Review:

Medicago truncatula as a model for understanding plant interactions with other organisms, plant development and stress biology: past, present and future

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Abstract. Medicago truncatula Gaertn. cv. Jemalong, a pasture species used in Australian agriculture, was first proposed as a model legume in 1990. Since that time M. truncatula, along with Lotus japonicus (Regal) Larsen, has contributed to major advances in understanding rhizobia Nod factor perception and the signalling pathway involved in nodule formation. Research using M. truncatula as a model has expanded beyond nodulation and the allied mycorrhizal research to investigate interactions with insect pests, plant pathogens and nematodes. In addition to biotic stresses the genetic mechanisms to ameliorate abiotic stresses such as salinity and drought are being investigated. Furthermore, M. truncatula is being used to increase understanding of plant development and cellular differentiation, with nodule differentiation providing a different perspective to organogenesis and meristem biology. This legume plant represents one of the major evolutionary success stories of plant adaptation to its environment, and it is particularly in understanding the capacity to integrate biotic and abiotic plant responses with plant growth and development that M. truncatula has an important role to play. The expanding genomic and genetic toolkit available with M. truncatula provides many opportunities for integrative biological research with a plant which is both a model for functional genomics and important in agricultural sustainability.

Additional keywords: abiotic stress, biotic stress, Jemalong 2HA, legumes, nodulation, regeneration.

Introduction

Medicago truncatula Gaertn. (barrel medic) is now well accepted as a model legume species, and, together with Lotus japonicus (Regal) Larsen, has helped to bring legumes into the position of being able to access many of the contemporary tools of genetics and functional genomics. Two recent demonstrations have emphasised the success obtained by a focus on model and functional genomics. Two recent demonstrations have emphasised the success obtained by a focus on model and functional genomics. Two recent demonstrations have emphasised the success obtained by a focus on model and functional genomics.

Medicago truncatula cv. Jemalong, agriculture and biotechnology

Annual Medicago species (annual medics) of Mediterranean origin have been particularly important in southern Australian agriculture because of their role in the wheat/sheep rotation. Cereal crops are alternated with legume pastures. The value of M. truncatula was recognised as early as 1939 and Jemalong was a commonly used cultivar (Crawford et al. 1989). This is an excellent example of sustainability with the pasture providing forage for livestock and symbiotically fixed nitrogen becoming available for subsequent crops (Loi et al. 2000). The annual medics germinate following the softening of hard seeds set in previous years (Crawford et al. 1989). More recently, a second generation of annual pasture legumes have been introduced to cater for a wider range of farming systems including grain legumes and oil seeds in phase farming systems (Loi et al. 2005). With the advent of somatic hybridisation (Carlson et al. 1972) and then Agrobacterium-mediated transformation in 1983 (Bevan et al. 1983; Fraley et al. 1983; Herrera-Estrella et al. 1983; Murai et al. 1983), biotechnology was directed to many agricultural species including legumes. Regeneration was a requirement for these techniques. Regeneration from cultured tissues or protoplasts was conducted with the perennial, allogamous and autotetraploid Medicago sativa L., which proved amenable to regeneration via somatic embryogenesis from tissue explants (Saunders and Bingham 1972) and...
protoplasts (Johnson et al. 1981; Rose et al. 1986). Regeneration was restricted to special lines, notably Regen S, which was bred for regenerability (Bingham et al. 1975). Annual Medicago species were difficult to regenerate, but work by Bingham and co-workers indicated that the Medicago genus included species with ‘regenerability’ genes. M. truncatula, an annual autogamous diploid, is more attractive for transformation work and the associated genetics than the allogamous autotetraploid M. sativa. The cultivar Jemalong proved amenable to regeneration but at extremely low frequency. What was surprising was that the few plants that regenerated, when used as explants, had a huge increase in regenerability (500×) via somatic embryogenesis and this was inherited (Nolan et al. 1989) and lead to the development of the highly regenerable Jemalong 2HA (2HA) (Rose et al. 1999). Using 2HA, transformation (Thomas et al. 1992; Chabaud et al. 1996; Wang et al. 1996), regeneration from protoplasts (Rose and Nolan 1995), asymmetric somatic hybridisation (Tian and Rose 1999) and transfer of agriculturally important genes such as viral resistance genes was feasible (Jayasena et al. 2001). Use of transformation as a tool for analysing gene function became crucial as whole-genome sequencing, large scale mutant isolation and the era of functional genomics began.

**Medicago truncatula as a model legume**

The foremost plant model Arabidopsis thaliana (L.) Heynh has many advantages for plant genomics, with its small size, short generation time, large numbers of offspring and small nuclear genome. Sequencing commenced in 1996; and its sequence was published in December 2000 (The Arabidopsis Genome Initiative 2000). The value of Arabidopsis mutants for functional analysis was shown some years ago by the isolation of mutants of C3 species with defects in CO2 assimilation and photorespiration (Somerville and Ogren 1979). The ability to transform Arabidopsis by the floral dip method (Clough and Bent 1998) has greatly facilitated research in Arabidopsis by enabling genome-wide insertional mutagenesis with T-DNA insertions (Alonso et al. 2003) to potentially enable knockouts of all genes. Arabidopsis is a dicotyledonous but non-nitrogen-fixing plant, making it important to develop a legume model with its ability to establish symbiotic interactions with rhizobia and mycorrhizae. With more than 18 000 species, legumes are the third largest family of higher plants (Young et al. 2005) does not regenerate via somatic embryogenesis but can be transformed by organogenesis procedures (Trieu and Harrison 1996; Zhou et al. 2004). The recent Zhou et al. (2004) procedure requires dissecting out cotyledonal nodes from emerging seedlings and rigorous selection procedures to avoid untransformed shoots (Zhou et al. 2004).

What has been a very valuable transformation adjunct in M. truncatula and the study of the rhizobia and the arbuscular mycorrhizal (AM) symbiosis has been the use of Agrobacterium rhizogenes to form transformed roots on composite plants (Boisson-Dernier et al. 2001). This protocol involves the inoculation of sectioned seedling radicles which form the hairy roots of the composite plants. Antibiotics such as kanamycin can be used to select for co-transformation of hairy roots with introduced constructs. The hairy roots successfully form nodules after inoculation with Sinorhizobium meliloti and can be colonised by AM fungi. The transgenic roots can be generated rapidly in 2–3 weeks which has been a great aid to M. truncatula symbiosis research.

Medicago truncatula was a focus for several meetings and workshops in the United States and Europe in the 1990s to establish it as a model and initiate the development of the necessary genetic and genomic tools (Cook et al. 1997; Cook 1999). Expressed sequence tags (ESTs) were rapidly developed, beginning with the M. truncatula root hair enriched cDNA library (Covitz et al. 1998) producing 899 ESTs. There are now 227 000 M. truncatula ESTs on the The Gene Index Project database (http://compbio.dfci.harvard.edu/tgi/, accessed 15 December 2007). The first steps towards sequencing were taken when
Nam et al. (1999) produced the first BAC clones from Jemalong A17. The first published genetic map of *M. truncatula* was produced by Thoquet et al. (2002) using two homozygous lines selected from Jemalong (Jemalong 6 or J6) and the Algerian natural population DZA315. Thoquet et al. (2002) noted that the three Jemalong lines A17, J5 and J6 could be considered to have an identical genotype (but different to the highly regenerable Jemalong genotype 2HA). BAC clones were mapped to *M. truncatula* A17 pachytene chromosomes by fluorescent *in situ* hybridisation (FISH), (Kulikova et al. 2001; Choi et al. 2004a; Kulikova et al. 2004). In the Choi et al. (2004a) study the mapping population was derived from A17 × A20. The A20 genotype is an *M. truncatula* ecotype with nodulation characteristics similar to A17 and a dominant leaf spot phenotype (Penmetsa and Cook 2000). From this work, it was inferred that gene-rich regions were located in the euchromatin rich chromosome arms, with the heterochromatin located in the centromere and pericentromeric regions This meant that if BAC clones could be identified as gene rich then most of the genespace could be obtained by BAC-by-BAC sequencing (Young et al. 2005). This latter strategy of anchored, clone-by-clone sequencing (as opposed to whole-genome shotgun sequencing) has been pursued. Genome sequencing began in 2002 (Young et al. 2005) and has continued until the present time (http://www.medicago.org/ genome/, accessed 15 December 2007). In the *M. truncatula* genome assembly version 1.0 (Mt1.0) nearly 2000 BACs have been sequenced representing ~186.2 Mbp of non-redundant genome sequence, about two-thirds of the gene rich space. The completed sequencing of the ~186.2 Mbp of non-redundant genome sequence, about two-thirds of the gene rich space is expected by the end of 2008. This latter information can be accessed on the publicly available databases (http://www.medicago.org/, accessed 15 December 2007). Physical and genetic maps are available on this latter site. The reference mapping population is A17 × A20 (Ané et al. 2008). Genome conservation between *M. truncatula* and crop and other legumes, including *Lotus japonicus*, has been examined (Choi et al. 2004b; Cannon et al. 2006). There is substantive synteny between *M. truncatula* and *M. sativa* (Thoquet et al. 2002; Choi et al. 2004a) and *M. truncatula* and *Pisum sativum* L. (Choi et al. 2004b; Aubert et al. 2006). These mapping studies will provide valuable information for fundamental research and translational research with crop legumes.

**Genetic and genomic tools available in *M. truncatula***

As outlined above there are now large numbers of ESTs, an increasing amount of gene rich genome sequence and physical and genetic maps available for *M. truncatula*. The EST information (227,000 ESTs) has been assembled into 18,612 TCs (tentative consensus sequences) and 18,238 singleton ESTs (http://compbio.dfc.libharvard.edu/cgi/, accessed 15 December 2007). Current estimate of gene number from the sequencing program in *M. truncatula* is 42,358 (http://www.medicago.org/, accessed 15 December 2007). The *M. truncatula* chloroplast genome, which contains only one copy of the inverted repeat, is also an active area of study (Shaver et al. 2008).

For functional genomics it is necessary to develop forward and reverse genetics tools. The first mutations affecting nodulation phenotypes were obtained in *M. truncatula* using γ-rays (Sagan et al. 1995) and ethylmethane sulfonate (Benaben et al. 1995; Penmetsa and Cook 1997). Mutants have been crucial to the progress in understanding the mechanism of nodulation. Several developmental mutants, other than nodulation, have also been isolated by Penmetsa and Cook (2000). Reverse genetic strategies to infer gene function based on induced variation within a specific gene sequence are also being pursued in *M. truncatula*. These are RNAi, TILLING (Targeting Induced Local Lesions in Genomes), *Tnt1* insertional mutagenesis (Ratet et al. 2006), and a fast neutron deletion mutagenesis based system. Using *Tnt1* retrotransposon-tagged mutants a leaf development gene SINGLE LEAFLET1 has been identified (Wang et al. 2008). The current status of these reverse genetic strategies has recently been discussed by Ané et al. (2008). We have recently used dexamethasone inducible RNAi (Mantiri et al. 2008) to identify SOMATIC EMBRYO-RELATED FACTOR1 (MISER1). Transcriptomics have been facilitated by the development of microarrays following the completion of the large scale EST projects. A spotted 16 K microarray of 70-mer oligos (http://www.noble.org/medicago/NSF/NSF.activities.html, accessed 15 December 2007) available in 2003 has been used in gene expression studies, for example in AM development (Hohnjec et al. 2006). The Affymetrix Medicago GeneChip became available in 2005 and includes 32,167 *M. truncatula* ESTs and 18,733 gene predictions from *M. truncatula* genome sequences, 1896 cDNAs from *M. sativa* and 8305 gene predictions from *Sinorhizobium meliloti* (Dandgeard de Lajudie et al. 2006). Udvardi and colleagues (2007) have also developed high-throughput quantitative reverse transcriptase (qRT-PCR) analysis of transcription factors, which is more sensitive than DNA array hybridisation methods. These authors also discuss methods to identify transcription factor target genes in a non-biased, high throughput manner.

In parallel with the development of the genetic, genomic and transcriptomic resources has been the development of an increasing amount of proteomic data for *M. truncatula* (e.g. Mathesius et al. 2001; Watson et al. 2003; Imin et al. 2004, 2005). This latter technology relies on protein separations and mass spectrometry. Proteins separated on two dimensional gels and trypsin digested proteins can be characterised by peptide mass fingerprints by MALDI-TOF-MS and then identified from available sequence information on publicly available databases for *M. truncatula* (Imin et al. 2004). Using LC/MS/MS protein spots can be fragmented into peptides and sequence information generated from an individual peptide (Imin et al. 2004). High throughput LC/MS/MS is an alternative to gel separation of proteins (Millar et al. 2005).

With all the approaches now available for functional genomics there is increasing reliance on bioinformatic resources and there are recent publications that have tabulated these resources (Cannon et al. 2005; Stacey et al. 2006).

**The interaction of *M. truncatula* with other organisms**

The *M. truncatula* model has been central to the progress in unravelling the legume-rhizobia symbiosis but is also proving valuable in other areas. Outlined below are several areas where
the *M. truncatula* system is proving useful in enhancing understanding of how plants interact with symbionts, pests and pathogens.

The rhizobia–legume symbiosis

The focus on the two legume models, *M. truncatula* and *L. japonicus*, has enabled substantive progress in the understanding of how Nod factors are perceived and the signalling pathway that ultimately leads to nodule formation. As the focus of this review is *M. truncatula*, the schematic in Fig. 1 draws attention to key *M. truncatula* mutants in the developing understanding of nodulation. Progress on the mechanism of nodulation has been reviewed in recent times (Riely et al. 2004; Oldroyd and Downie 2006; Stacey et al. 2006) and includes the *Lotus* mutants. Identifying the receptors and linking the signalling through to the Ca\(^{2+}\) spiking and the apparent phosphorylation of the GRAS-type NSP1 and

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**Fig. 1.** Simplified diagram showing the contribution of key *Medicago truncatula* mutants to understanding the mechanism of nodulation. Information from Cullimore and Dénaire (2003) Riely et al. (2004), Udvardi and Scheible (2005), Oldroyd and Downie (2006), Stacey et al. (2006) and Oldroyd (2007). *nfp* is the *M. truncatula* NFP (NOD FACTOR PERCEPTION) locus (Amor et al. 2003), which mutates the LysM receptor-like kinases thought to be the Nod Factor Receptor NFR1/NFR5. *dmi2* (doesn’t make infection mutant 2) mutates a plasma membrane located Leucine Rich Repeat Receptor Like Kinase (Limpens et al. 2005) which acts upstream of Ca\(^{2+}\) influx, Ca\(^{2+}\) spiking and root hair curling. *dmi1* mutates a ligand-gated cation channel (Ané et al. 2004). *dmi3* mutates a gene downstream of calcium spiking and is a calcium- and calmodulin-dependent protein kinase (Lévy et al. 2004). *nsp1* and *nsp2* mutate NODULATION SIGNALLING PATHWAY 1 (Smit et al. 2005) and 2 (Kaló et al. 2005), transcription factors of the GRAS family. *bit1–1* and *bit1–2* mutate the ERF transcription factor ERN REQUIRED FOR NODULATION (ERN) (Middleton et al. 2007). *sunn* mutates the SUNN gene which causes the hypernodulation phenotype (Schnabel et al. 2005).
2 transcription factors provides a strong framework for understanding nodule development. There are many steps in Fig. 1. to complete, particularly the integration of the development of the infection thread and the setting up of the symbiosomes associated with the morphogenesis of the nodule. Development of current thinking can be seen in several recent commentaries (Cullimore and Dénarié 2003; Udvardi and Scheible 2005; Oldroyd 2007).

The work on understanding signalling in noduleation is not only significant for understanding the mechanism of noduleation but also facilitates understanding of the AM symbiosis as well as interaction with pests such as nematodes. Further, the *M. truncatula* nodule is indeterminate producing an apical meristem. This provides a useful model to study organ morphogenesis and cell differentiation (Brewin 1991).

The arbuscular mycorrhizal legume symbiosis

*Meditago truncatula* has proven a useful model for investigating the arbuscular mycorrhizal symbiosis with the obligate biotroph *Glomus* spp. (Liu et al. 2003; Hohnjec et al. 2006). The AM symbiosis association enables a carbon supply for the fungus and an enhanced supply of mineral nutrients, notably phosphorous, to the plant (Liu et al. 2003; Hohnjec et al. 2006). The AM symbiosis as for the rhizobia symbiosis has important implications for ecology and sustainable agricultural systems. Using a legume model has the advantage of comparative studies of nodulation and mycorrhisation. Studies of mutants defective in nodulation have shown mechanistic similarities between nodulation and mycorrhizal infection (Stacey et al. 2006). Several *M. truncatula* mutants are defective in both the rhizobia and the AM symbiosis and the impaired genes are known as *SYM* genes (Parmiske 2004). An example of these genes are the DMI1, DMI2 and DMI3 genes (Fig. 1), involved in both Nod factor and Myc factor signalling (Ané et al. 2004; Stacey et al. 2006). Though the rhizobia and AM have some common signalling components, the NFR1 and NFR5 Nod-factor receptors are not the AM receptors (Parmiske 2004). Transcriptional profiling studies using microarrays reveal that several hundred genes are upregulated during AM development and how these genes relate to current understanding of signalling remains to be explored (Hohnjec et al. 2006).

Harrison and co-workers (Harrison et al. 2002; Javot et al. 2007) have defined a *M. truncatula* phosphate transporter (MtPT4) that is essential for both the acquisition of phosphate delivered by the AM fungus and is a requirement for the AM symbiosis. The *Javot et al.* (2007) study used RNAi strategies to downregulate MtPT4, and MtPT4 loss-of-function mutants were identified by TILLING. It was shown that MtPT4 is a low affinity phosphate transporter and is a member of a unique clade of phosphate transporters (Pht1, subfamily 1) which are only expressed in the AM symbiosis. The MtPT4 transporter is present in the *M. truncatula* periarbuscular membrane which forms a continuum with the plasma membrane of the *M. truncatula* cortical cell (Harrison et al. 2002).

The nematode-plant interaction

As noted above, there is overlap in the signalling pathways between rhizobia and AM symbionts and it is of interest to widen this comparison to include other microbe interactions such as with pathogenic nematodes (Mathesius 2003). The model legumes *M. truncatula* and *L. japonicus* can act as hosts for nematodes, which enables comparisons with the plant recognition of rhizobia and AM fungi and the subsequent signalling events (Bird 2004). Root knot nematodes (RKN) induce giant cells in the vascular cylinder where the RKN feed. There is evidence that nematode signalling at the root surface is influenced by mutations in Nod factor receptors (Bird 2004; Weerasinge et al. 2005).

Plant–aphid interactions

Plant breeders have recognised for some time that annual *Medicago* species have a range of insect resistances and that there is a range of aphid resistance within *M. truncatula* cultivars and genotypes (Lake 1989). Development of *M. truncatula* as a model legume opened up genetic and molecular studies in this area (Klingler et al. 2006; Gao et al. 2008). Different resistance genes exist for spotted alfalfa (*Theroioaphis trifolii* (Monell) f. *maculata*), pea (*Acrhythosphon pisum* Harris) and blue green aphid (*Acrhythosphon kondoi Shinni*) (Klingler et al. 2006; Gao et al. 2008). Using *M. truncatula*, Klingler et al. (2005) identified a single dominant gene which conferred resistance to the bluegreen aphid which is flanked by NBS-LRR resistance gene analogues. Salicylic acid- and ethylene-responsive genes were induced in both resistant and sensitive plants (Gao et al. 2007). However, 10 genes associated with jasmonate signalling were only induced in the aphid resistant line.

*Meditago truncatula* and pathogenic fungi

*Meditago truncatula* is also being increasingly used as a model to study pathogen resistance mechanisms (Ellwood et al. 2006b; Tivoli et al. 2006; Foster-Hartnett et al. 2007; Samac and Graham 2007). The value and basis of *M. truncatula* as a model for necrotrophic pathogens has been reviewed by Tivoli et al. (2006). The group at the Australian Centre for Necrotrophic Fungal Pathogens is making use of the World’s largest collection of *M. truncatula* accessions, curated by SARDI at the University of Adelaide Waite Campus. The collection has been shown to be highly diverse, with over 90% of individuals showing discrete genotypes (Ellwood et al. 2006a). Sources of resistance to *Phoma medicaginis* Malbr. & Roum. have been identified in the SARDI collection (Ellwood et al. 2006b). Legume powdery mildew caused by the biotroph *Erysiphe pisi* DC. has been investigated by Foster-Hartnett et al. (2007) using *M. truncatula* genotypes with different levels of resistance. This latter study includes microarray analysis of gene expression in three genotypes with moderate and high resistance, and susceptibility. This microarray analysis joins others on nodulation (Lohar et al. 2006) and AM infection (Hohnjec et al. 2006) and such data being accumulated will be valuable to link to each other and the mutant and single gene molecular studies. *M. truncatula* is also a useful pathosystem for root rot diseases caused by *Aphanomyces euteiches* Drechsler (Gaulin et al. 2007), for *Phytophthora* (Salzer et al. 2000) and for the soilborne bacterial wilt pathogen *Ralstonia solanacearum* (Smith) Yabuuchi et al. (Vailleau et al. 2007).
**Medicago truncatula** and plant development

**Nodulation**

As well as being a structure that houses the rhizobia the legume nodule enables a suitable environment for nitrogenase to conduct symbiotic biological nitrogen fixation (Oldroyd 2007) and provides an interesting model for plant cell differentiation and morphogenesis. The nodule is initiated opposite xylem poles from differentiated cells of the inner cortex (Beveridge et al. 2007) which means that these cells have to dedifferentiate and re-enter the cell cycle. A primordium ultimately forms and a mature nodule is produced. In the case of *M. truncatula*, the indeterminate nodule forms an apical meristem from which the nodule develops. As with plant development in general the hormones auxin and cytokinin are key players in nodule morphogenesis (Beveridge et al. 2007). Evidence from *M. truncatula* RNA interference studies against the cytokinin receptor CRE1 has shown that cytokinin acts as a positive regulator of nodulation (Gonzalez-Rizzo et al. 2006). In *L. japonicus*, a gain of function mutation in the cytokinin receptor Lotus histidine kinase 1 triggers spontaneous nodule formation in the absence of rhizobia (Tirichine et al. 2007). An LHK1 loss of function mutant fails to initiate cortical cell divisions in response to rhizobial signalling, but infection thread formation occurs (Murray et al. 2007). It is argued that cytokinin is necessary and sufficient for dedifferentiation and cell proliferation leading to root nodule formation (Tirichine et al. 2007); so how is auxin involved in nodule development? Auxin is thought to be involved at different stages of nodule formation and is required for differentiation of the vasculature (de Billy et al. 2001). Auxin accumulates at the site of nodule initiation and it has been shown (Pii et al. 2007) that an increased rhizobia auxin synthesis promotes the formation of indeterminate nodules (as in *M. truncatula*) but not in determinate nodules.

**Lateral root formation**

Lateral root formation has been extensively studied in *Arabidopsis* (Casimiro et al. 2003), but the use of legumes provides an interesting perspective as lateral root formation has some similarities to nodule morphogenesis (Beveridge et al. 2007). Lateral root primordia originate from pericycle cells and then the lateral root produces a meristem at the apex, as do indeterminate nodules (Beveridge et al. 2007). Auxin can stimulate lateral root formation in *Arabidopsis* (Hirotta et al. 2007), and recent studies in *M. truncatula* with IAA-overproducing nodules showed increased lateral root formation (Pii et al. 2007). There may be an overlap between the early events in nodulation and lateral root formation (Beveridge et al. 2007); however, the response to cytokinin differs in nodulation and lateral root formation in *M. truncatula*. In the case of CRE1 RNAi knockdowns where nodulation did not occur there is strong additional evidence for cytokinin control of nodulation (Gonzalez-Rizzo et al. 2006), but in the case of lateral roots MtCRE1 RNAi roots showed enhanced lateral root density. Clearly, though there are conceptual similarities and some specific overlap between meristem ontogeny, the specific type of meristem produced ultimately requires the expression of a different gene set.

**Somatic embryogenesis and organogenesis in vitro**

*M. truncatula* forms an indeterminate nodule and conceptually it illustrates the ability of plant cells to dedifferentiate, re-enter the cell cycle and differentiate into an organ containing a meristem. *In vitro* systems in plant biology function in a conceptually similar way and offer the opportunity to investigate cellular differentiation mechanisms and to experimentally modify the system in different ways (e.g. through media additives). In the development of transformation techniques for *M. truncatula* lines were developed, such as 2HA, with a greatly enhanced somatic embryogenesis compared with wild type. By modifying the hormones it is possible to obtain adventitious roots in both 2HA and wild type. These developmental outcomes in response to hormones and genotype are shown in Fig. 2.

**Somatic embryogenesis**

Somatic embryogenesis, like nodulation, offers fundamental insights into differentiation mechanisms. Somatic embryogenesis is a natural occurring asexual reproductive process in some plants (Garcès et al. 2007). Regeneration by somatic embryogenesis is an important pathway for transformation in many species but as in *M. truncatula* is often restricted to a specific cultivar, although the reason for this is unknown. The understanding of somatic embryogenesis will also enhance our understanding of apomixis (asexual seed formation, where the genotype is the same as the mother plant) as well as zygotic embryogenesis. In this latter case, an important example is the SERK1 gene, a leucine rich repeat receptor-like kinase, discovered in somatic embryogenesis (Schmidt et al. 1997) and expressed in apomictic (Tucker et al. 2003) and zygotic embryogenesis (Hecht et al. 2001). *MtSERK1* is important in somatic embryogenesis and also in *in vitro* auxin-induced root formation in *M. truncatula* (Nolan et al. 2003).

**Regulation of in vitro meristem formation in Medicago truncatula**

**Fig. 2.** Diagram of *in vitro* development of meristems in Medicago truncatula wild type Jemalong (Jem) and the Jemalong 2HA (2HA) embryogenic mutant. Cell proliferation of cultured leaf explants is initiated by the stress of excision and plating of the explant and increases in response to auxin, and is maximal in response to auxin plus cytokinin. Auxin stimulates root development in Jemalong and 2HA. The addition of cytokinin to the auxin medium inhibits root formation causing only proliferating callus in Jemalong and the bipolar embryos in 2HA callus. Based on Nolan and Rose (1998), Rose et al. (1999, 2006), Nolan et al. (2003) and Mantiri et al. (2008).
An unusual biological aspect of somatic embryogenesis in *Medicago truncatula* is that all the very highly regenerable genotypes have been derived after a cycle of tissue culture. These are Jemalong 2HA (Nolan et al. 1989; Rose et al. 1999), R108 (Hoffmann et al. 1997) and M9-10a (Araújo et al. 2004). This suggests that the regeneration process has consistently selected for a somatic cell with a somatic embryogenesis capacity, which is then inherited. As a cycle of tissue culture is always enough to enhance regenerability (Nolan et al. 1989), it suggests that the frequency is too high for a mutation and may be an epigenetic effect.

Somatic embryogenesis in *Medicago truncatula* requires a special genotype such as 2HA, auxin plus cytokinin plus the stress of the culture process, and research is in progress to define the signalling pathways involved (Rose and Nolan 2006). Isolated *Medicago truncatula* mesophyll protoplasts can regenerate via somatic embryogenesis and cell dedifferentiation and the first cell division cycle can be readily studied in these cells (Sheahan et al. 2005). In both *Medicago truncatula* and *Arabidopsis* massive mitochondrial fusion followed by fission is characteristic and relates to mitochondrial genetics (Sheahan et al. 2005).

*Adventitious root formation in vitro*

Adventitious root formation can be induced in cultured *Medicago truncatula* leaf explants by auxin in both 2HA and wild type (Nolan and Rose 1998; Fig. 2). Although this *in vitro* response has been known for 50 years in other species (Skoog and Miller 1957), it provides a useful system to study organ differentiation in the *Medicago truncatula* context. Histological examination of this system shows that root meristems are initiated *de novo* from procambial-like cells in the vasculature (Rose et al. 2006; Imin et al. 2007) and auxin-induced root formation is promoted in ethylene transduction mutants such as *sickle* which is most likely an *ein2* mutation. It is of interest to compare *in vitro* root formation regulation with that of nodules in *Medicago truncatula*. With the *sickle* mutant, there is stimulation of nodule number formation (Prayitno et al. 2006) which suggests that there is some commonality in committing pluripotent cells to a developmental pathway. In this later case ethylene is thought to modulate auxin transport (Prayitno et al. 2006). The value of organogenesis in an *in vitro* system is similar to that for embryogenesis in that it lends itself to media manipulation and the ready collection of material for high throughput analysis.

*Seed development*

Given the economic importance of grain legumes, seed development is an important area of investigation in this group of plants. A proteomic study of seed development in *Medicago truncatula* (cv. Jemalong line 35) at different stages of seed filling indicated the value of *Medicago truncatula* as a model for the analysis of seed filling in legumes (Gallardo et al. 2003). Studies by Djemel et al. (2005) on seed development and composition also concluded that *Medicago truncatula* was a suitable model for genomic approaches to seed development in grain legumes. During maturation protein and oil accumulated at fairly constant rates (Djemel et al. 2005), but the major protein groups were shown to accumulate in a specific temporal order (Gallardo et al. 2003). Firnhaber et al. (2005) have conducted microarray studies in flower pods and found more than 700 genes to be developmentally regulated.

*Medicago truncatula* and stress biology

*Medicago truncatula* is a valuable species to study how a plant interacts with its environment. It has the capacity to recognise signals from benefit organisations and to have appropriate developmental responses. It also has to combat disease, pests and environmental stressors by recognising and responding to these signals to enable continuing growth and development. Soil salinity is a significant stressor for crop plants and adaptation of root development has been studied by Merchan et al. (2007) in *Medicago truncatula*. An AP2 transcription factor MtZpt2–1 when overexpressed in *Medicago truncatula* plants carrying *Agrobacterium rhizogenes* transformed roots allowed sustained root growth under salt stress conditions. Another interesting feature of the study was that two genes homologous to cytokinin receptors were induced in the salt recovery phase. Hormones are known to be critical in root system growth and architecture and are important in responding to abiotic stress (Malamy 2005). In legumes it is interesting that cytokinin has a key role in nodule formation (Gonzalez-Rizzo et al. 2006) but inhibits root formation *in vitro* (Nolan and Rose 1998) and cytokinins in *Medicago truncatula* are negative regulators of lateral root formation (Gonzalez-Rizzo et al. 2006).

Drought tolerance investigations have also been carried out utilising *Medicago truncatula* genes. Zhang et al. (2005) have overexpressed *WXP1* a putative AP2 domain-containing transcription factor gene. This gene when overexpressed increased the cuticular wax and enhanced drought tolerance. It is noteworthy that MtZpt2–1 is also an AP2 transcription factor and has a single Ap2 domain which places it in the Dreb sub-family of the AP2/ERF super family. The AP2/ERF transcription family has some particularly interesting features as its members include genes related to abiotic and biotic stress and development (Nakano et al. 2006) which is suggestive of evolutionary forces connecting stress to development. Mantiri et al. (2008) have also recently found that a member of the ERF family of transcription factors is essential for somatic embryogenesis, possibly linking the stress of the culture process to development (Mantiri et al. 2008).

Transcription factors are clearly important regulators of both development and abiotic stress tolerance. In addition to the examples given, of MtZpt2–1 and *WXP1* in relation to salt and drought stress, other legume transcription factors have been implicated in abiotic stress tolerance (Udvardi et al. 2007). As Udvardi and coauthors have pointed out (Udvardi et al. 2007), evolution has endowed plants with the ability to ensure their growth and development while fixed in space and subject to environmental extremes. Less than 1% of the more than 2000 transcription factors (TFs) in the model legumes (*Medicago* and *Lotus*) have been functionally characterised, so there is much scope to discover new strategies for using genetic means to influence stress tolerance (Udvardi et al. 2007). The Udvardi et al. (2007) legume transcription factor update has a lists of legume TFs that have been genetically characterised and those that have been characterised biochemically and
molecularly. Also there is a useful guide to domain shuffling between *Medicago* TF families in this latter article.

**Conclusions and future prospects for research using *M. truncatula***

From the research discussed it can be seen that *M. truncatula* has emerged as an important model legume which has facilitated advances in the legume symbioses and opened up new areas of research into biotic stress, plant responses to pests and pathogens and plant development. There is a platform for continued progress in these areas. The completion of sequencing to capture most of the genes and the continued evolution of the bioinformatics is clearly important. As genome science progresses it seems likely that there will be a need to have a complete genome sequence, which given the advances in gene sequencing should be an attainable goal. *Arabidopsis* research has greatly benefited from the availability of insertional mutants which has made the identification of the function of all genes a realistic proposition. Though advances have been made, really high throughput transformation is still not possible in *M. truncatula*. There has been a promising recent study in *M. sativa* (Weeks et al. 2008) on *in planta* transformation directed at the apical meristem of the seedling, which are cut at the seedling node. Some specific areas where future *M. truncatula* research could provide further insights into legume and plant biology are highlighted below.

**Can symbiosis be engineered?**

With the increasing understanding of the signalling pathways involved in nodule and arbuscule signalling (Oldroyd and Downie 2006) and the demonstration that gain-of-function mutations in *CCaMK* and *LHK1* genes can cause spontaneous nodule formation in the absence of rhizobial bacteria (Gleason et al. 2006; Tirichine et al. 2007); the possibility of transferring symbiotic processes into other plant species has again been raised (Oldroyd 2007). Today, this possibility, with current genetic and genomic tools, increasingly looks a more realistic goal.

**Small RNAs**

There is another level of regulatory control that needs to be considered in understanding the regulation of plant processes. Gene expression can be regulated by RNA-induced gene silencing involving micro-RNAs (miRNAs) or short interfering RNAs (siRNAs) 21–24 nt in length (Jones-Rhoade et al. 2006; Axtell et al. 2007; Brosnan et al. 2007). The siRNAs and miRNAs guide argonaute-like proteins to mediate mRNA degradation, translational repression or transcriptional silencing (Jones-Rhoade et al. 2006; Brosnan et al. 2007). In plants, mRNA silencing can be transmitted from cell to cell and from roots to shoots (Brosnan et al. 2007). Many of the miRNAs regulate developmental processes (Jones-Rhoade et al. 2006). Small RNAs have received minimal attention in legumes.

**Connecting plant growth and development to the abiotic and biotic environment**

As highlighted in a recent opinion article (Potters et al. 2007), plants have to ultimately grow and develop out of trouble caused by environmental stressors. Legumes have this fascinating ability to have both symbiotic and defence responses leading to some different insights into the control of the diverse signalling pathways that contribute to the life of the legume plant. There are useful comparisons and overlaps in the area of plant–microbe interaction as well as in legume development (Beveridge et al. 2007). The biology of receptors is an area where functional overlaps are providing different perspectives. An example here is the SERK family of receptors (SERK3 is synonymous with BAK1) with roles in development, brassinosteroid reception and innate immunity (Chinchilla et al. 2007). One group of transcription factors that have interesting signalling connections when thinking of functional integration is the AP2/ERF superfamily of transcription factors (Alonso et al. 2003; Nakano et al. 2006). In *M. truncatula*, this family is involved in nodulation (Middleton et al. 2007), abiotic stress (Zhang et al. 2005) and development (Mantiri et al. 2008). In other species the role of the AP2/ERF superfamily in pathogen defence signalling is well established (Thatcher et al. 2005).

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**References**


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