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Molecular Genetics of the Model Legume *Medicago truncatula*

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***Medicago truncatula* is a diploid legume plant related to the forage crop alfalfa. Recently, it has been chosen as a model species for genomic studies due to its small genome, self-fertility, short generation time, and high transformation efficiency. *M. truncatula* engages in symbiosis with nitrogen-fixing soil bacterium *Rhizobium meliloti*. *M. truncatula* mutants that are defective in nodulation and developmental processes have been generated. Some of these mutants exhibited altered phenotypes in symbiotic responses such as root hair deformation, expression of nodulin genes, and calcium spiking. Thus, the genes controlling these traits are likely to encode functions that are required for Nod-factor signal transduction pathways. To facilitate genome analysis and map-based cloning of symbiotic genes, a bacterial artificial chromosome library was constructed. An efficient polymerase chain reaction-based screening of the library was devised to fasten physical mapping of specific genomic regions. As a genomics approach, comparative mapping revealed high levels of macro- and microsynteny between *M. truncatula* and other legume genomes. Expressed sequence tags and microarray profiles reflecting the genetic and biochemical events associated with the development and environmental interactions of *M. truncatula* are assembled in the databases. Together, these genomics programs will help enrich our understanding of the legume biology.**

Keywords : BAC library, *Medicago truncatula*, model legume, molecular genomics, nodulation mutants.

The legume family is one of the most important agricultural taxa worldwide. Legumes provide an important source of protein and oil for humans and animals, and nitrogen for soil improvement. In addition, legumes offer unique research opportunities in plant-microbe interactions such as symbiotic nitrogen fixation, mycorrhizal interactions and legume-pathogen interactions (Albrecht et al., 1999). However, traditional crop legumes have had a relatively minor impact on our understanding of the legume-rhizobial symbiosis. Molecular genetic analyses in pea, soybean and alfalfa are difficult due to large genome size, polyploidy and inefficient transformation and regeneration. Recently, diploid autogamous legumes such as *Lotus japonicus* and *Medicago truncatula* have emerged as model legumes (Cook et al., 1997; Handberg and Stougaard, 1992). These species are not crop plants themselves but offer many advantages that facilitate molecular and genetic analyses (Table 1). The findings emerging from studies in the model legumes can eventually be used to improve leguminous crops.

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Medicago truncatula as a Model Legume

Medicago truncatula ("barrel medic") is a plant species of Mediterranean origin. It is closely related to the agronomically important forage legume alfalfa (*Medicago sativa*). Several attributes of *M. truncatula* make it suitable as a model plant for legume biology and symbiosis research. It has a small diploid genome (~4.5×10^8 bp) and annual and self-fertile growth habits. Its life cycle is short and seed production is prolific. Additionally, it has high capacity for transformation and regeneration (Barker et al., 1990). These features are comparable to the most widely used model plant *Arabidopsis* (Fig. 1). A large collection of *M. truncatula* ecotypes is available in France, Australia and the U. S. A. A selected few ecotypes are used to generate mapping populations through effective artificial hybridization.

Of importance to research on symbiotic processes, *M. truncatula* is efficiently colonized by model microbial symbionts such as *Rhizobium meliloti* (Mylona et al., 1995). Many *R. meliloti* mutants and purified Nod factors are available as convenient tools (Long, 1996). In addition, various pathogen systems have been characterized in *M. truncatula*. Screening of *M. truncatula* germplasm for fungal

Table 1. Characteristics of legumes used in molecular genetic studies in comparison with *Arabidopsis* and rice

Plant species	Ploidy	Genome size[a] (Mbp/1C)
Arabidopsis thaliana	2n	145
Glycine max (soybean)	2n[b]	1,115
Lotus japonicus	2n	450
Medicago sativa (alfalfa)	4n	1,350
Medicago truncatula	2n	450
Oryza sativa (rice)	2n	430
Pisum sativum (pea)	2n	5,000

[a]Estimated from Arumuganathan and Earle (1991), Bennett and Smith (1976), and Blondon et al. (1994). Mbp/1C, megabase pairs per 1C.
[b]Considered as paleopolyploid.

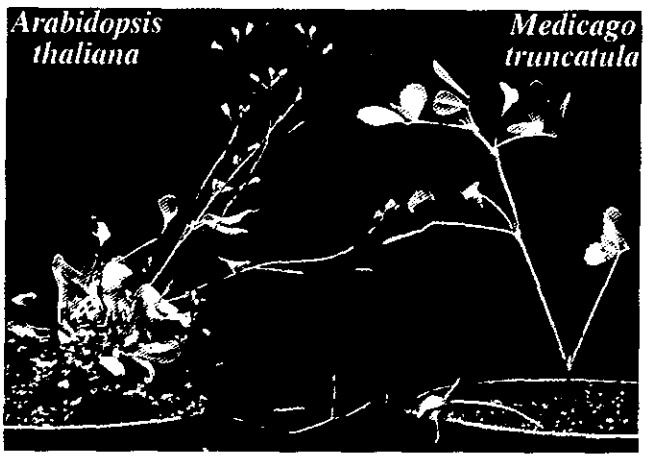

Fig. 1. Morphological distinction between model plants *Arabidopsis thaliana* and *Medicago truncatula*.

pathogens such as *Phytophthora medicaginis* and bacterial pathogens such as *Xanthomonas alfalfae* permitted identification of the compatible-susceptible and incompatible-resistant genotypes (Cook, 1999). Just as *Arabidopsis thaliana* has played a pivotal role in recent advances in our understanding of plant biology, *M. truncatula* is expected to serve as a model molecular genetic system for legume biology.

Nodulation Mutants

The roles of host genes required for symbiotic function can be studied using a genetically tractable legume through mutagenesis efforts. In *M. truncatula*, ethylmethyl sulfonate-induced mutagenesis led to the isolation of several mutants that are defective in nodulation as well as in other developmental processes. An example of such symbiotic mutants is *skl* ("sickle"), which exhibits at least 10-fold increase in the number of persistent rhizobial infections (Penmetsa and Cook, 1997). The same mutation also confers insensitivity to the plant hormone ethylene. These observations indicate that ethylene is a component of the signaling pathway controlling rhizobial infection of legumes. Other mutant genes affected in Nod-factor activated signal transduction pathway include *DMI1*, *DMI2* and *DMI3*, which lead to symbiotic responses such as root hair deformation, expression of nodulin genes, and cortical cell division (Catoira et al., 2000). Interestingly, only *DMI1* and *DMI2* genes appear to be required in common for calcium spiking, a characteristic response of Nod factor-elicited root hair cells, while DMI3 gene is not (Wais et al., 2000). These observations suggest that *DMI3* gene act downstream of calcium spiking upon ligand stimulation.

Mutants that are affected in growth and developmental processes have also been identified. The mutants defective in floral morphogenesis, shoot meristem initiation as well as exhibiting spontaneous necrotic lesion formation have been characterized (Penmetsa and Cook, 2000). The availability of these developmental mutants phenocopying *Arabidopsis* mutants will provide a basis for establishing orthologous gene function between *M. truncatula* and *Arabidopsis*.

The BAC Library

To facilitate genome analysis and map-based cloning of symbiotic genes, a bacterial artificial chromosome (BAC) library was constructed (Nam et al., 1999). The library consists of 30,720 clones with an average insert size of approximately 100 kb. With 16% of the clones apparently lacking inserts, the library represents about five haploid genome equivalents and a 99% probability of containing any sequence in the *M. truncatula* genome at least once. To screen the

Fig. 2. Screening of *Medicago truncatula* BAC library by colony hybridization. A high-density replica filter containing 1536 clones was prepared using a Biomek 2000 robot (Beckman, USA). Each clone was inoculated twice in a 3×3 grid. After bacterial cells were lysed and the DNA fixed with NaOH, the filter was hybridized with a labeled probe. Spots such as 12A22 indicate the addresses of the corresponding clones in the BAC library.

library, high-density replica filters prepared using robots were used for hybridization with labeled probes. This approach permitted identification of BAC clones corresponding to several single- or low-copy genes as well as to different types of repeated sequences (Fig. 2). To further expedite the screening procedure, a polymerase chain reaction-based strategy was developed. In this method, the entire library was pooled two-dimensionally into columns and rows, and the BAC DNA was isolated from each mixture and used to generate multiplex pools. This approach has effectively shortened the time required for library screening (Nam et al., 1999) and is expected to facilitate the construction of BAC physical contigs of overlapping clones spanning a specific genomic region.

Linkage Maps and Comparative Genomics

Linkage maps have been developed for *M. truncatula* using crosses between distantly related ecotypes. A map covering eight linkage groups including morphological, molecular and enzymatic markers is under construction in France (Huguet et al., 2000). Alternatively, another map is being developed with sequence-based PCR markers that utilize a separate mapping population. In this approach, sequence information obtained from expressed sequence tags (EST) or BAC ends is directly used to design co-dominant PCR markers to distinguish alleles from either parental ecotype (Cook, 1999). Together, these efforts indicate rapid progresses being achieved toward making a saturated linkage map of *M. truncatula*.

Genome comparisons among various legume species revealed significant levels of synteny. For instance, comparative mapping showed high levels of macro- and microsynteny between alfalfa and *M. truncatula* genomes. In addition, the soybean genome and *M. truncatula* genome share extensive microsynteny (Young et al., 2000). These observations indicate that comparative mapping can be exploited to investigate the extent to which different legume genomes are conserved structurally. Interestingly, syntenic relationships between genomes appear to extend beyond the legume family. Recent investigations suggest that at least some portions of the *M. truncatula* genome are microsyntenic with *Arabidopsis*, but in other regions similar relationships are not obvious (Cook, 1999). It remains to be determined how much proportions of *M. truncatula* genome will be microsyntenic with *Arabidopsis*. Nonetheless, continuing efforts in comparative genomics will help to elucidate the evolutionary relationships and conservation among plant genomes.

Resources for Functional Genomics

The EST resources are rapidly being constructed in *M. truncatula*. About 14,000 5 and 3 ESTs have been generated from *M. truncatula* roots, nodulated roots and arbuscular mycorrhizal roots (Journet et al., 2000). In addition, 17,000 ESTs in association with a diverse array of pathogens and symbionts and 27,000 from diverse *M. truncatula* tissues have been produced (VandenBosch et al., 2000). The EST sequence information is deposited in the public database (Bell et al., 2001). To analyze the global gene expression profiles associated with the development, symbiotic and environmental interactions of *M. truncatula*, the EST resources are also used in combination with DNA microarrays (Cook, 1999). As a large proportion of EST sequences appears to be unique (Harrison, 2000), comparisons to more extensive data sets developed from *Arabidopsis* should be very informative.

Two ecotypes of *Medicago truncatula* are transformed by *Agrobacterium tumefaciens* and regenerated into fertile plants (Hoffman et al., 1997; Trieu and Harrison, 1996). More recently, researchers reported vacuum transformation of *M. truncatula* (Trieu et al., 2000). The availability of such potentially powerful tools for tagging and insertional mutagenesis will provide support for reverse genetic approaches for elucidating functions of specific genes of *M. truncatula*.

Conclusion

Medicago truncatula is amenable to the majority of the molecular genetic tools used in *Arabidopsis*. Currently, *M. truncatula* is the subject of major genomics initiatives. Several international programs are under way, which aim to build the infrastructure upon which molecular genetic research on legumes and plant-microbe interaction can blossom. The National Science Foundation, the Noble Foundation (U.S.A.), and the European Union each support collaborative research projects performing comparative genomics, functional genomics and map-based cloning of symbiotic genes. The biological information to be garnered from *M. truncatula* research will enable us to accomplish efficient cloning and characterization of valuable genes and traits. Ultimately, these efforts will help to facilitate the development of improved crop varieties.

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