PlaMoM: a comprehensive database compiles plant mobile macromolecules

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ABSTRACT

In plants, various phloem-mobile macromolecules including noncoding RNAs, mRNAs and proteins are suggested to act as important long-distance signals in regulating crucial physiological and morphological transition processes such as flowering, plant growth and stress responses. Given recent advances in high-throughput sequencing technologies, numerous mobile macromolecules have been identified in diverse plant species from different plant families. However, most of the identified mobile macromolecules are not annotated in current versions of species-specific databases and are only available as non-searchable datasheets. To facilitate study of the mobile signaling macromolecules, we compiled the PlaMoM (Plant Mobile Macromolecules) database, a resource that provides convenient and interactive search tools allowing users to retrieve, to analyze and also to predict mobile RNAs/proteins. Each entry in the PlaMoM contains detailed information such as nucleotide/amino acid sequences, ortholog partners, related experiments, gene functions and literature. For the model plant *Arabidopsis thaliana*, protein–protein interactions of mobile transcripts are presented as interactive molecular networks. Furthermore, PlaMoM provides a built-in tool to identify potential RNA mobility signals such as tRNA-like structures. The current version of PlaMoM compiles a total of 17 991 mobile macromolecules from 14 plant species/ecotypes from published data and literature.

PlaMoM is available at http://www.systembioinfo.org/plamom/.

INTRODUCTION

Intercellular transport of macromolecules such as proteins and RNAs is thought to occur via a continuous cytoplasmic (symplasmic) network established by plasmodesmata (PD) between neighboring cells (1). Intercellular transport of macromolecules via PD depends on developmental stage of the tissue, the nature of transported macromolecules and the opening of the PD pores (1,2,3). For a number of intercellular or long-distant transported macromolecules their functions in the target tissues or organs were proposed in plant development and defense responses (4). While local (short-range) transport of mobile macromolecules can origin from single cells and move across 10–15 cells (Figure 1A) (5), long-distant transport occurs either from surrounding tissues via companion cells or companion cells connected to sieve elements (phloem) deliver the mobile macromolecules from source tissues to distant sink tissues (Figure 1A) (6).

Molecular, physiological, developmental and defense related functions in distant tissues were shown for mobile siRNA moving from shoot to root where they trigger asymmetrical cytosine (CHH, H any nucleotide except G) DNA methylation of root tissue genome via the RNA-dependent DNA methylation (RdDM) pathway (7). In a similar way, the phosphate-starvation-induced miR399 is allocated to roots triggering Pi uptake by downregulated *PHO2* expression, and, thus, allows the plants to adapt to phosphate shortage (8). Another example is the florigenic FT protein produced in leaves. FT is transported from sources leaves via the phloem to the shoot apex initiating floral development (9). Other homeotic factors moving
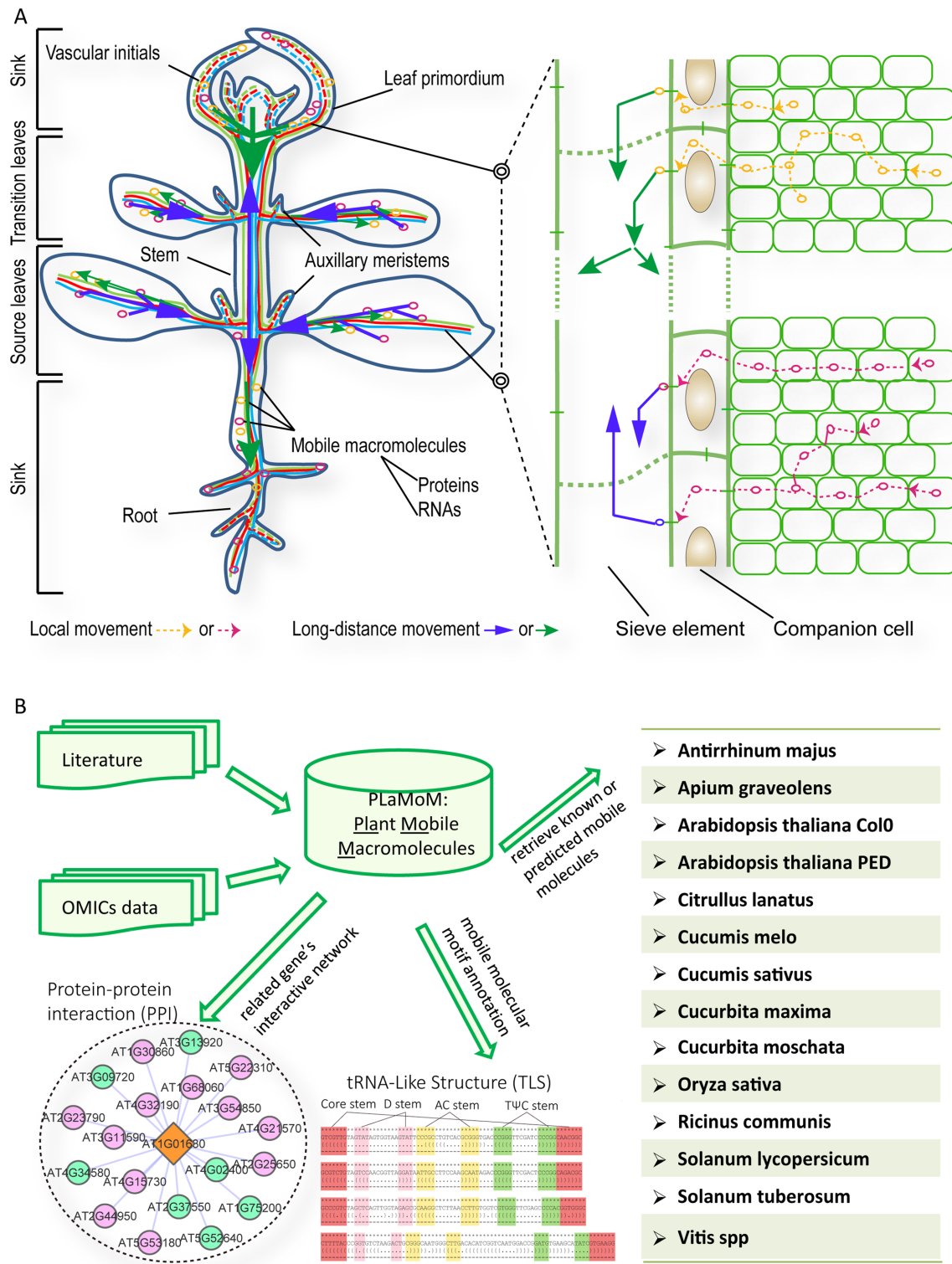
Figure 1. Schematic drawing of intercellular short-range and phloem-mediated long-distance movement of non-cell autonomous molecules and architecture of PlaMoM. (**A**) Long-distance transported mobile macromolecules (shown as green or blue arrows) can move from source (mature leaves) to sink (shoot apex or root apex and young non-functional leaves), and some even can move from sink to source. Locally transported macromolecules can move over 10–15 cells as illustrated by pink dash arrows. (**B**) Architecture of PlaMoM. 'Literature' refers to the origin of the mobile data, 'OMICs data' refers to proteomics, transcriptomics and microarray datasets. 'PlaMoM' is both, a collection of mobile macromolecules and a displaying platform on which users can retrieve known or predicted mobile molecules, scan mRNA sequences for 'tRNA-like structure (TLS)' motifs and inspect 'protein–protein interaction (PPI)' of mobile macromolecules.

over long distances are the transcription factor encoding *StBEL5* mRNA involved in potato stolon formation, and the light controlled *Arabidopsis thaliana HY5* mRNA regulating root formation (10,11).

Novel experimental and high-throughput sequencing technologies allowed for identifying numerous mobile macromolecules in diverse plant species. The identification of mobile RNAs and proteins is based on different methods, e.g. bleeding (12), grafting (13) and feeding (14), or on high-throughput Omics technologies (next generation deep sequencing, sanger-sequencing and microarrays). Consequently, biological functions of reported macromolecules are associated with their types, sampled plant species, different tissues, conditions as well as environmental factors. Although the signaling function in targeted cells or tissues of some mobile macromolecules has been elucidated, the majority of those identified by deep sequencing techniques and bioinformatic pipelines remain to be characterized. For the process of gene function characterization it is of great importance to know the properties of the investigated gene products such as cell-autonomy versus potential non-cell autonomy, presence of a mobile tRNA-like structure (TLS) motif and in which cellular pathway the transcripts or their products may be involved in. Therefore, to facilitate the study of the mobile macromolecules, it is necessary to collect and organize their enriched information from literature and published data sources.

Here, we present PlaMoM (Plant Mobile Macromolecules), a source for providing a convenient and interactive interface for plant biologists to retrieve mobile RNAs and proteins, to explore their associations with biological functions and to evaluate novel concepts and potential signaling functions of these mobile molecules. The PlaMoM database incorporates multiple sources of data mainly obtained by manual curation of literature and published high-throughput experimental reports addressing diverse plant species. PlaMoM provides a built-in tool that is able to identify potential RNA mobility signals such as TLS. Furthermore, the mobile transcripts can be linked to the protein–protein interaction (PPI) database of *A. thaliana,* which is constructed as an interactive molecular network module presenting an overview of mobile macromolecule–protein interactions. These interaction networks provide a basis for elucidating potential functions of these mobile molecules in plant signal transduction and developmental biology.

MATERIALS AND METHODS

Data sources and integration

The PlaMoM database contains three distinct categories of mobile macromolecules, namely mobile mRNAs, mobile non-coding RNAs (ncRNAs) and mobile proteins. The presented data was compiled from public data repositories, published references and in-house data. Currently, PlaMoM includes 672 identified mobile ncRNAs (∼3.7%), 16 928 mobile mRNAs (∼94.1%) and 389 mobile proteins (∼2.2%). The mobile macromolecules currently cover 14 different species and ecotypes, namely *A. thaliana Col-0*, *A. thaliana PED* (13), *Apium graveolens* (celery) (15), *Antirrhinum majus* (snapdragon) (16), *Ricinus communis* (cas-

tor bean) (17), *Cucumis melo* (melon) (18), *Citrullus lanatus* (watermelon) (19), *Cucumis sativus* (cucumber) (19), *Cucurbita maxima* (pumpkin) (20), *Cucurbita moschata* (winter squash) (21), *Vitis spp* (grapevine species) (22), *Oryza sativa* (rice) (23), *Solanum lycopersicum* (tomato) (24) and *Solanum tuberosum* (potato) (11). Identification of the mobile macromolecules was based on different experimental methods, such as analysis of the phloem exudate/sap (15,17,18,25,26), the presence of distinguishable transcripts found in parasitic plants and their host plants (13,14) or by single nucleotide polymorphisms (SNPs) in mRNAs from heterografted plants or from grafted mutant/wild-type plants (13,22,27,28). The mobile macromolecule data was derived from ESTs, RNA sequencing (RNA-seq), microarrays and proteomics. Lacking detailed annotations, EST sequences of mobile RNAs found in non-model plants were regarded as mRNAs. A summary of all presented data and the architecture of the platform is shown in Figure 1B.

Gene sequences and function information was retrieved from the respective databases. EST sequences of *A. graveolens*, *R. communis* and *C. melo* were obtained from NCBI. Sequences of *A. thaliana Col*-0 were extracted from TAIR10 (29). *A. thaliana PED* were retrieved from the 1001 genome project (30). Sequences of *Vitis spp.* were obtained from the EnsemblPlants (31), sequences of *C. lanatus* and *C. sativus* were extracted from Cucurbit Genomics Database (32). Sequences of other species were collected from their published reports. PPIs were collected from TAIR, BioGRID (33), AIM (34) and AtPIN (35). Additional information such as 'Source or tissue', 'Experiment approach', 'Data types', 'Summary', were manually curated from published literatures.

Mobile tRNA-like structure motif prediction

The molecular mechanisms enabling intercellular mRNA transport and the fate of transported mRNAs in target tissues remain poorly understood. A number of reports indicate that many positive-strand RNA viruses harbor conserved stem-loop structures in the 3′ untranslated region resembling those of canonical tRNAs. Such a viral TLS seems to play a crucial role in viral replication and infectivity (36,37). Viral TLS-mediated intercellular or long-distance transport of viral RNAs has been reported, suggesting that TLS might be a *bona fide* RNA mobility motif for endogenous transcripts (12). Some TLS motifs were identified in mobile mRNAs and described recently as mediating heterologous and endogenous mRNA transport over graft junctions in *A. thaliana* and *Nicotiana tabacum* (38). However, for most mRNA found in various species information on TLS motifs in mRNAs is not available. To facilitate such a TLS motif search for any mRNA, we developed a built-in tool capable of scanning mRNA sequences for TLS folding structures. This software tool integrates the RNA motif search algorithm and the default tRNA descriptor (39) and facilitates users to detect TLS motifs in the any given mRNA. Once submitting fasta-formatted sequences or uploading a fasta-formatted sequence file, the software will optionally add 50 'N' leading and trailing characters to the input sequences to enable the identification of TLS motifs at

5′ or 3′-untranslational regions besides scanning the middle of mRNA sequences.

Implementation

PlaMoM uses web-based HTML interactive interfaces combined with PHP, javascript and Pascal. All data is stored in a MySQL environment. The protein interaction network display is presented by using Cytoscape web (40). PlaMoM does not require excessive computing on client side.

RESULTS

Browse, search and download

PlaMoM provides four tabular-organized browse panels to retrieve mobile macromolecules, either presenting all mobile macromolecules, mobile mRNAs, mobile ncRNAs and mobile proteins. In addition to the 'original ID' obtained from published literatures and databases, each mobile macromolecule has a 'PlaMoMid' identifier for each of the browsing categories. These IDs are convenient for accessing mobile macromolecules independent from potential changes to external databases. The 'PlaMoMid' is an entry-based identifier corresponding to a specific experimental approach. Thus, the 'PlaMoMid' differs from the gene identifier provided under 'original ID'. For instance, the 'original ID' AT4G10340 refers to 4 PlaMoMids as it was described by 4 different experimental approaches. This allows us to add more data in the future without interfering with the structural integrity of our database and to keep all data in a reproducible state. Following the original ID in the tabular browser, we designed an '[source]' link to guide users to existing resources. Each mobile macromolecule entry lists origin (plant species/ecotypes), category (ncRNAs, mRNAs and proteins), functions, experimental validation methods (e.g. phloem exudate, grafting and feeding), data types (EST, RNA-seq, microarrays and proteomics) and the PMID (Pubmed ID with hyperlink). The PlaMoMid and original ID are connected to a detailed annotation page, where sequence information, PPI context, gene orthologs, GO annotation and relevant references are shown. The detailed annotation page also exhibits experimental approaches used for the mobile macromolecular identification, the PlaMoMid, observed movement direction, functional description and functional annotations (Figure 2A and B).

PlaMoM provides a 'search' interface to query detailed information on each mobile macromolecule and its associated functions (Figure 2C). Users can search by submitting a PlaMoMid or original ID (e.g. PLAMOM10016, AT1G01640, SYD0673, GSVIVG01003996001, etc.), or by a query term from selectable drop-down lists of species/ecotypes (e.g. *A. thaliana*, *C. lanatus*, etc.), category of mobile macromolecule (e.g. protein, mRNA or ncRNA), experimental approaches (e.g. phloem exudate, grafting or feeding) and data types (e.g. EST, RNA-seq, microarrays or proteomics). Furthermore, users can search mobile macromolecules according to their pre-set sequence length. GO ids and GO terms (keyword search) also can directly be used to search for mobile macromolecules from model plants.

The fuzzy search engine offers users to obtain a complete set of biological context information for the mobile macromolecule record without knowing the exact name documented in the database. In the PlaMoMid or original ID input box, PlaMoM will display an auto-completed list of selectable entries matching to the user's search term, for example showing all entries starting with the characters 'AT' on input. The search engine also allows for combined queries. For instance, to find how many mobile ncRNAs were identified by RNA-seq analysis in *C. sativus,* the user should select the '*C. sativus*' in the species/ecotype drop-down box, select 'ncRNAs' in the 'mobile macromolecules type' drop-down box and select the 'RNA-seq' in the 'data type' drop-down box. The final result page would display 672 ncRNAs. Furthermore, all the mobile macromolecules of PlaMoM can be downloaded using the download page of the associated species.

TLS motif scanning

Our built-in TLS motif scanning web server scans the user-provided mRNA sequences to detect potential mobility motifs. In the TLSfinder panel users can enter or upload their fasta-formatted sequences. The parameter selection box allows to adjust the 'minimum free energy cutoff'; the default value is −10. In addition, users also can set 'Add N in both terminals' whether they want to enable scanning the 5′ or 3′ ends. Optionally, users also can type in their email address to obtain alerts on their search results once the computation has finished. A periodically updated refresh page will show while calculating the TLS motifs. After around 10 s, the web interface will exhibit the secondary structures of assayed TLS if the input mRNA sequences harbor such a clover-like TLS structure. To get started with this feature, users might use the provided example data by clicking 'load example', then clicking 'submit' button. The final results will be displayed as shown in Figure 3.

Construction of mobile molecular interaction networks for flowering time

In plants, day length alteration is accompanied with seasonal changes and is perceived in leaves, which initiate long-distance signaling that induces flowering at the shoot apex (9). A set of flowering time proteins such as FT, CO, SOC1, LFY, SVP and GI, are in the core facility of flowering time pathway (41). FT protein is well documented for its mobility from leaves to shoot apex, where it facilitates flowering (42). Other flowering-related transcripts have recently been found as mobile molecules (13). In PlaMoM, we detected 16 flowering-related mRNAs of *A. thaliana* that are mobile and graft transmissible. As most of the mobile flowering mRNAs are identified by deep sequencing with grafting or feeding experiments, it is important to further investigate the roles of these mobile mRNAs in flowering time regulation. The PlaMoM database established a built-in PPI dataset of *A. thaliana* that allows linking these mobile mRNAs to protein interaction networks. For FT, the protein as well as the transcript were found in phloem sap, although evidences suggest that the mobile FT protein is one of the factors to initiate flowering (43). When searching or brows-
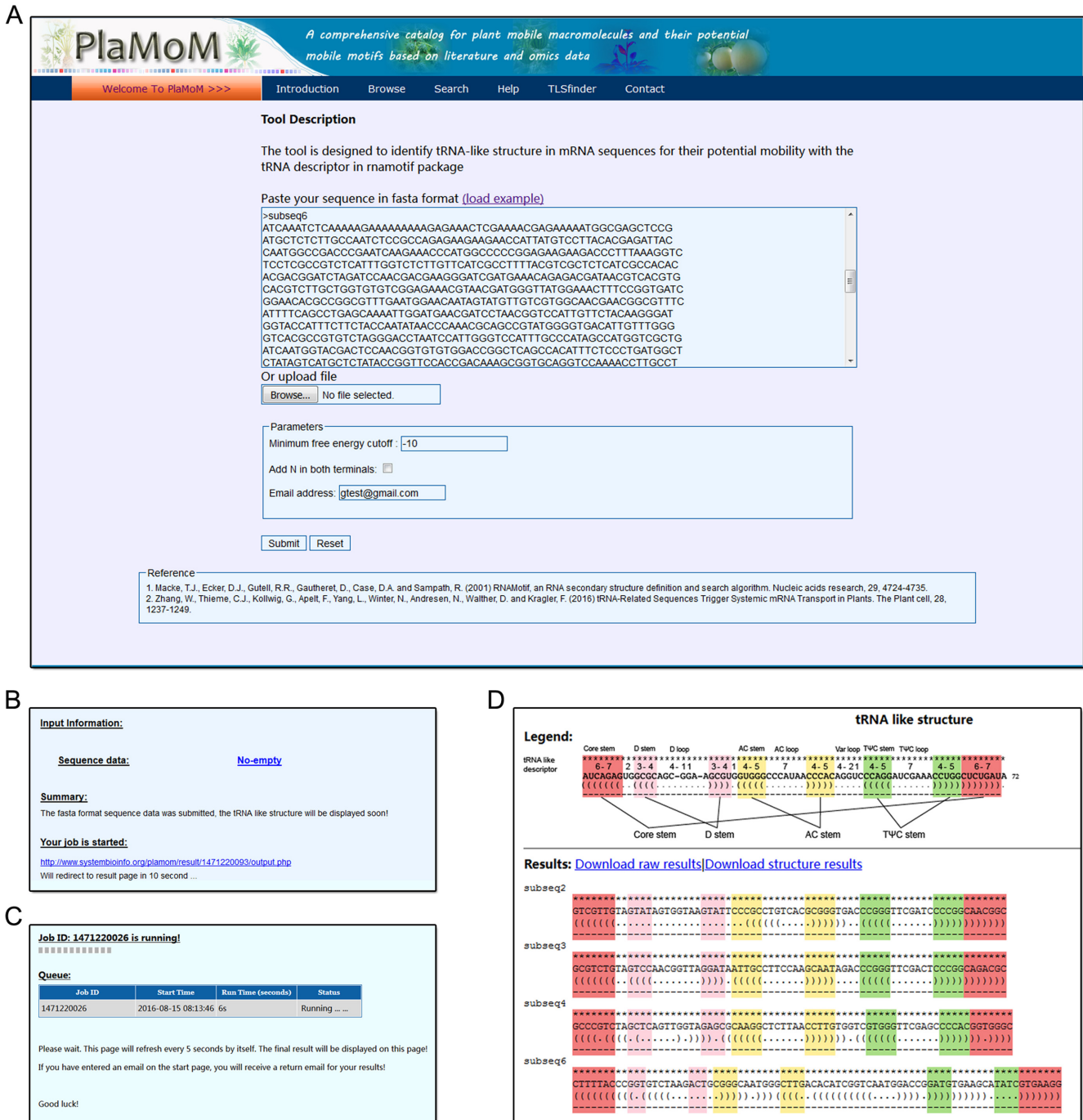
Figure 2. The browsing interface, detailed annotation page and search panel of mobile macromolecules in PlaMoM. (**A**) Screenshot of the browsing interface of PlaMoM listing all or subsets of mobile macromolecules. (**B**). Detailed annotation of browsed/searched mobile macromolecules, including sequence, interaction networks, orthologs, GO annotation, TLS motifs, experimental approaches, functional annotation, summary and references. (**C**) Search interface of PlaMoM.

Figure 3. An example and guide for TLSfinder usage. (**A**) The input form for the TLSfinder. (**B**) The interface for checking data submission status. (**C**) The interface when waiting for the calculation results of TLS scanning. (**D**) An interface when showing an identified TLS motif. The colors in red, pink, yellow and green refer to structure elements of the RNA, namely the 'Core stem', 'D stem', 'AC stem' and 'TΨC stem', respectively.

ing, users not only can retrieve FT from the mobile macromolecular database, but also can find flowering time-related transcripts such as GI, SVP and GRF3 as mobile molecules in the FT interaction network (Figure 4). These proteins are key components in FT-mediated flowering pathways, indicating that our database can reproduce biological meaningful mobile molecular networks and, thus, provides new insights by guiding further studies on signaling functions of mobile macromolecules.

CONCLUSIONS AND PERSPECTIVES

PlaMoM is the first comprehensive web-based resource allowing searching and retrieving known mobile mRNAs as well as to detect mobility-related TLS motifs. For model
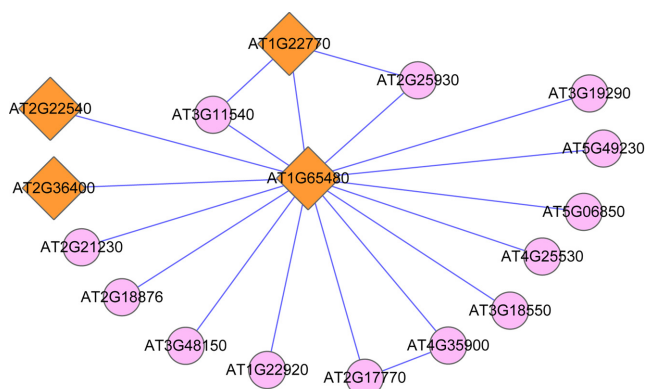
Figure 4. A mobile molecular interactive network of FT protein. Diamonds represent proteins whose transcripts are mobile, and Circles autonomous proteins respectively.

plants, the database not only enables to easily retrieve information about mobile RNAs, but also presents the related interactive molecular network and known mobile orthologs of these genes. PlaMoM offers interpretable, reusable and multi-layered information to the user and aims for full coverage of published mobile RNAs and proteins. The improvements of sequencing technologies lead to an increasing number of mobile macromolecules identified in distinct plant species/ecotypes which will be continuously integrated into this database in the future. Upon identification of new mobile macromolecules and improvement of algorithms for detecting mobile motifs, we will update the database frequently. We are convinced that the compiled data in combination with the presented annotations will be a useful source for further research on mobile plant macromolecules. Our PlaMoM platform will assist in identifying novel mobile macromolecules, potential mobility motifs and may provide a basis for characterizing their potential function(s) in distant tissues and cells.

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