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Proteomic insights into floral biology☆

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ABSTRACT

The flower is the most important biological structure for ensuring angiosperms reproductive success. Not only does the flower contain critical reproductive organs, but the wide variation in morphology, color, and scent has evolved to entice specialized pollinators, and arguably mankind in many cases, to ensure the successful propagation of its species. Recent proteomic approaches have identified protein candidates related to these flower traits, which has shed light on a number of previously unknown mechanisms underlying these traits. This review article provides a comprehensive overview of the latest advances in proteomic research in floral biology according to the order of flower structure, from corolla to male and female reproductive organs. It summarizes mainstream proteomic methods for plant research and recent improvements on two dimensional gel electrophoresis and gel-free workflows for both peptide level and protein level analysis. The recent advances in sequencing technologies provide a new paradigm for the ever-increasing genome and transcriptome information on many organisms. It is now possible to integrate genomic and transcriptomic data with proteomic results for large-scale protein characterization, so that a global understanding of the complex molecular networks in flower biology can be readily achieved. This article is part of a Special Issue entitled: Plant Proteomics — a bridge between fundamental processes and crop production, edited by Dr. Hans-Peter Mock.

1. Introduction

Many flowers owe their unique appearance to their evolutionary success, which has in turn contributed to their valuable ornamental and economic importance to humans. The complex flower structure in angiosperms is essential for their reproduction. A standard flower arises from the receptacle, and consists of four whorls of tepals, which are arranged as sepals, petals, stamens (androecium), and pistils (gynoecium) from the outermost to innermost whorl. Sepals surround and protect the flower before it blooms. Petals are generally colorful and often attract pollinators. Stamens and pistils make up the male and female reproductive organs, respectively. The flower has been studied in many respects in order to understand how the information contained in genes manifests in the phenotypes. At present, the two main strategies for studying floral biology consist of a traditional genetic strategy and a parallel profiling strategy. Traditional genetic strategies mainly focus on a few genes at a time [1]. For example, the floral development studies of homeotic mutants of two model eudicot species: *Arabidopsis thaliana* and *Antirrhinum majus* [2,3] have established the quartet model "ABCDE model" of flower development. In this model, five classes of MADS-box genes interact with each to determine identity of these floral tepals [3,4]. Studies on class B MADS-box genes in monocots have expanded this model. For example, in orchid, duplicated class B-AP3/DEF-like genes with differential expression patterns associate with the unique labellum and gynostemium morphology [5–8]. In *Lacandonia schismatica*, central stamens are surrounded by a peripheral gynoecium, which is a homeotic inversion of their position [2,9,10]. In addition, flavonoids and carotenoids pathways for floral color have been characterized extensively. The function of core enzymes in flavonoids pathway, i.e. chalcone synthase (CHS), chalcone isomerase (CHI), flavanone 3-hydroxylase (F3H), dihydroflavonol 4-reductase (DFR) or anthocyanidin synthase (ANS) has been validated by transgenic technology in many species such as carnation, chrysanthemum, cyclamen, gentian, gerbera, lisianthus, petunia, rose, tobacco, torenia [11,12], as well as key enzymes of carotenoid pathways, such as phytoene synthase (PSY) and phytoene desaturase (PDS) [13].

In addition to the traditional genetic strategies, highly parallel profiling strategies have been developed for biologic research over the last decade. Such parallel profiling technologies, including transcriptomics, proteomics and metabolomics, can simultaneously probe many genes,

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transcripts, proteins or metabolites, which have helped elucidate the complex regulatory network involved in flower biology. Such examples were widely seen with Arabidopsis in which transcriptomic analysis revealed unique pollen genes [14,15], and genes playing novel/greater roles in Brassicaceae floral development [16], as additional examples were seen with rice (*Oryza sativa*) as well with genes responding to heat stress in floral organs [17], male development under drought stress [18] and genes related to panicle development [19]. In *Gerbera*, transcriptome and cDNA microarray have been utilized to identify marker genes associated with floral organ differentiation [20]. In *Cymbidium ensifolium*, the transcriptome has been profiled for all of the transcripts during floral development and has identified numerous genes associated with floral development and coloration [21,22]. Also, transcriptome sequencing has been used to characterize the patterns of gene expression during floral development in bamboo (*Dendrocalamus latiflorus*) [23], to identify floral odor related genes in *Phalaenopsis* [24], the genes associated with flowering time in *Oncidium* [25], the genes responsible for a specific floral organ "column" in Pigeon orchid (*Dendrobium crumenatum*) [26], and genes associated with anther and pollen in *Brassica napus* [27], chili peppers [28] and garlic (*Allium sativum* L.) [29]. Although gene expression at the transcriptional level gives important information on various biological processes, a large number of genes have also exhibited inconsistency between transcriptional and translational levels [30–32]. Such inconsistencies are often due to uncoupling of transcriptional and translational control and regulation in the biological system [33]. A single gene can produce a variety of protein species through various mechanisms including alternate splicing/processing of mRNA, or proteolysis/post-translational modifications (PTMs) of the protein. The proteins themselves serve as the final determining factors for most biochemical processes. Proteomics focuses on accumulative changes and modifications of proteins in biochemical processes at a given time and assists in understanding the mechanism underlying phenotypes [34]. Many proteomic studies have been performed on floral biology. Here, we discuss a variety of proteomic approaches and the recent advances in proteomic applications to floral studies, which have yielded new insights into the molecular mechanisms of floral traits at the protein level.

2. Proteomic strategy

2.1. Gel-based approaches

Two dimensional electrophoresis (2DE) is one of the mainstream methods for floral proteomics (Table 1), and has been widely applied to flower organs, such as the androecium [35–46], the gynoecium [47–52], and petals [32,53–56]. 2DE has the advantages of identifying isoforms and posttranslational modifications (PTMs) [57,58], which is often difficult in gel-free techniques where peptides from different protein isoforms or PTMs are mixed prior to liquid chromatogram (LC) (Would liquid chromatography be more appropriate to say here?) separation. Disadvantages of 2DE include low resolution, poor separation of "extreme" protein (proteins with high/low mass or pI). However, results have improved since immobilized pH gradients with a wide range (pH 2.5–12) and narrow overlapping isoelectric points (pI = 0.001) have been introduced into the first dimension [59]. Another significant improvement on reproducibility has come about through labeling techniques such as isotopic and fluorescent labeling.

Isotopic labeling is a sensitive detection method for proteins separation on 2DE gels which has been around prior to the advent of silver staining methods (Fig. 1). For example, 125I/131I multiphoton detection (MPD) technology can be used either for in vitro labeling of the sample or in vivo [60]. However, it is limited by the lengthy time needed for image capture and the need for costly instrumentation. Two other labeling approaches have been developed; one is a double labeling method based on in vivo metabolic labeling of samples with (14C) and (3H) leucine [61], and the other is dual channel imaging, combining in vivo metabolic (35S) methionine labeling with direct silver staining [62]. These two techniques can separate two samples on a single 2DE gel, and superimpose the images perfectly. Thus, it is possible to search for differentially expressed proteins efficiently. Currently, differential in-gel electrophoresis (DIGE) is one of the most popular fluorescent labeling methods (Fig. 1), which has been applied to explore the proteins involved in pollen germination [42].

2.2. Gel-free approaches

The power of proteomics has been greatly enhanced by the development of relative and absolute quantitative proteomic methods which require only small sample volumes while maintaining high throughput capabilities and consistent reproducibility. Most of the quantitative methods are gel-free and typically divided into four categories, i.e. chemical labeling, enzymatic labeling, metabolic labeling and label-free (Fig. 1). These methods increase sensitivity significantly, compared to gel-based methods. In chemical labeling, proteins or peptides are tagged through chemical reactions. Two chemical labeling tags, i.e. isobaric tags for relative and absolute quantification (iTRAQ) and tandem mass tags (TMTs), are used at the peptide level, where labeling takes place after the digestion of protein with specific endoproteinases. iTRAQ reagents label N-termini and ε-amino groups of lysine side chains, and allow the comparison of up to ten samples at once in MS/MS-based relative quantitation. The technology has been used to characterize the proteomes of pollen grain and pollen tube in *Lilium davidii* [44], and the pollen proteome in tomato [46]. The cysteine active cysTMT reagents enable selective labeling and relative quantitation of cysteine containing peptides from up to six samples. Recently, a novel cysteine-reactive, iodoacetyl TMT (iodoTMT) reagent is increasingly being used. The iodoTMT reagent labels peptides via an irreversible thioester bond, which results in a more stable tagging modification under reducing environments than cysTMT [63].

In enzymatic labeling isotope-coded affinity tag (ICAT) labeling takes place at the protein level where labeling and pooling occur prior to protease treatment. The proteins containing cysteine residues are labeled either with light or heavy isotopes, and then, are pooled and proteolytically cleaved. Although ICAT-labeling reduces sample complexity, the cysteine specific labeling also reduces protein sequence coverage in the same scale [64]. To overcome this limitation, isotope-coded protein labeling (ICPL) has been proposed, where lysine residues and N-termini are isotopically labeled with a deuterium containing (heavy) or deuterium free (light) tag. This approach increases the level for N-termini labeling. Moreover, ICPL labeling can occur at both the protein level (before digestion) [65] and the peptide level (after digestion) [66,67], and allows the comparison of up to three samples by using two heavy tags and one light tag. The main drawback of this method is the isotopic effect of deuterated tags that interfere with the retention time of labeled peptides during LC. Dimethyl labeling is a simple chemical labeling method based on the dimethylation of lysine residues by stable isotope labeled formaldehyde and cyanoborohydride. This approach allows duplex and triplex relative comparisons, and achieves almost 100% labeling efficiency at a relatively low cost [68,69].

Metabolic labeling allows protein labeling at the time of protein synthesis during cell culture (labeling at the protein level), and eliminates a technical bias derived from labeling after protein extraction. In stable isotopic labeling with amino acids in cell culture (SILAC), cells are cultured in medium containing $^{13}C_6$-lysine and/or $^{13}C_6$-arginine. However, SILAC appears not to be very suitable for flower quantitative proteomic studies as plants are capable of synthesizing all amino acids from inorganic nitrogen [70]. 14N/15N labeling is introduced to samples through the growth medium as well. Samples can easily be labeled metabolically via 15N-labeled inorganic salts, such as $K^{15}NO_3$ [71], which result in more than 98% incorporation, which is much higher