens of cells [82]. However, cell function and gene expression vary over smaller resolutions than this within the complex structures of the seed [78,80,81]. Resolving patterns of gene expression in single cells of germinating barley seeds and mapping this information onto the spatial location of the underlying cells would, therefore, provide a more specific and comprehensive view of transcriptional activity over time. This would allow us to better understand the functions of known cell types and to identify cell types previously undiscovered. The integration of metabolomics and proteomics within this single-cell spatial transcriptomics framework would enable us to improve structural and functional models of cereal seeds (Figure 3).

Seed germination is strongly influenced by environmental conditions, with abiotic stresses able to have negative impacts [83–85]. One such abiotic stress is soil salinity, which prevents adequate water uptake and drives excessive ion uptake by the seed, leading to toxic effects. This can in turn inhibit or delay seed germination

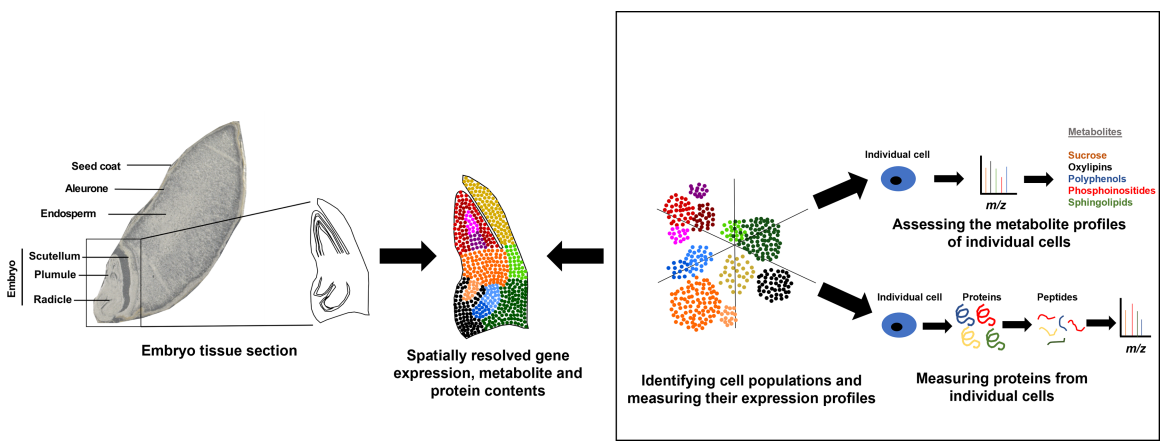

Figure 3. Spatial resolution of single-cell transcriptomics, metabolomics and proteomics in germinating barley seeds.
A spatial barcoded map of the barley embryo is obtained using spatial transcriptomics to capture the mRNA transcripts across the embryo tissue section. In parallel, the embryo tissue undergoes tissue dissociation for single-cell RNA sequencing (scRNA-seq), followed by gene expression measurements, dimensionality reduction and clustering of various cell types that constitute the scutellum, plumule and radicle. The single cells are also surveyed for their metabolite and protein contents. Finally, the various single-cell 'omics measurements are integrated with spatial information to annotate the cell subpopulations [20]. Image adapted from [76] with permission from John Wiley and Sons.

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and cause massive yield losses [85]. Unfortunately, the transcriptional regulation of cereal seed germination under salt stress remains poorly understood. Barley is exceptionally salt tolerant relative to other cereal crops and an excellent model for the study of this process [83]. Yousefirad et al. [84] recently investigated the effect of salt stress on seedlings of salt tolerant barley mutants using RNA-seq of bulk tissues. This provided insight into the mechanisms by which established seedlings cope with salt stress, but did not consider the effects of such an exposure during the very early stages of germination nor the cell-type specificity of tolerance mechanisms. In roots, different cell types are essential for tolerance of different abiotic stresses, employing individual strategies to sense and respond [86]. Termed the 'Gatekeeper Concept', it is conceivable that similar specialisations exist in seeds of individual cell types to particular stress responses, but this has not been investigated. By identifying these specialisations, defining their roles and characterising the underlying molecular mechanisms, we would be able to better engineer cereal crops with improved performance in challenging environments.

Challenges of multi-omics and cell-specific strategies for translational plant research

The application of individual omics' technologies in plant research has undoubtedly been very successful, especially in the last decade [3–7,17,38]. However, each technology comes with its own technical and data analysis challenges and these are further exacerbated at the single-cell level. For instance, each plant cell consists of a polysaccharide cell wall that must be removed to release the cell for scRNA-seq. This occurs via a process called protoplast isolation [12,87,88]. However, plant cell walls differ in thickness and composition across species, tissues, developmental stages and abiotic stresses, making protoplast isolation challenging whenever a new experimental system is examined [12,88]. Furthermore, protoplast isolation can induce unwanted transcriptional or metabolic changes to the cells [12]. This, coupled with the generally larger size of plant cells (100 μm) relative to mammalian cells (10–30 μm) and the variations in cell sizes among plant species, organs and tissues, may prevent some cell types from being captured and profiled by the current, most commonly used single-cell platforms and thereby introduce biases to experiments [12,13,87–90]. Optimised protocols for tissue and cellular dissociation that are applicable to a range of plant cell types must be developed for scRNA-seq analysis to become more widespread and accessible to the research community. Recent efforts to apply fixation or to isolate nuclei prior to profiling have shown promise in overcoming these obstacles [91–93].

Analysis of single-cell data is complicated by many factors including the inherent sparsity of the data, amplification biases caused by the minuscule amounts of starting cellular RNA and batch effects that occur during sample processing or library preparation [12,94,95]. While these issues can potentially be addressed using the 1000 or so scRNA-seq analysis tools currently available, it is important to remember that these tools have almost exclusively been developed based on mammalian datasets [96]. There may be as yet unrealised challenges associated with plant single-cell datasets or different analysis modes required, warranting the creation of new tools and databases. Furthermore, transcriptomics studies (bulk and single-cell) are reliant on high-quality genome assemblies and annotations for read mapping, gene-level quantification and downstream analyses [97]. These are currently lacking for the vast majority of plant species, including many medicinal plants, limiting the application of the technologies to more common model species. It is very important that we address this challenge if we are to fully investigate the diversity of plant life and efforts are underway through initiatives such as Genomics for Australian Plants and the Earth Biogenome Project, but a task of such magnitude will take substantial time [98,99].

Metabolomics and proteomics are powerful approaches for understanding the complete biotic state of biological systems, but they are not without challenges [100–102]. Proteins and metabolites cannot be amplified which limits detection sensitivity, a problem that becomes more significant in single-cell analysis [101,103]. As a result, highly efficient extraction techniques are required to isolate metabolites and proteins from single cells [101,102]. Mass spectrometry imaging (MSI) has been useful for spatially resolving cellular metabolomes and proteomes [102,104]. A highlight of this technique is its applicability to whole tissue sections, preserving spatial context and circumventing the need to digest plant cell walls [102]. However, current MSI approaches only cater to a handful of proteins and must be improved to provide a wider coverage of single-cell proteomes [102]. It will also be important to devise novel sample preparation protocols to account for the effect of post-translational modifications (PTMs) which modulate protein function and play crucial roles in a wide range of plant biological processes [105,106]. Single-cell analysis of PTMs remains challenging due to their highly

dynamic nature and the considerable amount of starting material required currently for PTM enrichment relative to total protein [102].

The adoption of multi-omics strategies is becoming routine in genomics projects. Earlier studies often analysed 'omics datasets independently then combined the results to provide better insight into the biological system being studied [107]. However, these processes can be represented more accurately by true integrative analysis of these individual modalities [107–111]. This is challenging at many levels and requires the careful consideration of several factors, such as the computational burden associated with the storage and analysis of various data types and the lack of heterogeneity across 'omics technologies [107–109]. The increasing interest in single-cell analyses and the tremendous opportunity that single-cell multi-omics represents pose additional analysis challenges such as the preponderance of missing values and noise across modalities [107]. While specialised software and workflows are continuously being developed and refined to address these issues, it is unclear how these will perform with plant single-cell datasets and how they will accommodate the analysis of emerging modalities for plant single-cell research.

Summary

- Individual cells and tissues are defined by their unique gene regulatory programmes. These drive the characteristic growth, development and physiology of cell types, enabling their particular function within the broader organism.
- Classically most 'omics approaches in plant biology have focused on bulk tissue samples, achieving organ-level resolution at best. However, recent advances in microfluidic cell handling and spatial analyses now enable transcriptome, proteome and metabolome analysis at a resolution of single to tens of cells.
- Gene regulatory network analyses can be applied to integrate data from these different modalities, enhancing our ability to understand the individual and concerted roles of genes, proteins and metabolites in the biology of cell types.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Author Contribution

M.G.L. and B.H. conceived and wrote the article. Both authors approved the final version.

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Abbreviations

BIA, benzylisoquinoline alkaloid; CNV, copy number variation; LCM, laser-capture microdissection; MSI, mass spectrometry imaging; PCA, Plant Cell Atlas; PTMs, post-translational modifications; scRNA-seq, single-cell RNA-seq.

References

1 Purugganan, M.D. (2019) Evolutionary insights into the nature of plant domestication. *Curr. Biol.* **29**, R705–R714 https://doi.org/10.1016/j.cub.2019.05.053
2 Ross-Ibarra, J., Morrell, P.L. and Gaut, B.S. (2007) Plant domestication, a unique opportunity to identify the genetic basis of adaptation. *Proc. Natl Acad. Sci. U.S.A.* **104**, 8641 https://doi.org/10.1073/pnas.0700643104
3 Yang, Y., Saand, M.A., Huang, L., Abdelaal, W.B., Zhang, J., Wu, Y. et al. (2021) Applications of multi-omics technologies for crop improvement. *Front. Plant Sci.* **12**, 563953 https://doi.org/10.3389/fpls.2021.563953
4 Zander, M., Lewsey, M.G., Clark, N.M., Yin, L., Bartlett, A., Saldierna Guzmán, J.P. et al. (2020) Integrated multi-omics framework of the plant response to jasmonic acid. *Nat. Plants* **6**, 290–302 https://doi.org/10.1038/s41477-020-0605-7
5 Crandall, S.G., Gold, K.M., Jiménez-Gasco, M.D.M., Filgueiras, C.C. and Willett, D.S. (2020) A multi-omics approach to solving problems in plant disease ecology. *PLoS One* **15**, e0237975. https://doi.org/10.1371/journal.pone.0237975
6 Wong, D.C.J. (2018) Harnessing integrated omics approaches for plant specialized metabolism research: new insights into shikonin biosynthesis. *Plant Cell Physiol.* **60**, 4–6 https://doi.org/10.1093/pcp/pcy230
7 Li, Q. and Yan, J. (2020) Sustainable agriculture in the era of omics: knowledge-driven crop breeding. *Genome Biol.* **21**, 154 https://doi.org/10.1186/s13059-020-02073-5
8 Wu, K., Wang, S., Song, W., Zhang, J., Wang, Y., Liu, Q. et al. (2020) Enhanced sustainable Green revolution yield via nitrogen-responsive chromatin modulation in rice. *Science* **367**, eaaz2046 https://doi.org/10.1126/science.aaz2046
9 Liu, H.-J., Jian, L., Xu, J., Zhang, Q., Zhang, M., Jin, M. et al. (2020) High-throughput CRISPR/Cas9 mutagenesis streamlines trait gene identification in maize. *Plant Cell* **32**, 1397–1413 https://doi.org/10.1105/tpc.19.00934
10 Kwon, C.-T., Heo, J., Lemmon, Z.H., Capua, Y., Hutton, S.F., Van Eck, J. et al. (2020) Rapid customization of Solanaceae fruit crops for urban agriculture. *Nat. Biotechnol.* **38**, 182–188 https://doi.org/10.1038/s41587-019-0361-2
11 Cole, B., Bergmann, D., Blaby-Haas, C.E., Blaby, I.K., Bouchard, K.E., Brady, S.M. et al. (2021) Plant single-cell solutions for energy and the environment. *Commun. Biol.* **4**, 962 https://doi.org/10.1038/s42003-021-02477-4
12 Shaw, R., Tian, X. and Xu, J. (2021) Single-cell transcriptome analysis in plants: advances and challenges. *Mol. Plant* **14**, 115–126 https://doi.org/10.1016/j.molp.2020.10.012
13 Ryu, K.H., Huang, L., Kang, H.M. and Schiefelbein, J. (2019) Single-cell RNA sequencing resolves molecular relationships among individual plant cells. *Plant Physiol.* **179**, 1444–1456 https://doi.org/10.1104/pp.18.01482
14 Rich-Griffin, C., Stechemesser, A., Finch, J., Lucas, E., Ott, S. and Schäfer, P. (2020) Single-cell transcriptomics: a high-resolution avenue for plant functional genomics. *Trends Plant Sci.* **25**, 186–197 https://doi.org/10.1016/j.tplants.2019.10.008
15 Satterlee, J.W., Strable, J. and Scanlon, M.J. (2020) Plant stem-cell organization and differentiation at single-cell resolution. *Proc. Natl Acad. Sci. U.S.A.* **117**, 33689 https://doi.org/10.1073/pnas.2018788117
16 Hasin, Y., Seldin, M. and Lusis, A. (2017) Multi-omics approaches to disease. *Genome Biol.* **18**, 83 https://doi.org/10.1186/s13059-017-1215-1
17 Rai, A., Saito, K. and Yamazaki, M. (2017) Integrated omics analysis of specialized metabolism in medicinal plants. *Plant J.* **90**, 764–787 https://doi.org/10.1111/tpj.13485
18 Jha, S.G., Borowsky, A.T., Cole, B.J., Fahlgren, N., Farmer, A., Huang, S.-S.C. et al. (2021) Science forum: vision, challenges and opportunities for a plant cell atlas. *eLife* **10**, e66877 https://doi.org/10.7554/eLife.66877
19 Rhee, S.Y., Birnbaum, K.D. and Ehrhardt, D.W. (2019) Towards building a plant cell atlas. *Trends Plant Sci.* **24**, 303–310 https://doi.org/10.1016/j.tplants.2019.01.006
20 Longo, S.K., Guo, M.G., Ji, A.L. and Khavari, P.A. (2021) Integrating single-cell and spatial transcriptomics to elucidate intercellular tissue dynamics. *Nat. Rev. Genet.* **22**, 627–644 https://doi.org/10.1038/s41576-021-00370-8
21 Marx, V. (2021) Method of the year: spatially resolved transcriptomics. *Nat. Methods* **18**, 9–14 https://doi.org/10.1038/s41592-020-01033-y
22 van Dam, S., Võsa, U., van der Graaf, A., Franke, L. and de Magalhães, J.P. (2017) Gene co-expression analysis for functional classification and gene–disease predictions. *Brief. Bioinform.* **19**, 575–592 https://doi.org/10.1093/bib/bbw139
23 Sun, Y. and Dinneny, J.R. (2018) Q&A: how do gene regulatory networks control environmental responses in plants? *BMC Biol.* **16**, 38 https://doi.org/10.1186/s12915-018-0506-7
24 Hurgobin, B., de Jong, E. and Bosco, A. (2018) Insights into respiratory disease through bioinformatics. *Respirology* **23**, 1117–1126 https://doi.org/10.1111/resp.13401
25 Livingston, S.J., Quilichini, T.D., Booth, J.K., Wong, D.C.J., Rensing, K.H., Laflamme-Yonkman, J. et al. (2020) Cannabis glandular trichomes alter morphology and metabolite content during flower maturation. *Plant J.* **101**, 37–56 https://doi.org/10.1111/tpj.14516
26 Zager, J.J., Lange, I., Srividya, N., Smith, A. and Lange, B.M. (2019) Gene networks underlying cannabinoid and terpenoid accumulation in cannabis. *Plant Physiol.* **180**, 1877–1897 https://doi.org/10.1104/pp.18.01506
27 Liu, W., Lin, L., Zhang, Z., Liu, S., Gao, K., Lv, Y. et al. (2019) Gene co-expression network analysis identifies trait-related modules in *Arabidopsis thaliana*. *Planta* **249**, 1487–1501 https://doi.org/10.1007/s00425-019-03102-9
28 Chen, D., Yan, W., Fu, L.-Y. and Kaufmann, K. (2018) Architecture of gene regulatory networks controlling flower development in *Arabidopsis thaliana*. *Nat. Commun.* **9**, 4534 https://doi.org/10.1038/s41467-018-06772-3
29 Van de Sande, B., Flerin, C., Davie, K., De Waegeneer, M., Hulselmans, G., Aibar, S. et al. (2020) A scalable SCENIC workflow for single-cell gene regulatory network analysis. *Nat. Protoc.* **15**, 2247–2276 https://doi.org/10.1038/s41596-020-0336-2

30 Pratapa, A., Jalihal, A.P., Law, J.N., Bharadwaj, A. and Murali, T.M. (2020) Benchmarking algorithms for gene regulatory network inference from single-cell transcriptomic data. *Nat. Methods* **17**, 147–154 https://doi.org/10.1038/s41592-019-0690-6
31 Iacono, G., Massoni-Badosa, R. and Heyn, H. (2019) Single-cell transcriptomics unveils gene regulatory network plasticity. *Genome Biol.* **20**, 110 https://doi.org/10.1186/s13059-019-1713-4
32 Bongers, F.J., Douma, J.C., Iwasa, Y., Pierik, R., Evers, J.B. and Anten, N.P. (2019) Variation in plastic responses to light results from selection in different competitive environments—a game theoretical approach using virtual plants. *PLoS Comput. Biol.* **15**, e1007253 https://doi.org/10.1371/journal.pcbi.1007253
33 Martín-Sanz, R.C., San-Martín, R., Poorter, H., Vázquez, A. and Climent, J. (2019) How does water availability affect the allocation to bark in a Mediterranean conifer? *Front. Plant Sci.* **10**, 607 https://doi.org/10.3389/fpls.2019.00607
34 Henn, J.J., Buzzard, V., Enquist, B.J., Halbritter, A.H., Klanderud, K., Maitner, B.S. et al. (2018) Intraspecific trait variation and phenotypic plasticity mediate alpine plant species response to climate change. *Front. Plant Sci.* **9**, 1548 https://doi.org/10.3389/fpls.2018.01548
35 Liu, N., Du, Y., Warburton, M.L., Xiao, Y. and Yan, J. (2020) Phenotypic plasticity contributes to maize adaptation and heterosis. *Mol. Biol. Evol.* **38**, 1262–1275 https://doi.org/10.1093/molbev/msaa283
36 Sobral, M., Sampedro, L., Neylan, I., Siemens, D. and Dirzo, R. (2021) Phenotypic plasticity in plant defense across life stages: inducibility, transgenerational induction, and transgenerational priming in wild radish. *Proc. Natl Acad. Sci. U.S.A.* **118**, e2005865118 https://doi.org/10.1073/pnas.2005865118
37 Ignea, C., Athanasakoglou, A., Andreadelli, A., Apostolaki, M., Iakovides, M., Stephanou, E.G. et al. (2017) Overcoming the plasticity of plant specialized metabolism for selective diterpene production in yeast. *Sci. Rep.* **7**, 8855 https://doi.org/10.1038/s41598-017-09592-5
38 Tiedge, K., Muchlinski, A. and Zerbe, P. (2020) Genomics-enabled analysis of specialized metabolism in bioenergy crops: current progress and challenges. *Synth. Biol.* **5**, ysaa005 https://doi.org/10.1093/synbio/ysaa005
39 Schuurink, R. and Tissier, A. (2020) Glandular trichomes: micro-organs with model status? *New Phytol.* **225**, 2251–2266 https://doi.org/10.1111/nph.16283
40 Ramos, M.V., Demarco, D., da Costa Souza, I.C. and de Freitas, C.D.T. (2019) Laticifers, latex, and their role in plant defense. *Trends Plant Sci.* **24**, 553–567 https://doi.org/10.1016/j.tplants.2019.03.006
41 Ngan, N.T.T., Hoang, N.H., Hien, N.T., Lan, N.N., Lien, N.T.K., Quang, T.H. et al. (2020) Cytotoxic phenanthrenes and phenolic constituents from the tubers of *Dioscorea persimilis*. *Phytochem. Lett.* **40**, 139–143 https://doi.org/10.1016/j.phytol.2020.10.005
42 Tanney, C.A.S., Backer, R., Geitmann, A. and Smith, D.L. (2021) Cannabis glandular trichomes: a cellular metabolite factory. *Front. Plant Sci.* **12**, 721986 https://doi.org/10.3389/fpls.2021.721986
43 Hurgobin, B., Tamiru-Oli, M., Welling, M.T., Doblin, M.S., Bacic, A., Whelan, J. et al. (2021) Recent advances in *Cannabis sativa* genomics research. *New Phytol.* **230**, 73–89 https://doi.org/10.1111/nph.17140
44 Kovalchuk, I., Pellino, M., Rigault, P., Van Velzen, R., Ebersbach, J., Ashnest, J. et al. (2020) The genomics of cannabis and its close relatives. *Annu. Rev. Plant Biol.* **71**, 713–739 https://doi.org/10.1146/annurev-arplant-081519-040203
45 Booth, J.K., Yuen, M.M., Jancsik, S., Madilao, L.L., Page, J.E. and Bohlmann, J. (2020) Terpene synthases and terpene variation in *Cannabis sativa*. *Plant Physiol.* **184**, 130–147 https://doi.org/10.1104/pp.20.00593
46 Jenkins, C. and Orsburn, B. (2020) The cannabis proteome draft map project. *Int. J. Mol. Sci.* **21**, 965 https://doi.org/10.3390/ijms21030965
47 Conneely, L.J., Mauleon, R., Mieog, J., Barkla, B.J. and Kretzschmar, T. (2021) Characterization of the *Cannabis sativa* glandular trichome proteome. *PLoS One* **16**, e0242633 https://doi.org/10.1371/journal.pone.0242633
48 Hillig, K.W. and Mahlberg, P.G. (2004) A chemotaxonomic analysis of cannabinoid variation in Cannabis (Cannabaceae). *Am. J. Bot.* **91**, 966–975 https://doi.org/10.3732/ajb.91.6.966
49 Welling, M.T., Liu, L., Shapter, T., Raymond, C.A. and King, G.J. (2016) Characterisation of cannabinoid composition in a diverse *Cannabis sativa* L. germplasm collection. *Euphytica* **208**, 463–475 https://doi.org/10.1007/s10681-015-1585-y
50 McKernan, K.J., Helbert, Y., Kane, L.T., Ebling, H., Zhang, L., Liu, B. et al. (2020) Sequence and annotation of 42 cannabis genomes reveals extensive copy number variation in cannabinoid synthesis and pathogen resistance genes. *BioRxiv*
51 Ran, F.A., Hsu, P.D., Wright, J., Agarwala, V., Scott, D.A. and Zhang, F. (2013) Genome engineering using the CRISPR-Cas9 system. *Nat. Protoc.* **8**, 2281–2308 https://doi.org/10.1038/nprot.2013.143
52 Bortesi, L. and Fischer, R. (2015) The CRISPR/Cas9 system for plant genome editing and beyond. *Biotechnol. Adv.* **33**, 41–52 https://doi.org/10.1016/j.biotechadv.2014.12.006
53 Schraivogel, D., Gschwind, A.R., Milbank, J.H., Leonce, D.R., Jakob, P., Mathur, L. et al. (2020) Targeted Perturb-seq enables genome-scale genetic screens in single cells. *Nat. Methods* **17**, 629–635 https://doi.org/10.1038/s41592-020-0837-5
54 Jackson, C.A., Castro, D.M., Saldi, G.-A., Bonneau, R. and Gresham, D. (2020) Gene regulatory network reconstruction using single-cell RNA sequencing of barcoded genotypes in diverse environments. *eLife* **9**, e51254 https://doi.org/10.7554/eLife.51254
55 Nemhauser, J.L. and Torii, K.U. (2016) Plant synthetic biology for molecular engineering of signalling and development. *Nat. Plants* **2**, 1–7 https://doi.org/10.1038/nplants.2016.10
56 Báez R, R., Buckley, Y., Yu, H., Chen, Z., Gallavotti, A., Nemhauser, J.L. et al. (2020) A synthetic approach allows rapid characterization of the maize nuclear auxin response circuit. *Plant Physiol.* **182**, 1713–1722 https://doi.org/10.1104/pp.19.01475
57 Pierre-Jerome, E., Moss, B.L., Lanctot, A., Hageman, A. and Nemhauser, J.L. (2016) Functional analysis of molecular interactions in synthetic auxin response circuits. *Proc. Natl Acad. Sci. U.S.A.* **113**, 11354–11359 https://doi.org/10.1073/pnas.1604379113
58 Qin, W., Xie, L., Li, Y., Liu, H., Fu, X., Chen, T. et al. (2021) An R2R3-MYB transcription factor positively regulates the glandular secretory trichome initiation in *Artemisia annua* L. *Front. Plant Sci.* **12**, 657156 https://doi.org/10.3389/fpls.2021.657156
59 Xie, L., Yan, T., Li, L., Chen, M., Ma, Y., Hao, X. et al. (2020) The WRKY transcription factor AaGSW2 promotes glandular trichome initiation in *Artemisia annua*. *J. Exp. Bot.* **72**, 1691–1701 https://doi.org/10.1093/jxb/eraa523
60 Hua, B., Chang, J., Wu, M., Xu, Z., Zhang, F., Yang, M. et al. (2021) Mediation of JA signalling in glandular trichomes by the *woolly/SlMYC1* regulatory module improves pest resistance in tomato. *Plant Biotechnol. J.* **19**, 375–393 https://doi.org/10.1111/pbi.13473
61 O'Malley, R.C., Huang, S.-S.C., Song, L., Lewsey, M.G., Bartlett, A., Nery, J.R. et al. (2016) Cistrome and epicistrome features shape the regulatory DNA landscape. *Cell* **165**, 1280–1292 https://doi.org/10.1016/j.cell.2016.04.038

62 Bartlett, A., O'Malley, R.C., Huang, S.-S.C., Galli, M., Nery, J.R., Gallavotti, A. et al. (2017) Mapping genome-wide transcription-factor binding sites using DAP-seq. *Nat. Protoc.* **12**, 1659–1672 https://doi.org/10.1038/nprot.2017.055
63 Blat, Y. and Kleckner, N. (1999) Cohesins bind to preferential sites along yeast chromosome III, with differential regulation along arms versus the centric region. *Cell* **98**, 249–259 https://doi.org/10.1016/S0092-8674(00)81019-3
64 Ren, B., Robert, F., Wyrick, J.J., Aparicio, O., Jennings, E.G. Simon, I. et al. (2000) Genome-wide location and function of DNA binding proteins. *Science* **290**, 2306–2309 https://doi.org/10.1126/science.290.5500.2306
65 Liu, Y., Zhu, P., Cai, S., Haughn, G. and Page, J.E. (2021) Three novel transcription factors involved in cannabinoid biosynthesis in *Cannabis sativa* L. *Plant Mol. Biol.* **106**, 49–65 https://doi.org/10.1007/s11103-021-01129-9
66 Singh, A., Menéndez-Perdomo, I.M. and Facchini, P.J. (2019) Benzylisoquinoline alkaloid biosynthesis in opium poppy: an update. *Phytochem. Rev.* **18**, 1457–1482 https://doi.org/10.1007/s11101-019-09644-w
67 Beaudoin, G.A. and Facchini, P.J. (2014) Benzylisoquinoline alkaloid biosynthesis in opium poppy. *Planta* **240**, 19–32 https://doi.org/10.1007/s00425-014-2056-8
68 Tamiru-Oli, M., Premaratna, S.D., Gendall, A.R. and Lewsey, M.G. (2018) Biochemistry, genetics, and genomics of opium poppy (*Papaver somniferum*) for crop improvement. In *Annual Plant Reviews*, **2**, pp. 1177–1219 https://doi.org/10.1002/9781119312994.apr0711
69 Onoyovwe, A., Hagel, J.M., Chen, X., Khan, M.F., Schriemer, D.C. and Facchini, P.J. (2013) Morphine biosynthesis in opium poppy involves two cell types: sieve elements and laticifers. *Plant Cell* **25**, 4110–4122 https://doi.org/10.1105/tpc.113.115113
70 Zhao, Y., Zhang, Z., Li, M., Luo, J., Chen, F., Gong, Y. et al. (2019) Transcriptomic profiles of 33 opium poppy samples in different tissues, growth phases, and cultivars. *Sci. Data.* **6**, 66 https://doi.org/10.1038/s41597-019-0082-x
71 Li, Q., Ramasamy, S., Singh, P., Hagel, J.M., Dunemann, S.M., Chen, X. et al. (2020) Gene clustering and copy number variation in alkaloid metabolic pathways of opium poppy. *Nat. Commun.* **11**, 1190 https://doi.org/10.1038/s41467-020-15040-2
72 Hong, U.V.T., Tamiru-Oli, M., Hurgobin, B., Okey, C.R, Abreu, A.R and Lewsey, M.G. (2021) Insights into opium poppy (*Papaver* spp.) genetic diversity from genotyping-by-sequencing analysis. *bioRxiv* 2021.09.28.462245
73 Hagel, J.M. and Facchini, P.J. (2013) Benzylisoquinoline alkaloid metabolism: a century of discovery and a brave new world. *Plant Cell Physiol.* **54**, 647–672 https://doi.org/10.1093/pcp/pct020
74 Wang, Z., Chen, F., Li, X., Cao, H., Ding, M., Zhang, C. et al. (2016) Arabidopsis seed germination speed is controlled by SNL histone deacetylase-binding factor-mediated regulation of AUX1. *Nat. Commun.* **7**, 13412 https://doi.org/10.1038/ncomms13412
75 Qu, C., Zuo, Z., Cao, L., Huang, J., Sun, X., Zhang, P. et al. (2019) Comprehensive dissection of transcript and metabolite shifts during seed germination and post-germination stages in poplar. *BMC Plant Biol.* **19**, 279 https://doi.org/10.1186/s12870-019-1862-3
76 Liew, L.C., Narsai, R., Wang, Y., Berkowitz, O., Whelan, J. and Lewsey, M.G. (2020) Temporal tissue-specific regulation of transcriptomes during barley (*Hordeum vulgare*) seed germination. *Plant J.* **101**, 700–715 https://doi.org/10.1111/tpj.14574
77 Gioria, M., Pyšek, P. and Osborne, B.A. (2016) Timing is everything: does early and late germination favor invasions by herbaceous alien plants? *J. Plant Ecol.* **11**, 4–16 https://doi.org/10.1093/jpe/rtw105
78 Topham, A.T., Taylor, R.E., Yan, D., Nambara, E., Johnston, I.G. and Bassel, G.W. (2017) Temperature variability is integrated by a spatially embedded decision-making center to break dormancy in *Arabidopsis* seeds. *Proc. Natl Acad. Sci. U.S.A.* **114**, 6629 https://doi.org/10.1073/pnas.1704745114
79 Narsai, R., Law, S.R., Carrie, C., Xu, L. and Whelan, J. (2011). In-depth temporal transcriptome profiling reveals a crucial developmental switch with roles for RNA processing and organelle metabolism that are essential for germination in Arabidopsis. *Plant Physiol.* **157**, 1342–1362 https://doi.org/10.1104/pp.111.183129
80 Narsai, R., Gouil, Q., Secco, D., Srivastava, A., Karpievitch, Y.V., Liew, L.C. et al. (2017) Extensive transcriptomic and epigenomic remodelling occurs during *Arabidopsis thaliana* germination. *Genome Biol.* **18**, 172 https://doi.org/10.1186/s13059-017-1302-3
81 Dekkers, B.J.W., Pearce, S., van Bolderen-Veldkamp, R.P., Marshall, A., Widera, P., Gilbert, J. et al. (2013) Transcriptional dynamics of two seed compartments with opposing roles in arabidopsis seed germination. *Plant Physiol.* **163**, 205–215 https://doi.org/10.1104/pp.113.223511
82 Espina, V., Wulfkuhle, J.D., Calvert, V.S., VanMeter, A., Zhou, W., Coukos, G. et al. (2006) Laser-capture microdissection. *Nat. Protoc.* **1**, 586–603 https://doi.org/10.1038/nprot.2006.85
83 Mwando, E., Angessa, T.T., Han, Y. and Li, C. (2020) Salinity tolerance in barley during germination- homologs and potential genes. *J. Zhejiang Univ. Sci. B* **21**, 93–121 https://doi.org/10.1631/jzus.B1900400
84 Yousefirad, S., Soltanloo, H., Ramezanpour, S.S., Zaynali Nezhad, K. and Shariati, V. (2020) The RNA-seq transcriptomic analysis reveals genes mediating salt tolerance through rapid triggering of ion transporters in a mutant barley. *PLOS One* **15**, e0229513 https://doi.org/10.1371/journal.pone.0229513
85 Zhang, C., Luo, W., Li, Y., Zhang, X., Bai, X., Niu, Z. et al. (2019) Transcriptomic analysis of seed germination under salt stress in two desert sister species (*Populus euphratica* and *P. pruinosa*). *Front. Genet.* **10**, 231 https://doi.org/10.3389/fgene.2019.00231
86 Henderson,, S.W. and Gilliham, M. (2015) The "Gatekeeper" concept: cell-type specific molecular mechanisms of plant adaptation to abiotic stress. In Laitinen, R.A.E. (ed.), *Molecular Mechanisms in Plant Adaptation*, John Wiley & Sons, Hoboken, NJ, USA, pp. 83–115
87 Jean-Baptiste, K., McFaline-Figueroa, J.L., Alexandre, C.M., Dorrity, M.W., Saunders, L., Bubb, K.L. et al. (2019) Dynamics of gene expression in single root cells of *Arabidopsis thaliana*. *Plant Cell* **31**, 993–1011 https://doi.org/10.1105/tpc.18.00785
88 Shulse, C.N., Cole, B.J., Ciobanu, D., Lin, J., Yoshinaga, Y., Gouran, M. et al. (2019) High-throughput single-cell transcriptome profiling of plant cell types. *Cell Rep.* **27**, 2241–7.e4 https://doi.org/10.1016/j.celrep.2019.04.054
89 Denyer, T., Ma, X., Klesen, S., Scacchi, E., Nieselt, K. and Timmermans, M.C. (2019) Spatiotemporal developmental trajectories in the Arabidopsis root revealed using high-throughput single-cell RNA sequencing. *Dev. Cell* **48**, 840–852.e5 https://doi.org/10.1016/j.devcel.2019.02.022
90 Zhang, T.-Q., Xu, Z.-G., Shang, G.-D. and Wang, J.-W. (2019) A single-cell RNA sequencing profiles the developmental landscape of Arabidopsis root. *Mol. Plant* **12**, 648–660 https://doi.org/10.1016/j.molp.2019.04.004
91 Long, Y., Liu, Z., Jia, J., Mo, W., Fang, L., Lu, D. et al. (2021) FlsnRNA-seq: protoplasting-free full-length single-nucleus RNA profiling in plants. *Genome Biol.* **22**, 66 https://doi.org/10.1186/s13059-021-02288-0
92 Sunaga-Franze, D.Y., Muino, J.M., Braeuning, C., Xu, X., Zong, M., Smaczniak, C. et al. (2021) Single-nucleus RNA sequencing of plant tissues using a nanowell-based system. *Plant J.* **108**, 859–869 https://doi.org/10.1111/tpj.15458

93 Conde, D., Triozzi, P.M., Balmant, K.M., Doty, A.L., Miranda, M., Boullosa, A. et al. (2021) A robust method of nuclei isolation for single-cell RNA sequencing of solid tissues from the plant genus Populus. *PLoS One* **16**, e0251149 https://doi.org/10.1371/journal.pone.0251149
94 Lähnemann, D., Köster, J., Szczurek, E., McCarthy, D.J., Hicks, S.C., Robinson, M.D. et al. (2020) Eleven grand challenges in single-cell data science. *Genome Biol.* **21**, 31 https://doi.org/10.1186/s13059-020-1926-6
95 Yuan, G.-C., Cai, L., Elowitz, M., Enver, T., Fan, G., Guo, G. et al. (2017) Challenges and emerging directions in single-cell analysis. *Genome Biol.* **18**, 84 https://doi.org/10.1186/s13059-017-1218-y
96 Zappia, L., Phipson, B. and Oshlack, A. (2018) Exploring the single-cell RNA-seq analysis landscape with the scRNA-tools database. *PLOS Comput. Biol.* **14**, e1006245 https://doi.org/10.1371/journal.pcbi.1006245
97 Jung, H., Ventura, T., Chung, J.S., Kim, W.-J., Nam, B.-H., Kong, H.J. et al. (2020) Twelve quick steps for genome assembly and annotation in the classroom. *PLOS Comput. Biol.* **16**, e1008325 https://doi.org/10.1371/journal.pcbi.1008325
98 Lewin, H.A., Robinson, G.E., Kress, W.J., Baker, W.J., Coddington, J., Crandall, K.A. et al. (2018) Earth BioGenome project: sequencing life for the future of life. *Proc. Natl Acad. Sci. U.S.A.* **115**, 4325–4333 https://doi.org/10.1073/pnas.1720115115
99 Genomics for Australian Plants [Internet]. 2018 Available from: https://www.genomicsforaustralianplants.com/
100 Schubert, O.T., Röst, H.L., Collins, B.C., Rosenberger, G. and Aebersold, R. (2017) Quantitative proteomics: challenges and opportunities in basic and applied research. *Nat. Protoc.* **12**, 1289–1294 https://doi.org/10.1038/nprot.2017.040
101 de Souza, L.P., Borghi, M. and Fernie, A. (2020) Plant single-cell metabolomics—challenges and perspectives. *Int. J. Mol. Sci.* **21**, 8987 https://doi.org/10.3390/ijms21238987
102 Clark, N.M., Elmore, J.M. and Walley, J.W. (2021) To the proteome and beyond: advances in single-cell omics profiling for plant systems. *Plant Physiol.* **188**, 726–737 https://doi.org/10.1093/plphys/kiab429
103 Ouyang, W. and Han, J. (2019) Universal amplification-free molecular diagnostics by billion-fold hierarchical nanofluidic concentration. *Proc. Natl Acad. Sci. U.S.A.* **116**, 16240 https://doi.org/10.1073/pnas.1904513116
104 Taylor, M.J., Lukowski, J.K. and Anderton, C.R. (2021) Spatially resolved mass spectrometry at the single cell: recent innovations in proteomics and metabolomics. *J. Am. Soc. Mass Spectrom.* **32**, 872–894 https://doi.org/10.1021/jasms.0c00439
105 Song, G. and Walley, J.W. (2016) Dynamic protein acetylation in plant-pathogen interactions. *Front. Plant Sci.* **7**, 421. https://doi.org/10.3389/fpls.2016.00421
106 Walley, J.W., Shen, Z., McReynolds, M.R., Schmelz, E.A. and Briggs, S.P. (2018) Fungal-induced protein hyperacetylation in maize identified by acetylome profiling. *Proc. Natl Acad. Sci. U.S.A.* **115**, 210 https://doi.org/10.1073/pnas.1717519115
107 Tarazona, S., Arzalluz-Luque, A. and Conesa, A. (2021) Undisclosed, unmet and neglected challenges in multi-omics studies. *Nat. Comput. Sci.* **1**, 395–402 https://doi.org/10.1038/s43588-021-00086-z
108 Gomez-Cabrero, D., Abugessaisa, I., Maier, D., Teschendorff, A., Merkenschlager, M., Gisel, A. et al. (2014) Data integration in the era of omics: current and future challenges. *BMC Syst. Biol.* **8**, I1 https://doi.org/10.1186/1752-0509-8-S2-I1
109 Duan, R., Gao, L., Gao, Y., Hu, Y., Xu, H., Huang, M. et al. (2021) Evaluation and comparison of multi-omics data integration methods for cancer subtyping. *PLOS Comput. Biol.* **17**, e1009224 https://doi.org/10.1371/journal.pcbi.1009224
110 Walley, J.W., Sartor, R.C., Shen, Z., Schmitz, R.J., Wu, K.J., Urich, M.A. et al. (2016) Integration of omic networks in a developmental atlas of maize. *Science* **353**, 814–818 https://doi.org/10.1126/science.aag1125
111 Clark, N.M., Nolan, T.M., Wang, P., Song, G., Montes, C., Valentine, C.T. et al. (2021) Integrated omics networks reveal the temporal signaling events of brassinosteroid response in Arabidopsis. *Nat. Commun.* **12**, 5858 https://doi.org/10.1038/s41467-021-26165-3