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Phenotyping floral attractiveness to pollinators using volatilomics, 3D imaging, and insect monitoring

PLANTS, POLLINATORS, AND BIODIVERSITY: THE STATUS QUO

Plant–pollinator coevolution has played a crucial role in shaping the biodiversity of ecosystems that we know today. Moreover, plants and pollinators are key to agriculture, contributing to the production of most fruits and vegetables necessary for healthy human diets. Unfortunately, over the last decades, there is mounting evidence of pollinator decline all over the world, which constitutes a major threat to food security (European Commission, 2020). To pollinators, nectar and pollen are the main rewards: pollen is essentially their only source of proteins, lipids, and vitamins, while nectar is a carbohydrate-rich solution that they use to fuel somatic functions (Ollerton, 2021). However, nectar is much more than just a sweet solution: it comprises a plethora of secondary metabolites and volatiles of major importance for plant–pollinator communication. Unfortunately, by neglecting these traits and reducing genetic diversity, plant breeding has potentially increased the risk of losing the traits beneficial to pollinators.

Improving cultivated plants for pollinator attractiveness is complex and requires a multidisciplinary approach in phenotyping. Once considered particularly labor intensive, the task of plant phenotyping has become highly automated owing to the recent advancements in sensing technologies, automation, and machine learning (Hall et al., 2022). Consequently, the automation of plant phenotyping has greatly advanced plant breeding, whose challenge became the capacity to analyze and integrate multiomics data that are being rapidly generated with increasing complexity (Xu et al., 2022). In this paper, we discuss some of the representative case studies and propose the integration of three phenotyping approaches for studying flower attractiveness in the context of plant–pollinator interactions (PPIs; Figure 1).

VOLATILOMICS IN THE CONTEXT OF PPIs

Plants use diverse chemicals to communicate with the world around them, many of which are volatile organic compounds (VOCs). VOCs are typically lipophilic molecules with high vapor pressure that play a multitude of roles during the plant life cycle, including the attraction of pollinators. Being highly diverse in their structure, plant VOCs comprise fatty acid derivatives, terpenoids, benzenoids/phenylpropanoids, as well as volatile hormones (Majchrzak et al., 2020; Dötterl and Gershenzon, 2023). The major challenges in the identification of these compounds are due to their numerousness and low abundance, which makes it necessary to analyze them using sensitive, high-throughput analytical techniques (Majchrzak et al., 2020).

Over the last decades, significant developments have occurred in the different fields of omics technologies. Within metabolomics, analysis of VOCs gave rise to the so-called "volatilomics." To collect the VOCs, different setups exist. Glass tubes can be used to fully enclose the plants, with the disadvantage of creating an artificial environment. Alternatively, sampling of volatiles could be performed using small pumps to suck air away from a bagged flower or other organs (Figure 1). Both setups allow VOC capture using static (e.g., solid-phase microextraction) or dynamic (volatile traps) headspace sampling procedures. Once trapped, VOC identification and quantification can be achieved using various techniques, depending upon the biological question and the type of compounds of interest, and today, gas chromatography–mass spectrometry (GC–MS) is the predominant one. GC–MS has seen notable advancements, including the widely targeted volatilomics method, which employs a "targeted spectra extraction" algorithm, resulting in enhanced sensitivity, high annotation coverage, and improved reproducibility (Yuan et al., 2024). Furthermore, recent years have witnessed the development of direct-injection MS (DI–MS) techniques which enable real-time monitoring of VOCs from various sources, ranging from plant parts to entire ecosystems, facilitating a more comprehensive analysis (Majchrzak et al., 2020). Technically, DI–MS analyses could be performed using proton transfer reaction–MS or an electronic nose, both of which can rapidly detect and quantify mass features. While e-nose instruments are portable, the proton transfer reaction–MS equipment is cumbersome, which makes it inappropriate for a high-throughput phenotyping facility (Hall et al., 2022). On the other hand, the main disadvantage of DI–MS is that it cannot assign structural identity unless equipped with a high-resolution time-of-flight–MS or coupled to MS/MS detectors that are sufficiently portable. As DI–MS instruments are relatively recent, another limitation is the lack of open-access databases of plant metabolites that could be used on this instrument. The solution for overcoming this limitation would be to integrate DI–MS methods with omics platforms through coupling with other high-throughput techniques, which is the current trend (Majchrzak et al., 2020). In addition to GC–MS, liquid chromatography–MS is frequently used in the analysis of secondary metabolites owing to the easy sample preparation, wide application, and large detection range, while its drawbacks include limited commercial libraries and challenging standardization (Shen

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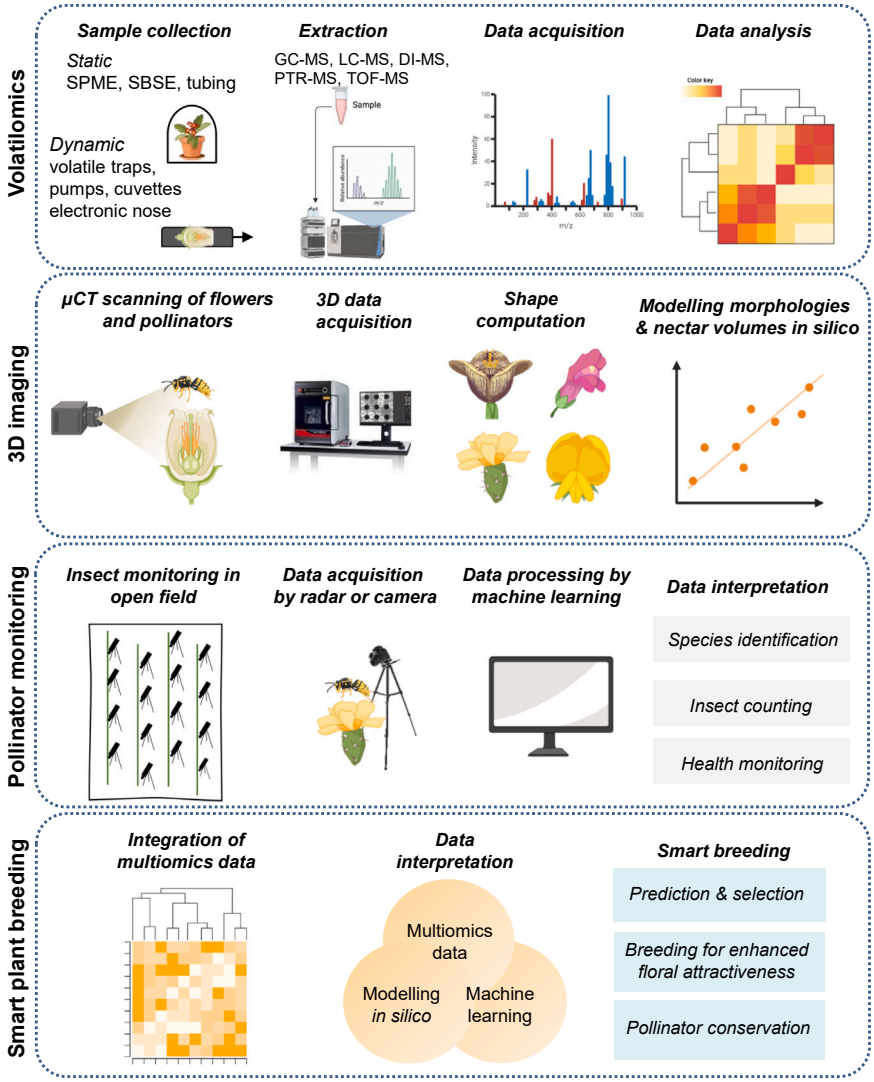

Figure 1. Phenotyping floral traits for enhancing PPIs.

Volatilomics. A typical metabolomics study workflow is as follows: (1) sample collection, (2) sample extraction, (3) data acquisition, and (4) data analysis. Volatile sampling could be done in different ways, such as by fully enclosing the plants or *in vivo*. GC, gas chromatography; LC, liquid chromatography; MS, mass spectrometry; PTR, proton transfer reaction; SPME, solid-phase microextraction; TOF, time of flight. 3D imaging and computation of flowers and pollinators was done using microcomputed tomography (μCT). Pollinator monitoring can be achieved in greenhouse conditions or in an open field using insect-counting devices—radars or cameras. Smart plant breeding and integration of phenotyping information including multiomics data, high-precision imaging, and pollinator monitoring.

et al., 2023). The advantages and disadvantages of other analysis techniques in metabolomics are reviewed in more details by Shen et al. (2023). For detailed practical information on these techniques, readers are also directed to the review by Hall et al. (2022).

COMPUTATION OF FLORAL MORPHOLOGIES USING MICROCOMPUTED TOMOGRAPHY

In addition to the volatile profile, floral morphology represents another crucial trait designed to attract and fit particular types of pollinators. Generally, polyphilous flowers, which are associated with generalist pollinators, have been reported to be mostly dish-, bell-, and tube-blossom types, while specialized (monophilous) flowers are flag, gullet, and trap blossom in shape (Ramirez, 2003).

To precisely compute plant structures, 2D microscopic imaging is oftentimes incomplete, as many structures have 3D features that are difficult to infer from 2D images. In particular, structures and shapes of organs, along with morphological metrics like volumes and curvatures, can offer valuable insights into the development and growth of an organism. X-ray microcomputed tomography (μCT) is a valuable tool for 3D imaging of living organisms. In the interaction between flowering plants and insect pollinators, μCT can allow a relatively rapid acquisition of complex 3D structural data of flowers and, as such, can be used to extract organ volumes and shapes as well as model the accessibility of pollinators to nectar and pollen (Begot et al., 2022). Advantages include large penetration depth, minimal sample preparation, and its non-invasive character. On the other hand, major challenges include attaining higher contrast for easier quantification, increasing the resolution for imaging subcellular features, and decreasing image acquisition and processing time for high-throughput phenotyping (Piovesan et al., 2021). Still, the main challenge that prevents automating organ measurements is the lack of color difference between the neighboring structures due to the lack of an X-ray absorption difference (Begot et al., 2022). This challenge could be overcome by using tissue-specific staining methods, which could allow the integration of machine learning into the pipeline and thus significantly facilitate high-throughput μCT scanning. Finally, modeling of the 3D structures of floral organs can further extend the horizon by enabling comprehensive simulation models and testing hypotheses completely *in silico*.

INSECT-TRACKING SYSTEMS FOR PHENOTYPING PPIs

Evaluation of floral attractiveness to pollinators could be carried out by identifying and counting the visiting insects and their time spent on a flower using insect-counting devices, most of which operate by using a type of camera or a radar. For example,

a recent case study reported that both scheduled and motion-activated cameras were proven effective and relatively inexpensive in detecting plant–insect interactions in various plant species (Naqvi et al., 2022). Similarly, radar systems have been reported to provide cheap and reliable insect counters while requiring less processing power and being more resilient to weather interference (Williams et al., 2023). Fully automated counting systems have not yet been developed, with most systems requiring human input or modifications (Williams et al., 2023). Data acquired from a camera or a radar could be used for insect counting or species identification, as in the case of zenith-pointing linear-polarized small-angle conical-scan radars, which have been used to classify insect species based on weight, wing beat, and body length-to-width ratio (Hu et al., 2018). Another effective method for a high-throughput evaluation of PPIs includes DNA metabarcoding of insect pollen, which consists of pollen collection, DNA marker amplification, and sequencing (Pornon et al., 2016) and can be easily integrated with other phenotyping facilities. When compared to the classical observation of visits, DNA metabarcoding revealed 2.5 times more plant species involved in PPIs, thus enlarging the spatiotemporal observation window and adding a new level of complexity (Pornon et al., 2016). Altogether, insect-counting devices and DNA metabarcoding have great potential to facilitate insect monitoring, colony health status, and insect species identification. Automation of these systems would rely on real-time data acquisition in field conditions or a greenhouse, secure transfer and data storage on a cloud, and the use of machine learning methods for analysis of large data sets. Lastly, full automation would enable simultaneous, large-scale capturing of bee activity patterns, providing vital data for ecological research, bee conservation, and smart plant breeding (Figure 1).

In summary, key challenges and optimizations still remain before we can fully integrate volatilomics, 3D imaging, and pollinator–flower visitation monitoring systems in order to allow high-throughput setup and obtain a true systems approach in phenotyping PPIs. In this respect, noninvasive imaging techniques have been most advanced, while key challenges prevail in the optimization of volatilomics. Regardless, the fast-paced progress of these techniques, development of other omics and phenotyping techniques, and data integration will undoubtedly play a key role in both fundamental and agricultural research.

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