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Review

Medicago truncatula resources to study legume biology and symbiotic nitrogen fixation

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Abstract *Medicago truncatula* is a chosen model for legumes towards deciphering fundamental legume biology, especially symbiotic nitrogen fixation. Current genomic resources for *M. truncatula* include a completed whole genome sequence information for R108 and Jemalong A17 accessions along with the sparse draft genome sequences for other 226 *M. truncatula* accessions. These genomic resources are complemented by the availability of mutant resources such as retrotransposon (*Tnt1*) insertion mutants in R108 and fast neutron bombardment (FNB) mutants in A17. In addition, several *M. truncatula* databases such as small secreted peptides (SSPs) database, transporter protein database, gene expression atlas, proteomic atlas, and metabolite atlas are available to the research community. This review describes these resources and provide information regarding how to access these resources.

Introduction

Symbiotic nitrogen fixation is an active and dynamic biological process mediated by legumes in a mutualistic process with soil bacteria, collectively rhizobia [1-4]. The legume family members that are commonly used as diet for human consumption purpose include crops such as soybean (*Glycine max*), peanut (*Arachis hypogea*), lentil (*Lens culinaris*), alfalfa (*Medicago sativa*),

chickpea (*Cicer arietinum*), and pea (*Pisum sativum*). These legume crops also form the crux in a variety of agricultural cropping systems owing to their ability to fix and restore nitrogen to soils in a regenerative way. Apart from the dietary benefits, they are also a good source of proteins, specialized metabolites and oils, which enhances their desirability. *M. truncatula*, a legume with a genome size of ~400 Mb, was chosen as a model to study fundamental biology of legumes in general and molecular mechanisms of symbiotic nitrogen fixation (SNF) in particular. Furthermore, the biparental inheritance of *M. truncatula* in comparison to the commonly occurring maternal inheritance in land flowering plants (angiosperms) also adds to the list of reasons to use as a model plant for flowering plants [5].

M. truncatula and *Lotus japonicus*, the two most popular model legumes share a genome synteny with other major legumes such as soybean, peanut, pigeon pea (*Cajanus cajan*), chickpea and others [6-12]. *M. truncatula* is a relative of the tetraploid forage crop, alfalfa, and can also be studied for drawing parallels to the auto polyploidy events [13]. Comparison of two close relatives *M. truncatula* and alfalfa reveals chromosomal re-arrangements and expansion of the gene families in alfalfa [13]. The relatively small size of the *M. truncatula* genome (~450 MB) makes it a guinea pig of legume genetics. The efficiency and ease of transformation combined with its short life cycle makes it ideal for legume crop genetic studies [6]. Two related, yet distinct ecotypes, Jemalong A17 and R108 representing two *M. truncatula* subspecies [14] are used as models to explore the *M. truncatula* genome. The distinctive nature of R108 from A17 was discovered through the SNP discovery process from a genome wide association study of diverse *M. truncatula* accessions [15]. The A17 accession is highly recalcitrant to transformation in comparison to R108, hence R108 became a pivot for legume genetics studies [16]. The first copy of the *M. truncatula* reference genome was published in the A17 accession due to the complexity of the R108 genome [17]. The ramp up in the next generation sequencing technologies expanded the genomic landscapes of currently studied models [7, 9-12, 18-20]. The advances in the availability of the genome information and the structural and molecular studies helped in the identification of key nodulation genes in legumes that can be attributed with a variety of molecular functions [21-26].

Tobacco *Tnt1,* a well characterized transposable element in the class of autonomous long terminal repeat (LTR) retrotransposons [27, 28], played a pivotal role in the insertional

mutagenesis of *M. truncatula* R108 [29]. Somatic embryogenesis via tissue culture was employed to generate 21,741 *Tnt1* insertion lines [1, 30]. Apart from *Tnt1* insertional mutagenesis, other mutagenic approaches like fast neutron bombardment (FNB) induced mutagenesis, RNAi knockdown, and CRISPR/Cas9-based mutagenesis platforms are also effective in *M. truncatula* [31, 32]. In this review, we describe the *M. truncatula* resources available for the research community.

Availability of genome sequences and differences among *M. truncatula* accessions

As previously described, the *M. truncatula* whole genome blueprint information is available for the popular and widely used two accessions, R108 and Jemalong A17. The first sequencing efforts were initially aimed at the *M. truncatula* A17 genome and it was sequenced using the conventional BAC end sequencing methodologies followed by the Illumina shotgun sequencing (Young et al. 2011). The supporting evidence and gap filling of the A17 draft genome in the earliest versions of the genome include data from expressed sequence tags (ESTs) (Bell et al. 2001) and gene expression datasets [33, 34]. The first reference genome sequence of the *M. truncatula* A17 accession was completed in 2011 and was used primarily in conjunction with R108 for gene functional studies [20]. The first version of the reference assembly for A17 genome was significantly improved with updates in 2014 (Mt4.0; [35] and more recently in 2018 (Mt5.0; [36]. The most recent *M. truncatula* assembly A17 v5.0, which is housed at https://medicago.toulouse.inra.fr/MtrunA17r5.0-ANR, is a more complete genome with 44,623 protein-coding genes, ~25K transposable elements and ~4K non-coding RNAs [36]. There were other studies that took aim at comparison of other *M. truncatula* accessions with an objective of identifying the single nucleotide polymorphism (SNP) variants [23, 37-40]. More than six million SNPs from across 226 accessions were identified [40]. With these SNPs, genome wide association mapping was performed to identify key genes controlling the phenotypic traits such as plant quality (height, trichome density, flowering time) and root associated nodulation [23, 37, 38, 40]. Li and his colleagues [41] performed a comparative structural variant analysis between the A17 genome and the R108 genome and identified large structural variants in the nodulation enhancing cysteine rich gene families and that accounted for differences in their phenotypic strengths to aluminum toxicity [41].

The first draft genome of R108 accession (R108 v1.0) was generated by Moll and colleagues through a combination of sequencing methodologies using long read sequencing (PacBio), long molecule sequencing (BioNano genomics) and through the use of high-quality genome assembly (Dovetail genomics) [17]. The first draft of the R108 assembly (~400 Mbp) has eight chromosomes and ~900 scaffolds with an average N50 of 5.93 Mb [17]. The annotation of R108 v1.0 resulted in the identification of 55,706 protein-coding genes relative to the 50,894 genes in the A17 draft of Mt4.0 or 48,704 genes in Mt5.0 showing the complex nature of the R108 genome.

Recently, Kaur and colleagues reported an updated complete chromosome length assembly of *M. truncatula* R108, generated by Hi-C data (MedtrR108_hic). The MedtrR108_hic genome assembly comprises of eight chromosomes with N50 metric of 51.86 Mb [42]. A complete re-annotation of MedtrR108_hiC genome assembly resulted in the identification of ~39K protein-coding genes, which are lower by 28% (55,706) in the R108 v1.0 (GenBank accession no. GCA_002024945.1) and 12 % lower (44,623) in A17 Mt5.0 assembly (GenBank accession no. GCA_003473485.2) [17, 36, 42]. The superiority of the genome assembly was identified through the gene space completeness assessment via Benchmarking Universal Single-Copy Orthologs (BUSCO) analysis. From the BUSCO analysis, higher percentage of complete BUSCOs (96.73%) were reported in MedtrR108_hic assembly than from the R108 v1.0 assembly [42]. All the MedtrR108_hiC data are maintained at the web portal: https://medicago.toulouse.inrae.fr/MtrunR108_HiC . The web portal also houses genome browser and sequence analysis tools like BLAST analysis, sequence extraction and extract features. A comparative analysis of the new MedtrR108_hic assembly with the A17 Mt5.0 assembly shows a complete collinearity and synteny of the gene information for the 39,027 predicted genes in the new MedtrR108_hic, 36,994 (94.79%) [42]. Recently, a more similar approach was used using Hi-C data and the genome assembly of R108 was improved [41]. The differences in the genome wide rearrangements between A17 and R108 was found to be correlated with the phenotypic display of resistance to certain metal toxicity, anthocyanin pigmentation, and mineral and nutrient deficiencies [41]. Interestingly, sequencing of a diploid progenitor of the autotetraploid alfalfa *M. sativa* spp. *caerulea* shows a larger genome (~800 Mb) with approximately ~47,000 protein-coding genes [13]. In comparison with the *M. truncatula*

genome, the genome duplication in alfalfa resulted from the expansion of repeat elements and expansion of gene families related to the pollen recognition and pollination [13].

Comparison between the *M. truncatula* genomes R108, A17, and others led to the confirmation and identification of a translocation event between chromosomes 4 and 8 [35]. The translocation event between the two accessions A17 and R108 can cause inaccurate and inappropriate synteny between *M. truncatula* and other legume genomes resulting in distorted analysis. This aberrant translocation is captured and previously observed issues such as aberrant recombinations involving genetic crosses between A17 and R108 and other accessions can now be resolved [36, 43]. With the availability of the new MedtrR108_hic assembly, genome synteny and collinearity were identified between A17 Mt5.0 and R108, with a syntenic region of ~12 Mb from 46.9 Mb region to 50 Mb region on A17 chromosome 4 and 32 Mb region to 50 Mb region on R108 chromosome 8 [41, 42]. Additionally, a syntenic region of ~17 Mb was identified from 37 Mb to 49 Mb region on A17 chromosome 8 and 41 Mb region to –35 Mb on R108 chromosome 4 [42]. Several syntenic blocks (59 blocks) of genes (25,548) were identified between A17 and R018 with each block containing several hundred genes [42]. One of the collinear blocks identified (block 54) encompasses ~2,500 genes [42]. In an identical study, Li and colleagues identified syntenic blocks that accounted for more than 84% between both the accessions [41]. Interestingly, out of the total genes identified in the syntenic regions (25,548), ~ 10% (2,676) were identified to be localized in the translocated regions: 1,143 in the ~12 Mb syntenic block and 1,533 in the ~17 Mb syntenic block [42].

The Medicago HapMap project was developed as an international consortium group project with contributions from the National Center for Genome Resources (NCGR), University of Minnesota, Boyce Thompson Institute (BTI), J. Craig Venter Institute (JCVI), Hamline University, the University of Southern California, INRA-Montpellier, ENSAT-Toulouse, and the Noble Research Institute. As a part of this project, 384 lines spanning a variety of accessions from *M. truncatula* were sequenced to identify variant classes such as SNPs, insertions/deletions (INDELs) and copy number variations (CNVs) from the *M. truncatula* accessions. This community resource is made available at https://medicagohapmap2.org. All these resources are combined to predict the genome wide association between the genotypes and phenotypes in *M. truncatula* and other accessions using recently built pipeline, GWASpro [44];

https://www.zhaolab.org/GWASPRO). Structural variants unlike SNPs and INDELs are larger variants that can range from ~50 bp to several KB regions, which often result in expansion or contraction of genomes that might alter the phenotypes [41, 45]. In one of the primary studies on structural variants, Zhou and colleagues compared 15 *M. truncatula* accessions, including R108 and A17 and identified approximately ~5 million SNPs with A17 as the reference genome [46]. SNP variants (~1.5 million) were also identified with other accessions in comparison to the A17 reference [46]. Other variant classes identified from the study using A17 as reference reflected on the presence of short INDELs (~1.5 million), large INDELs (~0.1 million), CNVs (~0.15 million) and ~12K translocations [46]. Apart from their genetic diversity, A17 and R108 accessions exhibit certain phenotypic plasticity to abiotic stresses, such as drought and salt stress [47] [48], aluminum toxicity and iron deficiency [47]. Similar observations were made in a recent study involving the novel upgraded assemblies of A17 and R108 [41]. Apart from their differences in their genetic makeup and their ability to respond to various stresses, some specificity exists in their interaction with different nitrogen fixing rhizobial strains [39]. In a study conducted to test different rhizobial strains of *Sinorhizobium meliloti* against *M. truncatula* accessions R108 and A17 [49], the strain FSM-MA was found to be effective on both accessions while the strain 102F34 was only effective on R108 accession [39]. The specificity of strains to nodulate a particular accession was thought to be controlled by genes *NFS1* and *NFS2*, previously identified as *Mtsym6* [50, 51]. With many similarities and differences in the popular accessions A17 and R108, R108 was a model of choice for genetic transformations and for conducting functional genomics as it scores higher in the test for transformation efficiency [16]. With many advantages of R108 accession over A17 and the availability of genomic information either through the syntenic and collinear relationship with A17 or through the more recently developed genome of R108 accession, R108 has become a crux of legume genetics.

Gene expression atlas and other genomic information available for *M. truncatula* and other legumes

From the time *M. truncatula* was chosen as a model plant, the studies were pivoted to focus on the wide range of biological aspects relevant to basic science and legume crop improvement. Several studies were focused on the *M. truncatula* gene expression between treatments or

between different genotypes. These studies were organized into a pivotal gene expression atlas for *M. truncatula* (https://mines.legumeinfo.org/medicmine/report.do?id=44783875) and heatmaps for group of genes can be visualized at https://mines.legumeinfo.org/medicmine/bag.do. The array data from MtGEA and RNAseq data are also housed at (https://medicago.toulouse.inrae.fr/MtExpress). Another database, LegumeIP v3, (https://www.zhaolab.org/LegumeIP/gdp/) is an integrative gene discovery platform designed to enhance the translational genomics in legumes that guides the discovery of novel key genes in legume plants [52]. LegumeIP hosts information on all legume genomes including *M. truncatula* as well as their transcriptomic data [52]. The integrated comparative genomic analysis pipeline will be a valuable tool for the identification of important genes involved in legume- specific biological pathways and in identification of traits in *M. truncatula* based on the GeneChip technology developed by Affymetrix [33]. The site also integrates the data from the Affymetrix chips for *M. truncatula* as an additional tool with other visualization tools integrated from the Medicago ePLant (http://bar.utoronto.ca/eplant_medicago/; [53]). Recently, *M. truncatula* RNAseq data collected from peer reviewed publications was presented as gene expression atlas at MtExpress (https://medicago.toulouse.inrae.fr/MtExpress; [54]). Users can quickly use this resource to obtain the expression profiles of genes of interest in a variety of organs, tissues and treatment conditions [55]. Another website dedicated to visualize the gene expression datasets is DiVenn (https://divenn.tch.harvard.edu/), an interactive and integrated web-based visualization tool for comparing lists of genes from any model organism and can be customized ([56]. The gene expression datasets of *M. truncatula* can be used with DiVenn for visualization [56]. Legume information system (LIS) is an integrated suite of tools for genomic data on members of the legume family. LIS provides data access to a variety of genetic and genomic information for all major crops and model legumes (https://legumeinfo.org/home; [57]. LIS currently maintains genomic data for ~15 legume species: alfalfa (*Medicago sativa*), chickpea (*Cicer arietinum*), common bean (*Phaseolus vulgaris*), cowpea (*Vigna unguiculata*), garden pea (*Pisum sativum*), lentil (*Lens culinaris*), mungbean (*Vigna radiata*), narrow-leafed-lupin (*Lupinus angustifolius*), pigeon pea (*Cajanus cajan*), white clover (*Trifolium repens*), and the two model species *Lotus japonicus* and *M. truncatula* [57]. Older versions of the *M. truncatula* and *M. sativa* are housed at https://v1.legumefederation.org/data/v2/Medicago/.

A comparative toolbox: Alfalfa Breeders Toolbox (https://alfalfatoolbox.org/) has been generated between *M. truncatula* and *M. sativa* (alfalfa) to compare the genomes and to provide a convenient access to alfalfa genomic, genetic and phenotypic datasets. An integrative suite of tools psRNATarget (https://www.zhaolab.org/psRNATarget/home) to detect plant regulatory small RNA targets (sRNAs) was developed and built with an objective of identifying targets for plant regulatory sRNAs that include microRNAs (miRNAs) and phased secondary small interfering RNAs (phasiRNAs) [58]. This can be used for *M. truncatula* and other plant species.

Medicago truncatula genetic resources

A variety of genetic resources are available to the *M. truncatula* research community apart from the availability of complete genome assemblies for various *M. truncatula* genotypes and the gene expression atlas. These resources include the availability of *M. truncatula Tnt1* insertion population and fast neutron bombardment (FNB) population.

Tnt1 insertion population

Insertional mutagenesis is used as a tool for gene function discovery through introduction of a foreign DNA fragment (T-DNA, transposon, or retrotransposon) into the genome of choice. This mutagenic approach typically leads to a loss-of-function mutation or a gain-of-function mutation depending on the purpose of the project [16]. This is often a quick way to perform a near-saturated mutagenesis on the entire plant genomes to develop a genetic resource for functional genomics [30]. Tobacco *Tnt1,* a well characterized plant autonomous LTR retrotransposon [27, 28], was used as a tool for insertional mutagenesis in *M. truncatula* cv. R108 [29, 30]. A single *M. truncatula* R108 transgenic line containing five *Tnt1* copies was selected as a parental material to generate *Tnt1* insertion lines through somatic embryogenesis [30]. With a 10-year effort, we have generated a large collection of *Tnt1* insertion lines (~21,000) in *M. truncatula* that nearly saturate the genome with more than 1,000,000 *Tnt1* insertions [2, 30, 59]. *Tnt1*-based insertional mutagenesis was applied in many plant species including *L. japonicus* [60], *M. truncatula* [30], *Brachypodium distachyon* [61], lettuce [62], soybean [63] and potato [64]. The legume research community greatly benefitted from the use of *M. truncatula Tnt1*-insertion population and made important discoveries by dissecting the pathways of symbiotic nitrogen

fixation, root nodulation, secondary metabolites, inflorescence and floral architecture, pollen development, seed development, and other aspects of legume biology [25, 26, 65-77]. All the identified *M. truncatula Tnt1* mutants and their phenotypic observations along with the flanking sequence tag (FST) analysis information are made publicly available through a database maintained at Oklahoma State University (https://medicago-mutant.dasnr.okstate.edu/mutant/index.php).

Three different methods were used to identify FSTs [2]. The three methods include the more common use of thermal asymmetric interlaced-PCR (TAIL-PCR) coupled with next generation sequencing technologies, whole genome sequencing (WGS), and sequence capture (Seqcap). A recent comprehensive review on *Tnt1* insertional mutagenesis details all the above methodologies for identification of FSTs [78]. The *Tnt1* database comprises of ~400,000 high confidence FSTs from a total of 21,741 *Tnt1* insertion lines generated in *M. truncatula*. All the high confidence FSTs were identified by TAIL-PCR followed by Sanger sequencing or Illumina sequencing [2]. The FST size varies based on the product recovered but averages to 363 bp in length [2]. The majority of the FSTs (~360,000) were identified by the next generation sequencing (Illumina) and approximately 10% of the FSTs (~33,000) were recovered from 2,650 *Tnt1* insertion lines using more traditional Sanger sequencing [2, 30]. The more recent next generation sequencing technologies aimed to sequence the FST regions directly through the use of whole genome sequencing (WGS) was demonstrated [79]. Recent methodology of FST identification using WGS demonstrated the identification and presence of substantially more *Tnt1* insertions in the mutant genomes [61, 79]. Seqcap, developed on the groundwork of exome capture, is more economical and faster than WGS. Seqcap was able to recover most of the *Tnt1* insertions in a given line and was comparable to WGS methodologies [2, 61].

Based on the natural distribution of the FSTs throughout the genome, it was estimated that the *Tnt1* retrotransposons inserts randomly throughout the genome, though that was not found to be the same in different *Tnt1* lines [2, 30]. Earlier conventional methodologies employed to identify the *Tnt1* insertions could not capture the entire landscape of the *Tnt1* insertions and hence the average insertions recovered per line were estimated at ~25, though they were profoundly higher than the average 4.76 *LORE1* insertions or the 3.37 *Tos17* in rice [16, 30, 59, 80-82]. The deployment of the more direct next generation sequencing methods as WGS

and Seqcap was able to capture the *Tnt1* insertion landscape with a higher threshold. A more complete profiling of the *Tnt1* insertions with WGS and Seqcap revealed an enhanced number of ~80 *Tnt1* insertions per line, clearly demonstrating the shortfalls of the previously used conventional methodologies (such as TAIL-PCR) in *Tnt1* insertion recovery [2]. With the new average of 80 *Tnt1* insertions per line, it is estimated that the entire mutant *Tnt1* collection might harbor ~1.7 million insertions [2]. Among the 221,275 high confidence FSTs about 92% (~200,000 FSTs) were anchored and mapped to the new *M. truncatula* R108 genome with greater than 90% identity [42]. The anchoring of the FSTs on to the new R108 Hi-C genome showed an even distribution of the *Tnt1* insertions across all eight chromosomes with a distribution of 25,178 insertions per chromosome and with a high- and low-density mapping to Chromosome 1 (27,902; 12.6%) and Chromosome 6 (16,433; 7.4%), respectively [42]. Given the small size of Chromosome 6, it is reasonable to have observed low density mapping to Chromosome 6 and affirms the previously held hypothesis of random *Tnt1* insertion mapping on to the MedtrR108_hic genome [42].

Fast neutron bombardment population

Physical mutagens, such as ionizing radiations/fast neutrons, were used to induce large scale chromosomal deletions and/or rearrangements in several model plant genomes, like Arabidopsis, rice, tomato, soybean, barley and *L. japonicus* [83-85]. In higher plant genomes, EMS or FNB mutagenesis can be used as an effective tool in inducing mutations in genes with short sequences. Briefly, the mutagenesis approach involves mutagenic treatment of wild-type seeds (M_0 seeds) of a plant by ionizing radiation or fast neutron bombardment. The resulting M_1 seeds contain a more unstable and chimeric mutations. Similar to the *Tnt1* insertional mutagenesis, dominant mutations (if present) display visible phenotypes in M_1 plants. More strong and heritable germline mutations remain less transitory and hence transmit to the next generations. The mixed chimeric mutations do segregate for recessive mutations that exhibit their trait phenotypes in appropriate screening experiments.

A comprehensive collection of FNB-derived deletion mutants of *M. truncatula* Jemalong A17 were developed as a joint effort between the Noble Research Institute and John Innes Center, UK [86, 87]. A database consisting of information regarding *M. truncatula* mutant M2 mutant lines from ~146,000 M1 lines obtained following fast neutron bombardment was

developed (https://medicago-mutant.dasnr.okstate.edu/mutant/aboutFNB.php). The ability to identify a visible phenotype from a FNB screen in *M. truncatula* is very low (~5% of the mutant populations; [87]. Through mutant screening workshops, Chen and colleagues have identified many putative mutants that might lead to discoveries in the realms of symbiotic nitrogen fixation and plant development [88]. All the information related to these mutants and the access to these lines are available at Oklahoma State University (https://medicago-mutant.dasnr.okstate.edu/mutant/aboutFNB.php).

The saturation FNB mutagenesis explorative statistics estimate ~150,000 mutant lines for targeting all gene disruptions in *M. truncatula*. The use of FNB mutants for gene discovery by forward or reverse genetics can be achieved through positional cloning and genetic linkage analysis [87, 88]. Cloning and gene discovery process is less complicated in FNB mutants than EMS mutants due to underwhelming nature of the background mutations [89]. Recently, a new approach was developed to recover the mutation sites of FNB mutants in *M. truncatula* using next generation sequencing approach, WGS [89].

M. truncatula small secreted peptides (SSPs) resource

Plant small secreted peptides (SSPs) are small peptides that are biologically active with an average length of ~5 to 50 amino acid residues and are more recently known to be involved with a horde of plant developmental process [90]. Apart from their roles in plant development, they have enhanced roles in other areas of plant biology such as stress responses (biotic and abiotic), nodulation biology, general growth and reproduction [24, 90]. The *M. truncatula* SSP database was developed at the Noble Research Institute as a part of the National Science Foundation project entitled "Genome-wide Analysis of Small Signaling Peptides in *M. truncatula*" that was aimed to understand the nodule biology in *M. truncatula* and other legumes. The *M. truncatula* SSP (MtSSP database) (https://mtsspdb.zhaolab.org/database/) was developed to host the experimental and predicted SSPs from the project. The MtSSP database integrates SSP data with transcriptomics data and has dynamic analytical tools built in to explore, analyze and visualize relevant SSPs [90, 91]. The MtSSP database now includes all the ~70,000 *M. truncatula* annotated genes along with the ~4000 small peptide genes and 2,455 novel SSPs [90]. Boscherio et al. classified known SSPs into 48 gene families in *M.*

truncatula [90]. Community can also benefit from the integrated data on the built-in custom peptide libraries (consist of 155 synthetic peptides) that were validated on *M. truncatula,* Arabidopsis and switchgrass for 24 root and nodule-related phenotypes [90, 91]. *M. truncatula* expression atlas that includes SSP-encoding genes (SSP-GEA) (https://mtsspdb.zhaolab.org/atlas-internal/3880/transcript/profile/0) hosts expression data from 17 RNA-seq experiments conducted with ~200 experimental conditions [90].

Phosphoproteins, transporter and metabolic pathway resources

Initiation of the symbiotic nitrogen fixation is dependent upon protein phosphorylation-mediated signaling [92]. All the protein phosphorylation data, ~3,400 proteins from *M. truncatula* root nodulation including phosphoprotein, phosphopeptide, and phosphosite data, were grouped and integrated into an online repository at Medicago PhosphoProtein Database (MPPD) [92, 93]. Analytical and experimental methods for phosphoprotein identification involved the total proteome isolation from whole and membrane fractions of *M. truncatula* roots. Utilizing the complementary fragmentation methods and tandem mass spectrometry technology, protein phosphorylation sites from *M. truncatula* roots were mapped [92, 93]. This can be a useful resource to the research community working on the legume nodulation biology. In addition, the *M. truncatula* proteome describes a quantitative proteome atlas of *M. truncatula* and its rhizobial symbiont *Sinorhizobium meliloti*, comprising of more than 23,000 proteins, 20,000 phosphorylation sites, and 700 lysine acetylation sites [94].

In addition to phosphoproteins, transporter proteins play a critical role in nutrient uptake and maintaining cellular homeostasis. A comprehensive database called *M. truncatula* transporter database (MTDB, http://genomics.cpolar.cn/MtTransporter/), was built to integrate information on the transporters in *M. truncatula* and houses a total of ~3,600 putative predicted transporters (http://bioinformatics.cau.edu.cn/MtTransporter/). The current information in MTDB shows a hierarchical and relational classification with at least 162 families according to the transporter classification system [95].

MedicCyc (https://plantcyc.org/typeofpublication/mediccyc) was generated using metabolic pathway reconstruction from more than 225,000 *M. truncatula* ESTs [96]. MedicCyc

currently aggregates greater than 250 pathways with related genes, enzymes and metabolites [96]. Metabolites in differential experiments were aggregated and stacked into a metabolite database and can be visualized to understand their roles in the pathways in *M. truncatula* roots [97]. The metabolite database was developed through the framework of *M. truncatula* metabolite atlas (http://artemis.cyverse.org/efp_medicago/cgi-bin/efpWeb.cgi), an adaptation from Arabidopsis Electronic Fluorescent Pictograph (eFP) Browser (http://bar.utoronto.ca/). Briefly, several *M. truncatula* accessions were analyzed for metabolites in various root tissues, such as border cells, root cap, elongation zone, mature root, and analyzed by UHPLC-QTOF-MS and GC-Q-MS for identification [97]. This database reveals localization of many metabolites that are spatially separated in the cell [97].

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Conflict of interest

The authors declare that they have no conflicts of interest in this work.

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