Medicago truncatula Stock centres

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1. Introduction

Stock centres dealing with *Medicago truncatula* germplasm in the world are based at four main centres, namely The South Australian Research & Development Institute (SARDI), Australia, The USDA National Plant Germplasm System (NPGS), USA The French National Institute for Agricultural Research (INRA), France, and The Samuel Roberts Noble Foundation, USA. A brief history and an overview of the activities of each of these centres are presented below including the future directions and the possibilities of integration of the activities.

2.1 Australian *Medicago* Genetic Resource Centre, SARDI, Australia

2.1.1 History

The Australian *Medicago* Genetic Resource Centre (AMGRC) is one of five Centres within the national network of Australian Genetic Resource Centres. The Centre is strategically important to Australian agriculture, being an integral component of national pasture plant improvement. The material is of immeasurable value with over 90% of accessions being unique to the Centre, many of which are irreplaceable following the desecration of their native environments (Hamilton *et al.*, 2000).

The AMGRC temperate pasture legume germplasm collection contains over 44,700 accessions representing 326 genera and 1,509 species. This includes the Worlds largest collection of *Medicago*, with over 27,602 accessions representing 73 annual and perennial species from 84, predominantly Mediterranean, countries. Also housed at the Centre are comprehensive collections of lesser known but increasingly important genera including *Lotus*, *Hedysarum*, *Melilotus*, *Onobrychis*, *Astragalus* and *Trigonella*. More recently the AMGRC has been actively supporting the development of native Australian species, and as a result has acquired a substantial collection of native fodder species, especially *Cullen*, *Atriplex*, *Swainsona*, *Lotus*, and *Trigonella* species.

2.1.1 Resources and Activities

The AMGRC has both national and international responsibilities and is responsible for the collection, introduction, quarantine, characterisation, preliminary evaluation, multiplication, storage, regeneration, documentation and distribution of mandated genera.

The *Medicago truncatula* collection.

Australia's contribution to the conservation of annual *Medicago* germplasm has arisen as a result of the recognition of the value of annual medics in legume-ley-farming systems (Crawford *et al*., 1989). Annual legumes are used extensively in temperate Australia as a means of increasing soil fertility, combating herbicide resistant weeds, and as a disease break. Annual species of the genus *Medicago* are better adapted than other annual legumes to the extensive calcareous soils of Southern Australia (Crawford, 1970). The barrel medic, *M. truncatula* Gaertn, is estimated to be grown on over 4.5 million hectares in Australia (Hill and Donald, 1998).

As a result of 128 collections and donations over a period of 58 years, *M. truncatula* is represented by 5,509 accessions from 40 countries. This includes 4,517 wild accessions of the three morphological varieties described by Heyn (1963), a core collection of 231 accessions, 788 breeding lines, 18 cultivars, 5 mutants, 2 landraces, 175 recombinant inbred lines. The

collection also contains 2,809 and 857 accessions respectively of the closely related species *M. littoralis* and *M. tornata.*

Propagation and storage

Between 1968 and 2004, 4,423 lines of *M. truncatula* were multiplied and characterised in nursery rows. These are currently being maintained to International standards. This involves slowly drying clean seed at 15^{o}C and 15% relative humidity until the optimum moisture content of around 5-6 % is attained. Seed is then tested for viability and packaged in heat-sealed aluminium foil packets and placed in cold storage. A minimum of 8,000 seeds, or up to 15gm, is placed in the 'Base' store at -20^{o}C. Seed in the 'Base' collection is preserved for the long-term future and comprises as close as possible one generation of multiplication of the original seed sample. Seed is not distributed from this store and is accessed only to monitor its viability and as necessary to regenerate seed for the 'Active' collection.

At least 12,000 seeds or up to 60gm are placed in the 'Active' store at 2^{o}C. Seed in the 'Active' collection is preserved for medium term storage. Seed is routinely monitored and regenerated as necessary. In line with international convention, 100 seeds per accession are freely available to *bone fide* researchers throughout the world.

Larger quantities of seed are stored in the 'Residual' store at 12^{o}C and 30% relative humidity. This store enables the immediate supply of larger quantities of seed to national programs saving costs associated with one generation of multiplication. A small duplicate sample of seed is sent off site to CSIRO in Canberra as black box storage.

The value of the collection not only lies in the physical preservation of the resources per se but also in the acquisition, storage and ready retrieval of the initial data obtained in the nursery row stage of characterisation and evaluation (Auricht *et al*.,1999). The 4,423 lines of *M. truncatula* have up to 64 descriptors recorded against them, with all lines having at least 30 detailed descriptions. A descriptor list, which details both the morphological and agronomic characteristics recorded in nursery stage evaluation has been published by the International Plant Genetic Resource Institute (IBPGR, 1991). The characterisation and preliminary evaluation datasets are utilised by the AMGRC as taxonomic aids and by subsequent programs to identify workable numbers of genotypes that possess, or are likely to possess, desired traits. Important agronomic traits essential for ultimate cultivar selection include: seedling vigour; winter herbage production; flowering time; seed production; pod spininess (Fig. 1), changes in seedcoat permeability, herbicide tolerance and resistance to insects and disease (Crawford, 1983, Nair, *et al.*, 2002).

Fig. 1. Range of pod spininess in *M. truncatula*.

The AMGRC *Medicago* core collection

The core collection was developed from 20,997 annual *Medicago* accessions (Skinner *et al*., 1999a). A core collection is a subset of accessions representing the genetic variability in the whole collection, which enables the variation in large collections to be more efficiently utilised. The core collection of is composed of 1,705 accessions, of which 231 are *M. truncatula*, and represents 74% of the extremes of 27 characters (Skinner *et al*., 1999b). The core is dynamic, as accessions representing the extremes of newly evaluated characters are routinely added. The *M. truncatula* core has recently been refined to an inner core of 61 accessions that maximises genetic diversity based on microsatellite genotyping (Ellwood *et al* 2006).

The core data sets, characterisation, evaluation, passport, digital images and seed inventory data are maintained in a fully relational database and are available upon request. Passport information pertaining to accessions and summary statistics of the collection can be accessed online through the Australian Plant Genetic Resource information System (AusPGRIS). http://www.dpi.qld.gov.au/extra/asp/auspgris

Future Directions

The role of annual medics in Australian farming systems has been declining over the last 10 years. As a result, recent emphasis of AMGRC activities has been focussed on alternative annual species and perennial legumes, particularly lucerne and native Australian fodder species.

The decline in annual medics can be attributed due to a change in farming systems, driven largely by the need for perennials to restore hydrological stability and a decline in wool prices. As a result, industry resources supporting improvement in annual *Medicago* have been limited since 1997. This has resulted in 6,284 accessions in a backlog to be evaluated, including 1,086 accessions of *M. truncatula*. However, wool prices are improving significantly and for many regions of Australia, annual medics are the best pasture option. Industry support to reduce the backlog and create a better balance of resources spent on pasture improvement is anticipated.

The AMGRC and SARDI breeding programs are actively collaborating with the Medicago research community utilising *M. truncatula* as a model legume. Nationally this includes CSIRO and Murdoch University, and internationally INRA (France), John Innes Centre (UK) and the Noble Foundation (USA). These programs have a common requirement for the

conservation and access to germplasm and have approached the AMGRC to be the Asia/Pacific node for the long term conservation, multiplication and distribution of hundreds of mutant lines, recombinant inbred lines (RILS) and single seed descent germplasm generated by these Centres. Funding opportunities to support this activity will need to be explored.

In mid-2006 the AMGRC plans to have available for distribution to the international community, single seed descent germplasm of the Australian *M. truncatula* core collection. Seed stocks are currently being multiplied for a minimum of 3 generations. In 2006 the AMGRC will also multiply, conserve and distribute 175 RILS - Jemalong 6 (SA 37612) x DZA 315-16 (SA 37442) obtained from Dr. Jean-Marie Prosperi, INRA France). SARDI is also generating a Jemalong A17 x A20 mapping population (in collaboration with Dr. John Klingler, CSIRO, WA) for the national and international research communities.

Acknowledgements
The Centre acknowledges the contribution of the many countries from which the germplasm collection was obtained. The efforts of officials and farmers in these countries have enabled this material to become available either through direct collection or through assisting international efforts.

Finally we would like to acknowledge the additional financial support towards the activities of the AMGRC provided by the Grains Research and Development Corporation, Australian Wool Innovation Pty Ltd and the CRC for Plant Based Management of Dryland Salinity.

2.2 USDA, ARS National Plant Germplasm System

2.2.1 History

The USDA National Plant Germplasm System (NPGS) germplasm collections date back to 1898, when the USDA Section of Seed and Plant Introduction was established and charged with collecting and introducing foreign crop germplasm for the benefit of U.S. agriculture. Today, the agency houses over 450,000 accessions representing 11,682 plant species (USDA, ARS, National Genetic Resources Program 2006). With few exceptions, our germplasm is freely available to the world (for further information see http://www.ars-grin.gov/npgs). The *Medicago* collection contains 8100 accessions representing 78 species from 93 countries (USDA, ARS, National Genetic Resources Program 2006). Most of this germplasm represents cultivated species, both improved and unimproved types. Bauchan and Greene (2002) provide a list of collection trips that have been made for alfalfa and other *Medicago* species from 1898 to 2000. *Medicago truncatula* was first received in 1950, when a commercial strain and 9 unimproved samples were received from J.R.A. McMillan from the University of Sydney, New South Wales, Australia. Currently the collection includes 324 ecotypes of *M. truncatula*, originating from 22 countries. Over 50 % of the accessions were collected in Algeria, Morocco and Tunisia. A collaborative collection was made in 1973 in Morocco by I. Forbes, from the USDA, ARS, Georgia, USA, and J.S. Gladstones, from the Department of Agriculture, South Perth, Australia. W. Graves, from the University of California, California, USA, made extensive collections in Morocco and Tunisia in the early 1980s. Approximately 55 % of the collection was received in the late 1980's, when the NPGS inherited the germplasm collection of the late Canadian taxonomist, Dr. Karlis A. Lesins. Most of the accessions (80 % of collection total) in the *M. truncatula* collection are unimproved or wild

germplasm. 15 % of the collection is either cultivars or breeding material. A large portion of the cultivated germplasm has been obtained from Australia.

2.2.2 Resources and Activities

The NPGS Medicago collection is housed and distributed from the Western Regional Plant Introduction Station located in Pullman, Washington. Distribution seed lots are stored at 4 ° C and 30 % relative humidity. Original and regeneration seed lots are stored at – 10 ° C. The germplasm is increased at the National Temperate Forage Legume Germplasm Resources Unit, in Prosser, Washington. Transplants are started in the greenhouse each spring. To minimize genetic change during the seed increase, 50-100 individual plants are used. For cross-pollinated species, individual accessions are covered with isolation cages, and leaf cutter bees (*Megachile rotundata*) are added as pollinators. Although *M. truncatula* is a self-pollinated species, it is also covered with isolation cages, since a small but genetically significant level of cross pollination has been suggested (Bonnin *et al*., 2001). Our goal is to preserve the genetic diversity inherent in collected germplasm. Although most of the annual medic species are self-pollinated, they are surprisingly heterogeneous, and we treat them as populations. To better support genomics research on, we are exploring the development of inbred lines for M. truncatula accessions that are unique to the USDA collection.

Characterization and evaluation have been important activities carried out on the Medicago collection. Since 1981 approximately one third of the perennial *Medicago* collection has been evaluated for 13 diseases, seven insects, chromosome number, 27 agronomic traits, seven feed quality traits and five abiotic stress tolerant traits (Bauchan and Greene 2002). Evaluations were carried out using standard tests developed by research scientists and approved by the North American Alfalfa Improvement Conference to evaluate new varieties. A description of the Standard Tests can be found at the North American Alfalfa Improvement Conference web site (http://www.naaic.org). The annual medics have also been evaluated for agronomic and morphological traits. Bauchan and Greene (2002) list the traits and percent of collection evaluated for each annual species. In 2003, we carried out an extensive morphological evaluation of the entire *M. truncatula* collection. Our objectives were to characterize the length of growth stages, as well as the morphological features of leaves, branches, flowers and fruit for each accession. Table _ and __ lists the traits evaluated.

Table 1. Growth stage evaluated for 323 accessions of *M. truncatula* in 2003.

Growth Stage	Number of days from sowing to:
1.0 Cotyledon	Fully expanded cotyledon
2.0 First leaves	
2.1 Unifoliate	First unifoliate leaf
2.2 First trifoliate	First trifoliate leaf
2.3 Second trifoliate	Second trifoliate leaf
3.0 Vegetative	Start of vegetative growth period
4.0 Flowering	
4.1 First flower	First plant with open flowers
4.2 Last flower	First plant with dried flowers
5.0 Fruiting	
5.1 Green pod	First plant with full green pod
5.2 Brown pod	First plant with brown pod
6.0 Senescence	90% of plants dead

Table 2. Morphological traits evaluated for 323 accessions of *M. truncatula* in 2003.

Cotyledon width	Flower length
Cotyledon length	Calyx length
Center leaflet width	Fruit diameter
Center leaflet length	Pods per plant
Leaflet mark position	Longest spine
Leaflet mark shape	Spine adpression
Leaflet mark color	Density of hair
Leaflet widest point	Fruit Peduncle
Number of basal branches	Subtending Peduncle
Branch length	Number of fruit coils
Stem diameter	Pods per plant
Base to third internode	Dry weight
Flowers per peduncle	

Evaluation data on individual accessions can be found in the Germplasm Resources Information Network (GRIN) for perennial species (http://www.ars-grin.gov/cgi-bin/npgs/html/crop.pl?68) and annual species (http://www.ars-grin.gov/cgi-bin/npgs/html/crop.pl?145)

Seed from the *Medicago* germplasm collection is available for distribution. Fig. 2 shows the number of seed packets that have been distributed in the last 5 years. GRIN (http://www.ars-grin.gov/npgs) provides a public website where users can find out what germplasm is available and request seed. GRIN also contains information on the accession origins and evaluation data. For further information and help using GRIN please contact the collection curator (greenes@wsu.edu).

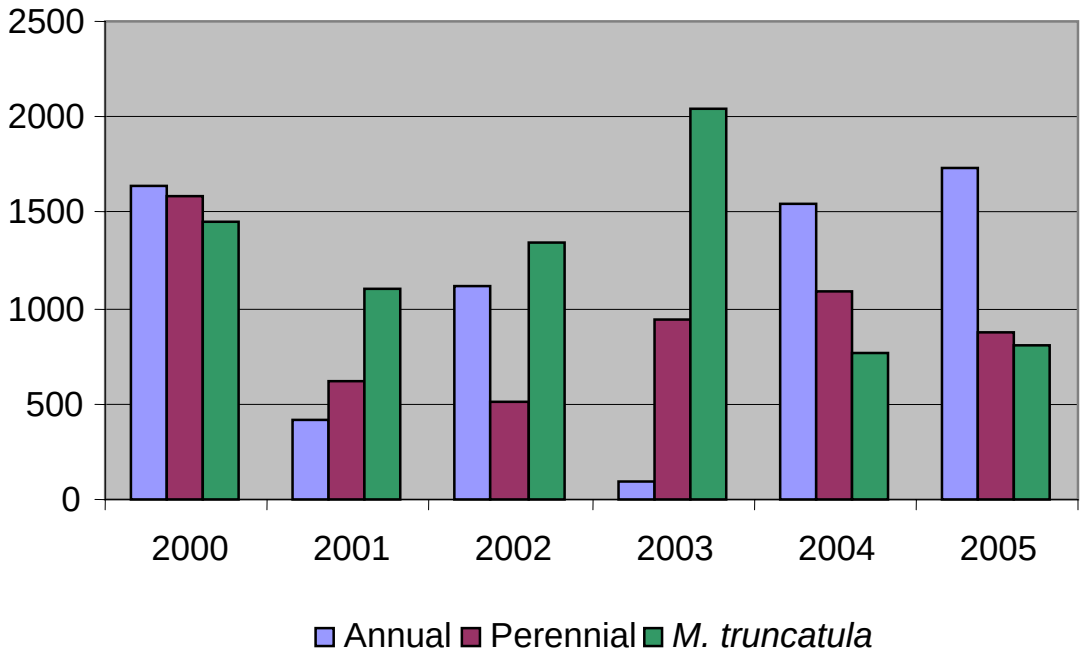

Figure 2. The number of seed packets of *Medicago* germplasm distributed in the last five years at NPGS, USA..

2.3 INRA France

2.3.1 History

The French National Institute for Agricultural Research (INRA) has large collections of genetic resources of agricultural crops and model species. The *Medicago* gene bank is located in Montpellier in the South of France on the campus of Montpellier Higher National Agronomic School, which is an integral part of the international Agropolis complex, comprising approximately 2000 scientists working in the field of Mediterranean and tropical agronomy, as well as 18 higher education and research establishments.

Since 1985, the Plant Breeding laboratory of Mediterranean crop forage has lead several surveys around the Mediterranean Basin (France, Algeria, Greece, Spain and Portugal) in order to collect natural populations of *Medicago* species. 3000 populations were thus collected, representing forty species of the genus *Medicago* (Prosperi *et al*., 2001).

The *Medicago truncatula* collection is currently organised and managed in a way to answer the needs of scientists in the domain of genomic researches, by offering several types of materials: firstly natural populations (i.e. plants collected on one site and including a large range of genetic variability); secondly fixed lines obtained by selfing individuals from populations in greenhouses (at least two cycles of selfings); and thirdly lines issued from mutagenesis programs.

The French *Medicago truncatula* collection contains more than 800 populations, of which some were introduced from the core collections of SARDI (Australia) and ICARDA (Syria), resulting in the second largest collection in the world after the Australian one.

According to the intra-population diversity one or more inbred lines have been created from each population. This results in the largest collection of inbred lines in the world with more than 900 inbred lines in 2005.

Some specific accessions such as manual crosses and recombinant inbred lines are also created for scientific purposes.

Large mutagenesis programs have been developed for *M. truncatula* by INRA and CNRS (National Center for Scientific Research). Material has been obtained using three techniques: gamma irradiation, mutagenesis using t-DNA and mutagenesis using transposon Tnt-1 of tobacco. More recently a program for production of mutants using the Tilling procedure has been developed. All these programs of mutagenesis are run on the referent accession ‘A 17’ (inbred line extracted from the Australian cultivar Jemalong). At present 1,750 lines are available: 200 lines from Tnt-1 transposon, 50 lines from gamma-irradiation and 1500 lines from t-DNA transfert. These programs for the production of mutants will develop, most notably, during the European FP6 program “Grain legumes” which will support the production of 9 to 10,000 Tilling lines and about 8000 Tnt1 mutant lines will be placed at the disposal of the wider scientific community.

2.3.2 Resources and Activities

INRA is in charge of introduction, creation, characterization, primary evaluation, multiplication, storage, distribution, regeneration and documentation of all seeds of *Medicago truncatula* collection. As part of Center for Biological Resources, the functions of production and characterization of the diversity are separated from the functions of distribution and management. Production and characterization of the natural diversity are under the

responsibility of Montpellier laboratory, while Dijon is in charge of such functions for mutant lines. Distribution and management of information (web-site) are under Montpellier's responsibility.

Resources: the *Medicago truncatula* French collection

Table 3. The French *Medicago truncatula* collection (by the end of 2005)

	M. truncatula	*M. littoralis*	*M. italica (tornata)*	*Other species*
Cultivated varieties	*16*	*2*	*3*	-
Natural populations	*638*	*174*	*21*	-
Inbred lines	*720*	*174*	*18*	*32*
F2 populations	*461*			
Rils	*5 populations of about 200 lines*			
Mutant	*1750*			

Cultivated varieties are mainly Australian. Only one cultivar 'Salernes' is a French variety. It is mainly used in the Mediterranean vineyards to prevent erosion caused by rainfalls.

Natural populations: One population does not refer to one inbred line but to a sample of pods collected from one site, in most cases a set of different lines. A variable level of polymorphism remains in each population as observed through several experiments carried out on natural populations (Bonin *et al*., 1996a; Bonin *et al*., 1996b; Bonin *et al*., 1997; Bonin *et al*., 2001). Collecting methodology is described in chapter "Wild accession / populations". Populations have been collected by INRA Montpellier in France, Spain, Portugal, Greece and Algeria.

Half of the **inbred lines** currently available in this collection come from the northern parts of the Mediterranean Basin (France, Spain, Portugal, Italy, Greece and some Mediterranean Islands) and the 'Maghreb' are well represented (mainly Algeria). Lines from Orient belong to Egypt, Iran, Israel, Jordan, Libya, Turkey and Syria (Fig. 3).

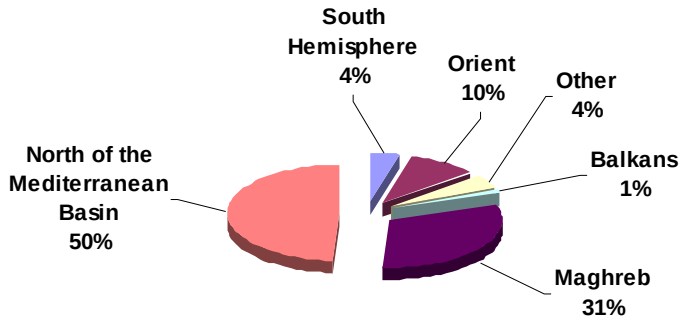

Fig. 3. Origins of the inbred lines at INRA, France.

We tried to quantify the neutral genetic diversity available in a subset of 346 unique lines from our collection with 13 microsatellites markers and test for the presence of an underlying population structure in broad sample. Results from these analyses were used to generate a set of nested core-collections for *Medicago truncatula*. This set of inbred lines

represents the bulk of the diversity segregating in *M. truncatula* and are publicly available. The nested core-collections consist in 8, 16, 32, 64 and 96 accessions [Ronfort *et al*., 2005 *submitted*]. These core-collections will help organize and coordinate current efforts in the *Medicago truncatula* community to study patterns of variation at both the phenotypic and the molecular levels.

Recombinant Inbred Lines (RIL):
Montpellier INRA is responsible for the multiplication and worldwide distribution of five Recombinant inbred lines:

LR 1: DZA 315-26 x DZA 45-6

LR 2: DZA 45-5 x DZA 315-16

LR 3: F83005-5 x DZA 45-5

LR 4: Jemalong 6 x DZA 315-16

LR 5: Jemalong 6 x F83005-5

Many other F_2 populations exist. Specific crosses and new RIL have also been created to work on specific themes such as flowering time (5 new RIL).

We continue to create new manual crosses involving the international referent line A17 and develop F_2 populations to be able to quickly start new recombinant inbred lines is necessary.

Concerning natural diversity, the 2005 INRA collection consisted in 833 populations, 944 fixed lines, more than 500 progenies at the F_2, F_3 or further generations, and 3 populations each of 200 RIL at generation F_6 to F_8. In the long term, the collection will include 1500 populations and more than 3000 fixed lines.

Mutant lines:
TILLING is a reverse genetic strategy to identify mutations throughout a genome and a screening method that facilitates localization of these mutations. The European *Medicago truncatula* TILLING project is funded by the GLIP (EU-Grain legumes Integrated Project). TILLING populations have been generated by INRA- URLEG (Grain Legume Genetics and Ecophysiology Research Unit) in Dijon. The establishment of a high-throughput TILLING service is a collaborative effort between the INRA-URLEG lab in Dijon (France) and the Genome Lab in Norwich (UK).
A first population "EMS1" of 4500 M_2 raised from 500 M_1 was available with DNA extracts in 2004. A second population "EMS2"of 4500 M_2 plants raised by single seed descent from M_1 plants is now available in the EU-GLIP project (seeds and DNA lots harvested in 2005). The first screenings are underway in INRA-URLEG and in the JI Genome lab in Norwich, accessible in priority to GLIP partners (http://www.eugrainlegumes.org).

http://tilling.fhcrc.org:9366/files/TILLING_publications.html for a list of TILLING-related publications.

Concerning mutagenesis programs, 9 to 10 000 Tilling lines and about 8000 Tnt1 mutant lines (Collaboration with ISV-CNRS, Gif sur Yvette, France) produced during the FP6 program "Grain Legumes" will be placed at the disposal of the wider scientific community.

Multiplication and identification

Cultivated varieties and **populations** are multiplied on 1m² boxes in greenhouses with fertilisation and isolation. Minimum number of plants per population is 32. This size allows maintaining diversity within the populations. Each population is identified by a bar code stating its accession label, species and year of production.

Inbred lines (and **RIL**) are multiplied as isolated plants. A first selfing is realized to produce the conservation stock, and a second one leads to the production of the distribution stock. Each line is identified by a bar code stating its accession label, number of selfings and year of production. The plant used for production of conservation stock is genotyped with 13 microsatellites. The leaves, pods and seeds are scanned. The genotype and the morphological description are verified on each distribution stock produced.

Morphological descriptions are based on IPGRI recommended list of descriptors for passport data, vegetative, inflorescence and fruit description or further evaluation (see chapter "Phenotyping").

Storage and distribution

Seeds from conservation and distribution stocks are stored in cold chambers at 5°C and 30% relative humidity (active stock). A sub-sample of the conservation stock is kept at -20°C (long term conservation). The conservation stock is only used for producing the distribution stocks. Distribution stock is shared into small samples used for experiments and for distribution to the scientific community. Quantities of seeds commonly sent are 25 seeds per lines and 250 seeds per population. Viability is regularly tested on both conservation and distribution stocks of seeds.

The collection is already being widely distributed to the international scientific community, with more than 1500 samples sent per year over the past six years. Fig. 4 below shows the number of accessions that have been distributed over the past 6 years.

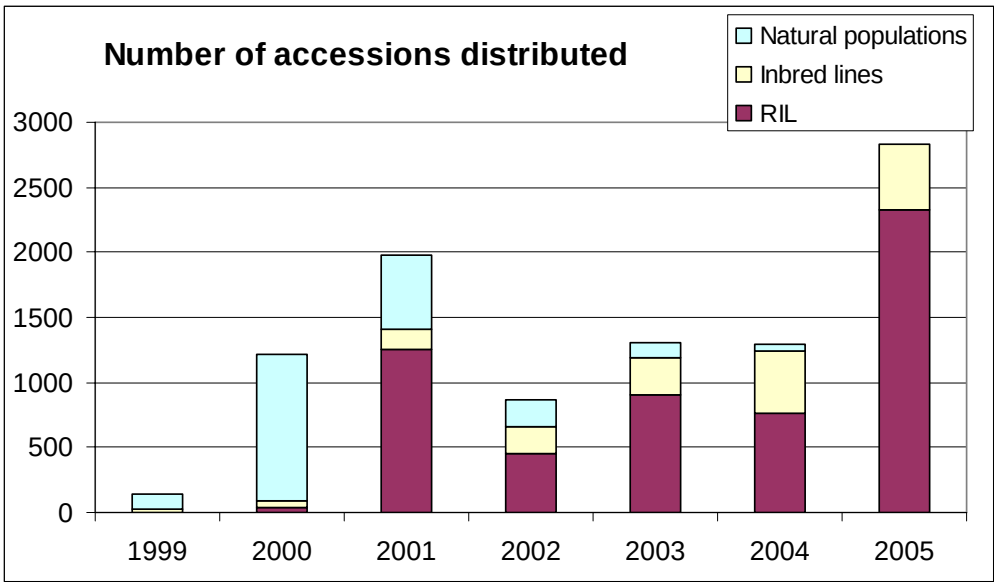

Fig. 4. Number of accessions distributed over the past 6 years by INRA, France.

Future directions

The emphasis is put on a larger use of *Medicago truncatula* as a model species in the international scientific community. This enforces the collaboration between the main collections (SARDI and CSIRO -Australia, Noble foundation-USA, John Innes Centre- UK and INRA- France) in sharing the responsibility of maintaining huge collections of natural and induced diversities and defining common requirements for conservation as well as for description and distribution of germplasms throughout the continents. In this system Montpellier INRA could act as the responsible center for the maintenance of the natural diversity and as a portal for shipment to Europe of the material coming from the natural diversity and the mutant lines produced in France, as shown in the Fig. 5 below.

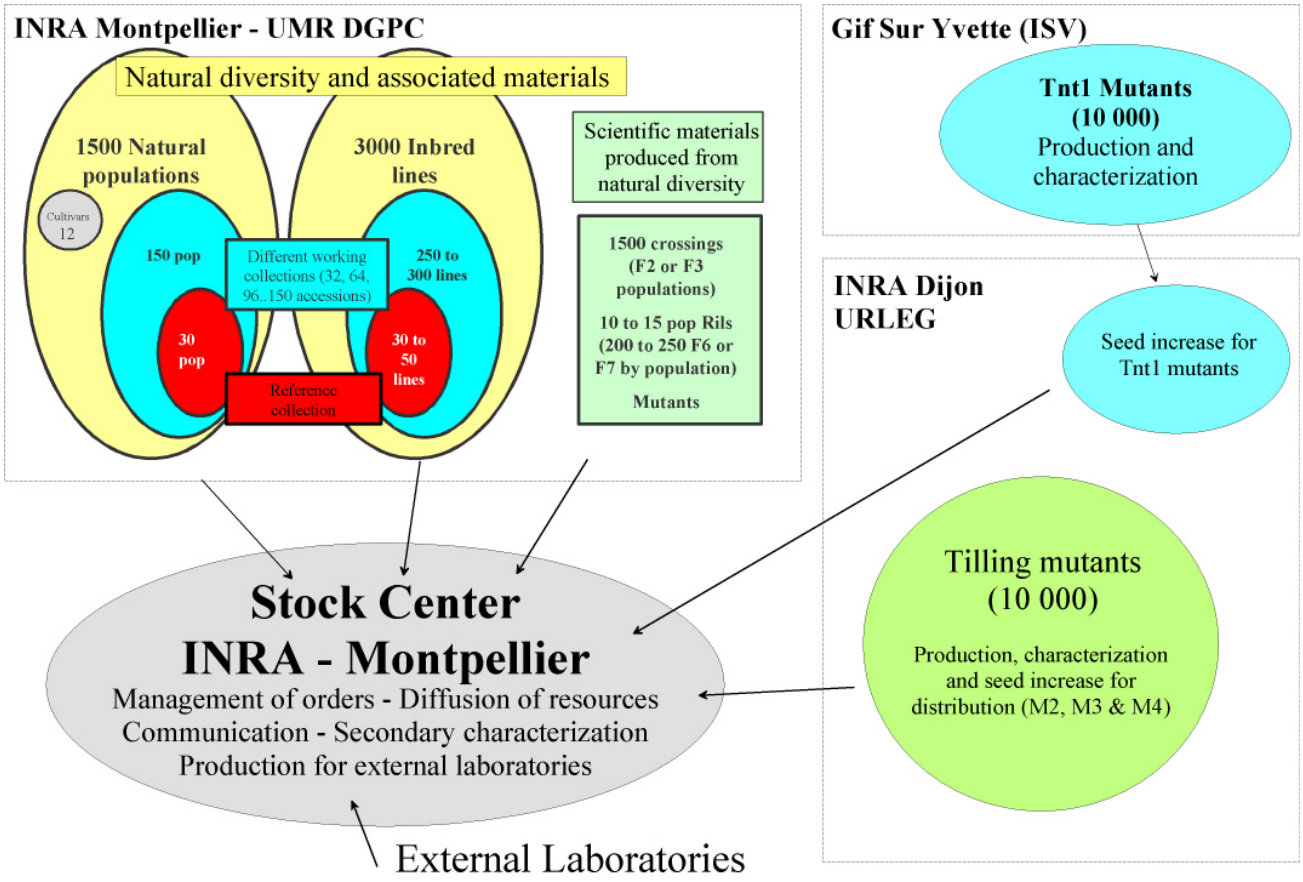

Fig. 5. Overview of INRA activities, France

A website presenting the Biological Resource Center, materials available and ordering on line is being created and will be available by end 2006.

We have also proposed for the official Biological Resource Center accreditation for the *Medicago* stock center. In France, quality control assurance is of highly importance and we think such an argument would enhance the value of the service provided.

Acknowledgements

The Center acknowledges the contribution of the many countries from which the germplasm collection was obtained.

2.4 Noble Foundation US

2.4.1 History

The Samuel Roberts Noble Foundation was founded in Ardmore, Oklahoma, USA in 1945. Today, the Foundation's operations are conducted through three operating divisions — Agricultural, Plant Biology and Forage Improvement.

The Plant Biology Division, established in 1988, conducts basic biochemical, genetic and genomic plant research for plant improvement, enhancement of human and animal health and production of novel products in plants. The model legume *Medicago truncatula* is used as a major plant material in most groups within the Plant Biology Division. A Center for *Medicago* Genomics Research was established within the division in the fall of 1999. Since then, a large scale EST population has been generated, sequenced and deposited to the TIGR EST databases. In 2000, the Noble Foundation made a $5 milllion grant to the Center for Advanced Genome Technology at the University of Oklahoma, Norman, to initiate the sequencing of the *M. truncatula* genome.

Researchers at the Noble Foundation have taken a global approach to functional genomics studies in *M. truncatula*. Collectively, these programs address the growth, development, environmental interactions and natural product biochemistry of *M. truncatula* and its related species alfalfa (*M. sativa*). The approaches employed include: large-scale EST and genome sequencing, gene expression profiling, the generation of *M. truncatula* mutant populations by fast-neutron bombardment and *Tnt1* retrotransposon insertion, and high-throughput metabolite and protein profiling. The resulting multidisciplinary databases developed in this program will be interfaced to provide scientists with an integrated set of tools to address fundamental questions pertaining to legume biology.

2.4.2 Resources & Activities

The *M. truncatula* collections at the Noble Foundation are housed and propagated on the Foundation's campus in Ardmore. The Foundation is responsible for both national and international distributions of its collections of molecular and biological materials.

Medicago truncatula EST clones

Since 1999, the Noble Foundation has generated a total of 129,696 EST clones from 14 distinct *M. truncatula* libraries (Table 1), accounting for more than 50% of the total ESTs from the *Medicago* research community. All these EST clones from the Noble Foundation were sequenced and the sequence information was deposited into the TIGR *M. truncatula* EST Database (http://www.tigr.org/tigr-scripts/tgi/T_index.cgi?species=medicago). These EST clones are stored as glycerol and plasmid stocks in the Plant Biology Division. Currently the EST clones are distributed free on request by signing a Materials Transfer Agreement (MTA) form. Since 2000, the Noble Foundation has sent out 322 EST clone requests to researchers in the United States and 158 EST requests to international researchers from 15 countries (Figure 6).

Table 4. *Medicago truncatula* EST clones and library distribution.

***Medicago truncatula* EST Library - Glycerol Stock**		
Library origin	**# of 96-well plates**	**Total number of clones**
Drought stressed	111	10,656
Elicited cell cultures	110	10,560
Flowers	104	9,984
Germinated seeds	110	10,560
Insects attacked	113	10,848
Irradiated	108	10,368
Leaves	113	10,848
Methyl jasmonate-treated	119	11,424
Nodulated roots	39	3,744
Phoma infected	97	9,312
Pi-starved leaves	120	11,520
Roots	35	3,360
Stems	120	11,520
Young stems	52	4,992
TOTAL	1351	129,696

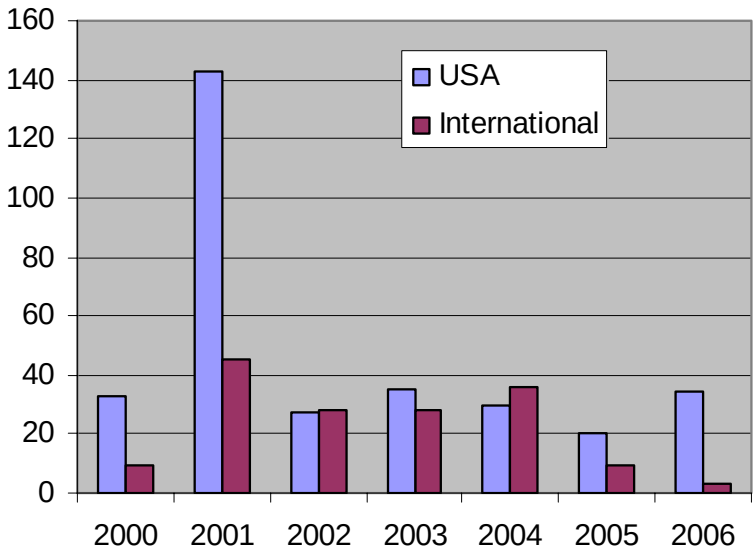

Fig. 6. *Medicago truncatula* EST clone requests from researchers in the United States and international community during 2000 to March of 2006.

Medicago truncatula mutant lines

Concomitant with the on-going *Medicago* genome sequencing project (see chapter "Bioinformatics resources for *Medicago truncatula*"), generation of loss-of-function mutants is of increasing importance in understanding functions of genes important for legumes. Due to a low transformation efficiency, generation of a large collection of mutants by T-DNA

insertional mutagenesis, as has been used successfullly for other plant species such as *Arabidopsis thaliana,* is not practical for *M. truncatula* at the present time. Three years ago, the Noble Foundation established an intramural project to generate large collections of deletion mutants in the Jemalong A17 background by fast-neutron bombardment (FNB), and insertion mutants in the R108 background by *Tnt1* retrotransposon mutagenesis. To date, we have collected M_2 seeds from more than 50,000 M_1 plants mutagenized by FNB with irradiation dosages ranging from 30 Gy to 40 Gy. A direct forward genetic screening was carried out to isolate numerous mutants exhibiting discernible shoot and root phenotypes, suggesting that the mutagenesis is effective (Wang *et al*., 2006). To develop a reverse genetic screening platform, a three-dimensional pooling strategy is being utilized to organize DNA pools and PCR-screens (Wang et al., 2006). Pilot experiments are currently being carried out to test the effectiveness of the screening strategy. In the next several years, genomic DNA templates will be prepared from all the mutant lines to allow the establishment of the reverse genetic screening platform. To increase the probability of recovering deletion mutants for genes of interest, a steady increase in the M_2 population size will be made in the coming years. The reverse genetic screening platform developed at the Noble Foundation is coordinated with Giles Oldroyd's group at the John Innes Center, Norwich, UK, which is developing a similar reverse genetic screening platform. Based on the mutation frequencies observed in other plant species mutagenized by FNB, and a similar frequency of *albino* plants observed, we estimate to have more than 500,000 deletion events in the current population.

Tobacco *Tnt1* is one of the best characterised plant autonomous LTR retrotransposons (Grandbastien et al., 1989). Our collaborator, Dr. Pascal Ratet at INRA, France, first introduced the *Tnt1* retrotransposon into *M. truncatula* R108 by *Agrobacterium*-mediated transformation (d'Erfurth et al., 2003). We obtained a transgenic line that harboured three *Tnt1* insertions from Dr. Ratet. From this transgenic line, we regenerated more than 2,000 independent lines between 2003 and 2005 (Tadege et al., 2005) and plan to regenerate ~1,000 additional lines each year for the next five years. Among the 2000 currently regenerated lines, there are on average 15-20 *Tnt1* insertions in each regenerated plant, totalling 30,000-40,000 *Tnt1* insertions in the *M. truncatula* genome. TAIL-PCR is being used to recover most of the flanking sequences of the *Tnt1* insertions. The ultimate goal is to create a web-based, publicly accessible *M. truncatula* mutant database with associated flanking sequences for the worldwide research community.

The *Tnt1* insertional mutants will be made available to the scientific community for collaborative screening. Scientists are welcome to participate in the annual mutant screening events at the Noble Foundation as long as their interests are complementary with ours and the screening assay is non-destructive. Unlike *Arabidopsis*, *M. truncatula* produces very little seeds, and seed production ties up an enormous amount of greenhouse space for long periods. At this time it is not feasible for us to distribute the whole set of mutant seeds to the scientific community. However, mutant seeds are sent to collaborators for free. Funding opportunities to support the broader distribution of mutant seeds to the community will be explored.

3. 1 International Integration of *M. truncatula* stock centres.

The *Medicago truncatula* research community is rapidly enlarging and this model legume is increasingly used to address a broad range of scientific questions. In reflection of this the genetic and genomic resources in *M. truncatula* are expanding and the demand for these resources is also expanding. The integration of worldwide *M. truncatula* stock centre

activities and the extension of these activities to include all *M. truncatula* genetic and genomic resources has been recognised as essential to meet the current and future needs of this research community.

As outlined in this chapter there are a number of activities currently underway that curate and supply a subset of the genetic and genomic resources in *M. truncatula*. While the activities of these centres are invaluable, they are not integrated and do not provide the full extent of resources available or in development. To better integrate and expand these activities a meeting was held at the John Innes Centre in the UK in October 2005. At this meeting it was agreed in principle that the groups currently involved in stock centre activities would work together to create a single virtual stock centre that integrated the diverse efforts around the globe. The current activities will be expanded to include single mutant lines, large mutant populations and the reverse genetic populations in development.

While the vision is for a single virtual stock centre the reality will be a diversity of stock centre activities across the globe. At the meeting in Norwich it was agreed that *M. truncatula* diverse lines and their associated RILs will continue to be curated by USDA, INRA and SARDI and these centres would work together to create common descriptors and common SSR markers for characterisation. Single mutant lines will be curated by the John Innes Centre and SARDI. The reverse genetic platforms will be curated by the groups currently developing them: INRA and the Noble Foundation will provide Tnt1 lines, INRA will provide the TILLING lines and the John Innes Centre and the Noble Foundation will provide fast neutron lines. It is hoped that large mutant populations or large amounts of single ecotype seed will be provided by a private company. In order to better facilitate the movement of *M. truncatula* seed internationally it was agreed that USDA would act as a portal for receiving material for the US, the John Innes Centre would act as a portal for Europe and SARDI would act as a portal for Australasia, analogous to similar roles played by the Nottingham Arabidopsis Stock Centre (UK) and the Arabidopsis Biological Resource Collection (US). The Plant Genomics facility in Toulouse will continue to provide genomic libraries, associated filters and single clones and the Noble foundation will continue to curate and supply cDNAs. Additional funding will be required to make the single virtual stock centre a reality and raising the necessary funds is the main challenge that the individual groups are currently facing. We believe that this single virtual stock centre will be a valuable resource for the plant scientific community and we hope that this vision will become a reality in the near future.

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