Wild accessions / populations

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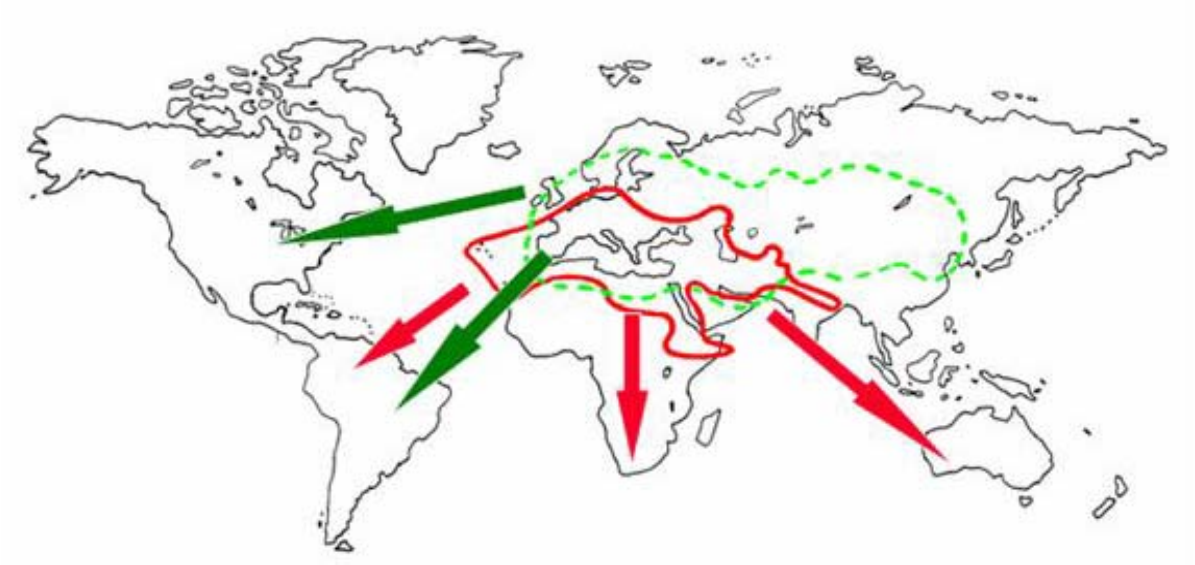
Introduction

The genus *Medicago* originates from the Fertile Crescent, covering the actual Turkey, Iran, Iraq, South of Caucasus and the circumference of the Mediterranean Basin. These species have conquered the whole Mediterranean countries and the neighbouring steppes (map 1). During XIXth century, they invade other parts of the world, in particular American and Australian continent in favour of several waves of colonisation (shown by the arrows on map 1).

Map 1: *Medicago* world distribution area.



Annual *Medicago* (medics) **Perennial *Medicago* (alfalfa)**

1. Wild accessions: representing the natural populations

About 10 out of the 55 *Medicago* species counted by Lesins & Lesins (1979) are cultivated and most of them are present in the pasture lands under Mediterranean climate. Accessions from center of origin and centers of diversity have been collected through many collections led by national or international research organisms.
The international collection effort through many years of collection, independently from the collector and the genetic resource centre in charge of its maintenance is given in Table 1.
A total number of 9453 have been listed through the four main collections (SARDI, INRA, ARS-USDA and LILM-CBBC): 5703 *M. truncatula*, 2887 *M. littoralis* and 863 *M. italica*. *Medicago truncatula* represent 60.4 % of 3 species collected whereas *M. littoralis* represents 30,9% of the collections and *M. italica* only 9 %.

Distribution among countries of origin and species is shown on table 1. These numbers do not consider accessions acquired by exchange between the different collections, but the only collected accessions.

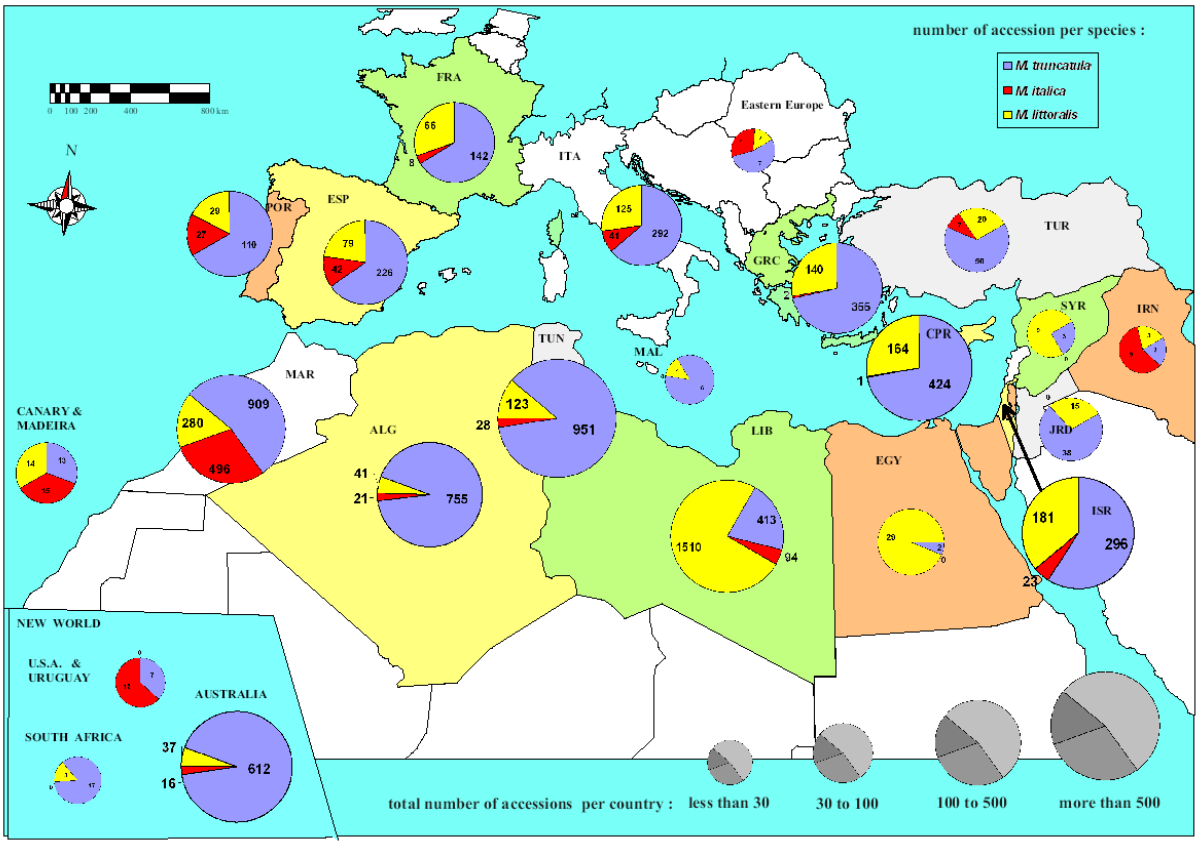
From now it is obvious that diversity coming from Libya, Morocco, and Tunisia is well represented (50.8% of the collected populations) whereas diversity from center of origin (and peculiarly South of Caucasus) and the North East of Mediterranean basin is under represented, as well as areas of late colonization (South Africa and America).

Representation of this distribution on a geographical map (map 2) shows evident differences of density of collection between areas of distribution. Size of pie-charts represented on the map is proportional to total the number of populations collected in each country. This map considers only populations belonging to the *Medicago truncatula*, *M. littoralis* and *M. italica* (ex *M. tornata*). Each species is represented by a color (*M.truncatula* is blue, *M. littoralis* is yellow, and *M. italica* is red).

Table 1: Distribution of the collected populations among countries of origin and species

COUNTRY	*M. truncatula*	*M. littoralis*	*M. italica*	total	percentage
Algeria	755	41	21	817	**8,6**
Tunisia	951	123	28	1102	**11,7**
Morocco	909	280	496	1685	**17,8**
Libya	413	1510	94	2017	**21,3**
Egypt	2	29	0	31	**0,3**
Jordan	38	15	0	53	**0,6**
Israel	296	181	23	500	**5,3**
Iran	3	3	9	15	**0,2**
Syria	3	9	0	12	**0,1**
Turkey	50	20	7	77	**0,8**
Cyprus	424	164	1	589	**6,2**
Malta	6	1	0	7	**0,1**
Greece	355	140	2	497	**5,3**
Italy	292	125	41	458	**4,8**
France	142	66	8	216	**2,3**
Spain	226	79	42	347	**3,7**
Portugal	110	29	27	166	**1,8**
Canary & Madeira	13	15	14	42	**0,4**
Turkmenistan et Azerbijan	3	5	3	11	**0,1**
Russia	1	0	5	6	**0,1**
Eastern Europe	7	2	4	13	**0,1**
Ethiopia	1	0	0	1	**0,0**
America	3	0	0	3	**0,0**
Australia	612	37	16	665	**7,0**
South Africa	17	3	0	20	**0,2**
Unknown	68	13	12	92	**1,0**
TOTAL	**5700**	**2890**	**853**	**9442**	100
percentage	60,4	30,6	9,0	100	

Map2: Distribution of collected *M. truncatula*, *M. littoralis* and *M. italica* by country of origin



Density of collection is not equal all around the Mediterranean Basin: 38.1 % of wild populations are native from **Maghreb** (Morocco, Algeria and Tunisia). These three countries provide the collections with 45.9 % of the whole *M truncatula* (MTR), 15.4 % of the whole *M littoralis* (MLI) and 63.2 % of the whole *M. italica* MIT. In this geographical area two points must be noted: *M. truncatula* is equally distributed over the three countries (15.9 % of the MTR comes from Morocco, 13.2 % from Algeria and 16.7 % from Tunisia) and at the opposite most of the *M. italica* comes from Morocco (with a total of 545 accessions of MIT out of a whole collection of 863, populations from Morocco represent 57.5 % of the wholly MIT collections).

Thirty-four percent of the wild populations come from 9 countries of **Eastern Mediterranean Basin** (north and South): Cyprus, Turkey, Iran, Iraq, Syria, Jordan, Israel, Egypt and Libya. From these 9 countries, Libya appears to be the most densely collected (21.3% of the whole collection comes from Libya, with an unbalanced ration of 7.2% of the MTR, 52.3% of MLI and 10.9% of the wholly MIT). Another point to be noted about is that 66.9% of the total MLI has been collected in the eastern Mediterranean Basin.

Six countries of the **Southern Europe** have provided 17.9% of the whole collection: Portugal, Spain, France (including Corsica), Italy (including Sicily and Sardinia), Greece (including Crete) and Malta. Over this area the proportions of the three species is quite balanced: 19.8% of the MTR, 15.2 % of the MLI and 13.9% of the MIT. Even if Greece and Italy is the most

representative for Southern Europe, they each represent less than 5% of the whole collection (458 accessions per each country over the three species considered).

Three other zones of origin can be commented upon: New world (Australia, South Africa and America) is not well represented with only 7.4% of the wild accessions originating from there. Another very poorly represented zone is the Eastern Europe to western Asia area (Bulgaria, Czechoslovakia, Hungary, Romania, Yugoslavia, Russia, Azerbaijan and Turkmenistan). The climate is of course less favorable to medics, but perhaps the collection effort has not been as important as in other countries? And at least, the Atlantic Islands (mainly Canary Islands) with less than 1% of the wild accessions, but with a notable equality between the three species considered (13 MTR, 15 MLI and 14 accessions of MIT).

Of course areas and climates are not similar between the geographical blocks considered, but the collection effort has not been the same everywhere and perhaps new collections could be considered by our community, possibly under the banner of the *Medicago truncatula* Stock Center?

2. Wild accessions / populations through the main world collections:

The main collections owning and maintaining natural diversity of *Medicago truncatula* and associates species participating in this chapter are:
AMGRC (Australian Medicago Genetic Resource Centre) ,
INRA (France, Institut National de la Recherche Agronomique),
ARS-USDA (Agricultural research Service,United States Department of Agriculture)
CBBC-LILM (Laboratoire Interactions Légumineuses Microorganismes, Centre de Biotechnologie, Technopole de Borj, Tunisia)
This chapter may be completed with other important collections in the second version.

This chapter aims to describe the collection effort of each institution and the peculiarity of each collection. First collection of wild populations in the world, AMGRC offers here many details of maintenance and multiplication of the collection. Unless otherwise advised this information is valuable for all collections.

2.1 Australian Medicago Genetic Resource Centre (AMGCR)

Objectives of the AMGRC

Each year to manage the AMGRC to International standards and continue the availability and effective utilisation of the germplasm collection to breeders both national and international.

AMGRC mission statement

To acquire, characterise, document, maintain, evaluate and distribute genetic resources of *Medicago, Lotus, Astragalus, Hedysarum, Melilotus, Onobrychis, Trigonella* and other pasture species adapted to saline and alkaline soils.

Responsibilities of the AMGRC

The AMGRC has State, National and International responsibilities.

a. State

- **To assist with the development of improved pasture legume cultivars for southern Australia through:**

- The provision of genetic resources of *Medicago* and associated forage species, which have been introduced from overseas either by collection or through exchange or other negotiation.
- The provision of taxonomic, morphological and agronomic data on these lines, which is collected at the Waite Research Precinct.
- Assisting departmental pasture researchers to plan and execute regional evaluation programs to ensure the best use is made of the collection.

- **To provide a permanent resource for future cultivar development programs through**:

- Storing and maintaining viable seed stocks of all mandate lines.
- Increasing the size of the collection to maximise its diversity and to conserve wild populations of mandate species under threat in their native environments.

- **To provide long-term storage for breeders seed of cultivars.**

  Thus the AMGRC provides a resource from which other pasture researchers develop cultivars following detailed regional evaluation and/or breeding, rather than directly undertaking these later stages of cultivar development itself. The continuing activities of the AMGRC are essential for the success of these other closely linked pasture research programs.

b. National and International

The functions of the Australian Network of Plant Genetic Resource Centres including the AMGRC were established by a working group convened by Standing Committee on Agriculture (SCA) to report on the conservation of plant genetic resources for the long term benefit of Australian agriculture. These functions were detailed at SCA 120 and are:

- **To establish comprehensive germplasm collections of each mandate species to meet foreseeable breeding requirements.**

- **To arrange the introduction and quarantine of germplasm in the mandate species.**
- **To supply material to breeders and other researchers in Australia and overseas.**
- **To establish close and personal contact with scientists working on the mandate species to facilitate a coordinated introduction program, keep them informed of new accessions to the collection and ensure maximum usefulness of the centre.**

- This includes establishing close liaison between the Centres, the International Plant Genetic Resource Institute (IPGRI) and national and international collections concerned with the same species.

- **To maintain, regenerate and document collections to internationally approved standards.**

- This includes storing of duplicates at a safe distance either in Australia or in cooperating international institutes.

Genetic resource centre activities

Figure 1 is a flow chart of the key activities undertaken by the Australian Medicago Genetic Resource Centre (AMGRC) from the initial collection and introduction of germplasm through to the selection, distribution and utilisation of germplasm for further research and development.

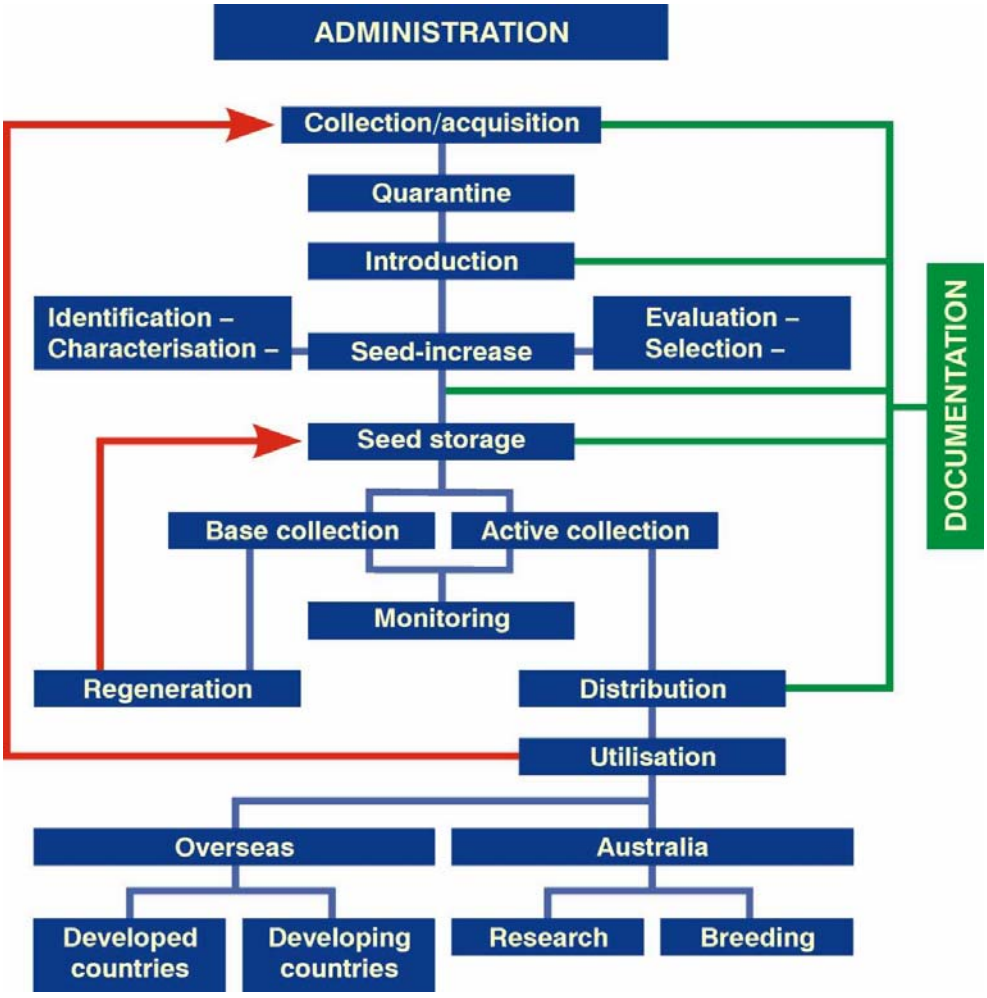


Figure 1: Flow chart of AMGRC activities.

GERMPLASM INTRODUCTION (collection / acquisition)

This activity is aimed to establish comprehensive collections of each mandate species to meet immediate or foreseeable breeding requirements either by collecting or targeted acquisition from International Genebanks.

The AMGRC collection has been assembled predominantly through direct collection of samples from natural centres of diversity. Other targeted accessions have been acquired either through receipt of seed from national breeding/evaluation programs or direct exchange with collaborative International Genetic Resource Centres

The collecting strategy adopted by the AMGRC for forage legumes has been variable over the last 50 years and could be categorised into 2 categories: Environmental and Species specific. Environmental collections have targeted specific environments and transects are conducted across regions within these environments and areas of environmental variation. The collection

will collect all variations in genera known to be pasture plants and occasionally new species unknown to agriculture.
Species specific collections usually target one or two species, occasionally genera and transects are made across environments where the species is known to occur and adjacent environments where the species may be expected to occur.
In practice there is an overlap between these 2 collecting strategies.

The sampling strategy aims to collect as many variants as possible in the target species. This involves maximising the environmental variation, the number of sites visited and plants collected within the time available.

The extent of environmental variation generally dictates the frequency of samples taken. In uniform environments, random samples are taken at 30 km (sometimes wider) intervals. In variable environments, samples are made more frequently.

The number of plants collected is determined by the frequency of the population at the collection site. As described by Marshall and Brown (1999), for annual *Medicago,* a pod from 100 plants is the optimal number required to represent 95% of genetic variability. However in reality, pod from 50 plants is more achievable. Although in many instances even this is not achievable and you can only collect all pod that is available.

In addition to seed, soil and associated root nodule bacteria are also collected. Comprehensive prevenance and habitat information is recorded for each accession collected and is maintained in the AMGRC passport database

QUARANTINE

This activity enables the Centre the capacity to conduct an efficient national plant import and quarantine facility to meet user requirements for temperate pasture legumes.

On arrival in Australia personnel accredited by the Australian Quarantine Inspection Service (AQIS) inspect accessions. The identity of samples is confirmed and any foreign soil or organic matter is removed and destroyed.

Upon meeting basic quarantine import requirements, germplasm to be introduced into the collection is cross-referenced with existing material for duplication. If the accession is unique it is then indexed with a genebank accession number prefixed with a "SA". Passport details are entered into the AMGRC database.

Germplasm with a Post Entry Quarantine (PEQ) requirement, eg *Medicago*, must be maintained and grown under AQIS accredited controlled conditions or be treated to AQIS approved standards.

GERMPLASM CONSERVATION

This activity involves the cleaning and storage of seed into the AMGRC cold rooms, consisting of an Original Seed and Base Store (-20degC), Active Store (2degC), Residual Seed Store (12degC) and a Plant Breeders Store of registered varieties (-20degC). These stores operate to International standards, minimise loss of viability and ensure the long-term preservation of the germplasm. It also involves off site black box storage, ensuring the safety of germplasm in the event of a disaster at the AMGRC.

Germplasm that has been introduced from a collection expedition or through exchange with International Centres are usually of low seed quantity. If not immediately required, this material is packaged in small heat sealed aluminium foil packets and placed in cold storage (-20degC) to await prioritisation for processing through quarantine, seed multiplication and associated activities at the Waite Research Precinct. Data on the accession, its quarantine status and number of seeds available is recorded and maintained in the AMGRC database.

Seed cleaning

Material harvested from the seed multiplication, characterisation or regeneration programs is stored in a bulk storage facility to await prioritisation for further processing. Processing is conducted in a specialised cleaning room equipped with a centralised dust extraction system that can be ducted to any piece of purpose built equipment, allowing a safe and comfortable working environment.

The AMGRC supports a wide diversity of different pasture and fodder species. This results in large differences in the shape and size of pod and seed. This has necessitated a range of specialised sorting and cleaning equipment. An experienced operator also inspects all lines under magnification. This activity is the final quality check, ensuring seed is true to type and free from contaminants.

Figure 2: Example of seed cleaning equipment.

Figure 3: Final stage of seed cleaning prior to storage.

Seed storage

Clean seed is placed in a drying room at 15ºC and 15% relative humidity for 2 to 3 months or until the seed has reached an optimum moisture content of around 5 to 6 %.

100 seeds of each line are then tested for viability prior to storage. Percentages of alive, dormant and dead seed are recorded onto the AMGRC database. Seed is then packaged in heat-sealed aluminium foil packets ready for placement into cold storage.

In line with international procedures a minimum of 8,000 seeds, or 15gms of each accession of annual *Medicago* are placed in the 'Base' store at -20oC. Seed in the Base collection is persevered 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 only accessed to monitor its viability and as necessary regenerate seed.

Figure 4: AMGRC Active Store.

At least 12,000 seeds or up to 60gm are placed in the 'Active' store at 2degC. Seed in the Active collection is preserved for medium term storage. Seed is routinely monitored and 1gram is made freely available for national and international distribution.

Larger quantities of seed, and for annual *Medicgao*, pod material, is stored in the 'Residual Seed Store' at 12degC and 30% relative humidity. This seed store supplements the 'Active' store when seed weights become critical and primarily serves the immediate supply of larger quantities of seed to National plant improvement programs.

Before a grant of an Australian Plant Breeder's Rights can be made on an application, the Plant Breeder's Rights Act 1994 states that propagating material of that variety must have been deposited for storage, in a genetic resource centre. In accordance with this requirement a current total of 77 commercial cultivars have at least 1kg of seed maintained at –20degC in the AMGRC PBR store.

In addition to the storage of seed within the AMGRC cold stores a one-gram seed sample is dispatched with associated data to the Commonwealth Scientific and Industrial Research Organisation (CSIRO) in Canberra, Australia for offsite duplicate or "black box" storage.

All storage seed inventory information is maintained on the AMGRC database.

GERMPLASM MAINTENANCE

Maintenance activities performed by the AMGRC involve the routine monitoring and regeneration of germplasm. Seed viability and seed numbers are the two principal factors monitored in order to maintain the genetic diversity and genetic integrity of material within the collection.

Monitoring

The health of the seed in the Active and Base collections is monitored at regular intervals of five to ten years respectively. When seed viability declines or seed resources fall below a critical level, the accession is flagged for regeneration.

Viability data is recorded on the AMGRC database. Monitoring of seed viability is an important and continuing function of the AMGRC.

REGENERATION (old germplasm)

There is always a need for the periodic regeneration of accessions and restocking of seed in cold storage. This is either due to loss in seed viability or to depletion of seed stocks as a result of use. Critical levels for both viability and quantity are established and when that level is reached, seeds are flagged for regeneration and then prioritised to be grown in a given year.

Accessions are planted under optimum agronomic conditions and regenerated. At least 50 plants are desired to maintain the genetic integrity of the line. Variation is monitored during regeneration to detect any genetic differences from the original characterisation datasets.

SEED MULTIPLICATION (New Germplasm)

The overriding objectives of this activity are to safeguard the germplasm for long term utilisation and to support the requirements of National clients by increasing the diversity of priority germplasm available for utilisation as a basis for cultivar development and associated research.

In collaboration with researchers in national and state pasture research programs, germplasm within the backlog of introductions, held as Original Seed, is prioritised to be grown for pure seed multiplication, characterisation, preliminary evaluation and taxonomic confirmation. The Original seed, increased for the first time, forms the basis of the AMGRC Active and Base stores, thus ensuring its long-term preservation.

National and State programs are also supported through the availability of priority true to type, high quality germplasm, free of contamination with associated technical information to enable informed decisions on the advancement of germplasm as the foundation for the development of improved cultivars and further research as required.

In addition detailed characterisation and larger seed multiplication plots are conducted as required.

The number of accessions grown in a given year is dependent on the resources available, the species targeted and the characters to be recorded

Additional Seed Increase

The AMGRC receives additional financial support from national breeding and molecular programs to supply them with large quantities of seed (beyond 1g) required to meet the needs of their research.

GERMPLASM CHARACTERISATION

The overriding objective of this activity is to document the diversity and range of variation within the germplasm. The data recorded provides the basis for selection of germplasm for further development. This activity is invaluable to breeding programs and enhances the effective utilisation of the collection.

All traits recorded are species and activity specific and importantly are determined in close collaboration with major breeders of the species and prioritised according to resources available.

Annual Medicago have up to 64 descriptors recorded against them, with all lines having at least 30 detailed descriptions. An Annual Medicago 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 the desired traits. Important agronomic traits essential for ultimate cultivar selection include: seedling vigour; winter herbage production; flowering time; seed production; pod spininess, changes in seedcoat permeability, herbicide tolerance and resistance to insects and disease (Crawford, 1983, Nair, *et al.*, 2002).

A core collection has been determined using 20,997 annual *Medicago* accessions (Skinner *et al*., 1999a). A core collection is a smaller subset of the large collection that is likely to contain accessions representative of the genetic variability of the collection. Its purpose was to improve and better utilise the data associated with the large AMGRC annual *Medicago* collection. Twenty-seven characters were utilised in assessing relationships based on evaluations carried out from 1968 and 1994. The result was a core collection of 1,705 accessions, including 231 *M.truncatula*, which represented 74% of the extremes of the 27 characters (Skinner *et al*., 1999b). The core is dynamic, as additional characters eg. insect resistance are evaluated, new accessions representing the extremes of these characters are being routinely added.

FIELD SITE DETAILS

The AMGRC characterisation and pure seed multiplication is conducted at the Waite Research Precinct and Turretfield Research Centre. The Waite land (Figure 5) occupies 3.5ha and is

located at the base of the Adelaide hills, approximately ten kilometers south east of central Adelaide, South Australia, GPS 34 58 56 S, 138 38 111 E.

Figure 5: Waite Research Precinct characterisation plots.

Topsoil at the Waite is uniform throughout, with the top 15-20 cm a red-brown clay-loam of pH 6.5 (H_20) on the surface dropping to around 6.0 below the first couple of centimetres. Below 20cm the pH rises back to 6.5 and the texture becomes heavier to medium clay. Rainfall is 600-800mm per year. The AMGRC also has 2ha of land based on Turretfield Research Centre located approximately 80km north of Adelaide, GPS 34 32 17.1 S, 138 50 22.1 E. Soils are a loamy red brown earth with a surface pH of 7.5 (water). Rainfall is around 400-600mm. Turretfield is largely utilised by the AMGRC for larger scale seed increase activities.

METHODOLOGY

The methodology adopted in characterisation and initial pure seed increase activity is well established and will vary annually according to the species grown and the objectives of the research.
Due to limited initial seed numbers and to maintain genetic integrity, 25-50 representative seed from each pod for each line of annual *Medicago* are grown as spaced plants, 15 cm apart in individual rows. These rows are all hand planted with seedlings, which have been pre-germinated in petri dishes and placed in the glasshouse in customised seedling cells germplasm. Annual *Medicago* are inoculated with the recommended rhizobial strain (AL strain for *M.littoralis* and *M.tornata* and AM strain for *M.truncatula* and other species).Common key steps in the seed multiplication/characterisation activity (Auricht *et al,* 1999) include the following:

- Selection of lines to grow. This is done annually in consultation with national programs. Lines to be characterised are prioritised from the backlog of material awaiting multiplication in the Original Seed database.

- Determination of characteristics to record (descriptors and descriptor states). The strategy adopted in recent years has been to target the characters to be recorded in consultation with researchers who have an interest in the material being grown in a given year. Examples include seedling vigour, winter herbage production (in comparison to relevant controls), time of flowering (providing suitable maturity period for given districts), grazing habit, low pod spininess (reducing vegetable fault in wool clips), seed size, ease of harvestability and seed production.
- Selection of comparators (control lines as standard benchmarks for analysis of preliminary evaluation data)
- Preparation of seed including scarification and counting.
- Acquisition and application of appropriate *Rhizobium* for each species. Supplied by SARDI rhizobiologists.
- Germination of between 50 and 100 seedlings in a germination cabinet.
- Transfer of germinated seeds to peat pellets.
- Maintenance of plants for six weeks in the glasshouse to ensure establishment.
- Paddock preparation, involving cultivation, rolling, laying and preparing plastic.
- Hand transplanting the plants to the field.
- Sowing cereal barriers to minimise contamination and the spread of virus' by insects.
- Maintenance of plants. Involves regular rouging, weeding, insect spraying and as required watering.
- Collection of data.
- Identification of off-types (mixtures) and as required the individual plant selection of desired traits within the population.
- Collection and pressing of herbarium specimens of selected accessions.
- Construction of controlled pollination cages for outcrossing insect pollinated species, eg Perennial Medicago.
- Placement and management of nucleus bee hives in individual cages
- Harvesting of pod (field), threshing of pod (lab).
- Machine and hand cleaning of seeds.
- Recording of pod and seed characteristics.
- Taxonomic identification or confirmation
- Storage of seeds. The material harvested from the characterisation program forms the basis of the genetic resource collection.

- The transfer of data onto the AMGRC database. Modifications are regularly required to accommodate for the specific characterisation and preliminary evaluation descriptors of different species.
- Analysis and reporting of results.
- The utilisation of the data collected. 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 the desired traits.
- Monitoring the progress of material.

DOCUMENTATION

The AMGRC has documented to the highest possible standard, passport data on all 5,509 accessions of *Medicago truncatula* currently maintained in the collection

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://www2.dpi.qld.gov.au/extra/asp/auspgris/
Currently AusPGRIS is only designed to meet general inquiries into Australia's germplasm collection and meet the requirements of politicians and decision makers. A strategy for the further development of AusPGRIS is currently being undertaken.

The core data sets, characterisation, evaluation, passport, digital images and seed inventory data associated with the collection are maintained in a fully relational database and are available upon request.

UTILISATION

This activity aims to supply seed to national and international researchers and assist national programs with the development of improved pasture legume cultivars for southern Australia.

Under current arrangements, one gram samples of seed and associated passport data are distributed free of charge for any legitimate research purpose to researchers throughout the world. Larger samples are provided to National programs through their provision of additional funding and can also be provided to other researchers under certain conditions and where sufficient seed stocks exist.

The AMGRC continues to support the requirements of national and international research and an average of 3,000 accessions are distributed each year.

Contact Details

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2.2 Institut National de la recherche Agronomique (INRA), France Biological Resource Center " *Medicago truncatula*"

Germplasm (collection / acquisition)
INRA wild populations' collection is based on a large program of collection mainly concentrated on the western part of the Mediterranean basin. In 1985, 12 collects were driven by INRA in Algeria, Spain, Portugal, Greece (including Crete) and France (including Corsica) and is rich of 142 populations of *M. truncatula*, 66 populations of *M. littoralis* and 8 populations of *M. italica*. The collections were dedicated to the Genus *Medicago*, so it was more than 3,000 populations that have been collected over these countries.

A wild accession does not refer to a line but to a population: a sample of pods collected from one site (50 to 500 pods are collected per population). So, these accessions include a variable level of within-diversity as we will see later. Moreover, several data are available for each accession: passport data, description of the site, soil characteristics, climatic data.

Germplasm availability:
INRA collection is the only one currently organised and managed in a way that can respond to the needs of researchers in the area of genomics, by offering a variety of materials: firstly populations (that is numbers of accessions collected on one site and including a large element of genetic variability); secondly, fixed lines that have been obtained by selfing individuals from populations though at least two generations in greenhouse (one of the most well-known inbred line is the international reference Jemalong A17); and thirdly lines derived from recombinant inbred populations (RILs).
These collections are being increased annually in order to improve the range of natural diversity of the species available mainly at the inbred line level.
Collections in 2005 consist of 800 populations, 700 fixed lines, 5 populations each of 250 RILs at generation F6 to F8, and a large number of F1 (resulting from manual crosses) between inbred lines (notably crosses involving the reference line Jemalong A17) and their descent in F2 or F3. The material is already being widely distributed to the international scientific community, with more than 1500 samples sent out per year over the past five years.

Seed production:

Production of populations in boxes under glasshouse: 80 plants per population are multiplied in order to avoid genetic drift.

Production of inbred lines: each plant is isolated in a cotton bag when beginning of flowering to avoid exchanges of pod and mixture when harvesting. The greenhouse is kept insect-proof to avoid crosses between lines.

Production of RILS: the single seed descend is made through a minimum of 3 selfing in small plugs embagged in polyethylene bags to avoid natural crosses and to allow individual harvesting.

Every accession (population, inbred line, SSD) is identified with a barcode.

Seed storage:
A hundred clean seeds of each line are tested for viability prior to storage. Percentages of alive, dormant and dead seed are recorded.
Clean seeds are kept in two locations: the active stock is in cold chamber (5°C – 30 to 40% HR), the long term stock in kept at -20°C.
Seeds and pods of the active stock are packaged in paper packets ready for placement into cold storage and are identified with a barcode.

Germplasm maintenance / monitoring
Accessions are characterised when multiplied under greenhouse. Some of the characteristics are also used to control identity through multiplications (foliage markers, earliness of flowering, pod coiling, micro satellites). Germination is checked before storage and randomly controlled during storage. Multiplication or regeneration is minded according to the inventory and the requests of scientific community.

Core collections:

A subset of core collections of inbred lines of *Medicago truncatula* (only var *truncatula* and var *longispina*) has been created (Ronfort *et al,*. 2006). The core collections were built with a stratified strategy using the genetic structure of an anonymous collection of 339 accessions based on allele frequencies of 13 micro satellites, eco-geography information and earliness of flowering.

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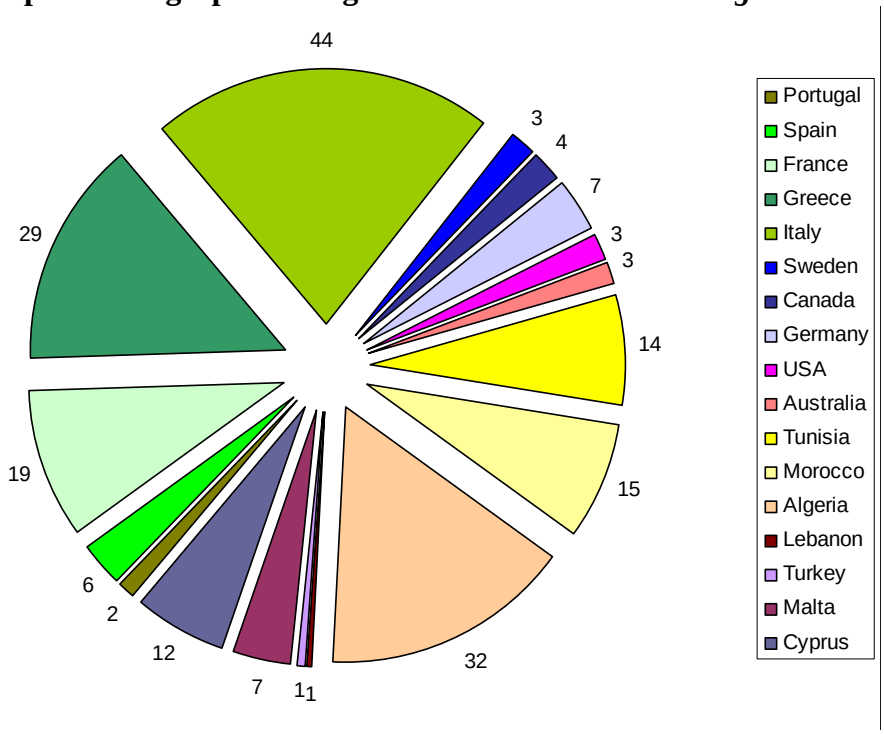
2.3 Agricultural Research Service (ARS) United States Department of Agriculture (USDA)

The USDA NPGS *Medicago truncatula* collection has 323 accessions collected or donated from the 1950's to present. Efforts are currently being made to expand our collection of significant genetic stocks and address geographic gaps in the collection. With the exception of 15 accessions that are either breeding material or cultivars, all accessions represented are wild populations. Accessions originate from 22 countries. Over 50 % of the accessions were collected in Algeria, Morocco and Tunisia. Sixty-two percent of our collection was once a part of Dr. Lesin's *Medicago* collection, which the USDA received from the University of Alberta in the late 1980's (graph 1). Twenty-four percent of the collection was obtained by US scientists during international collecting trips. Much of this material was collected in the wild, although some was given to collectors by donor institutes such as ICARDA, in Aleppo, Syria. About 10 % of the collection was donated by various institutes in Australia.

For most wild collected germplasm, passport data, including a description of the habitat and geographic coordinates of the collection site are included in the Germplasm Resources Information Network (GRIN) (http:/www.ars-grin.gov/npgs). Generally a large number of plants are sampled, although sampling strategies using transects or equalized pod harvest, are not routinely practiced. Collected germplasm is increased at Prosser, WA. A large number of plants are used to minimize genetic drift, and the plots are covered to prevent any cross pollination. Seed of the collection is freely available to support research efforts. We are currently developing a core subset based on morphological and molecular markers (micro satellites).

Graph 1 : Geographical origin of the Dr. Lesin's *Medicago* collection



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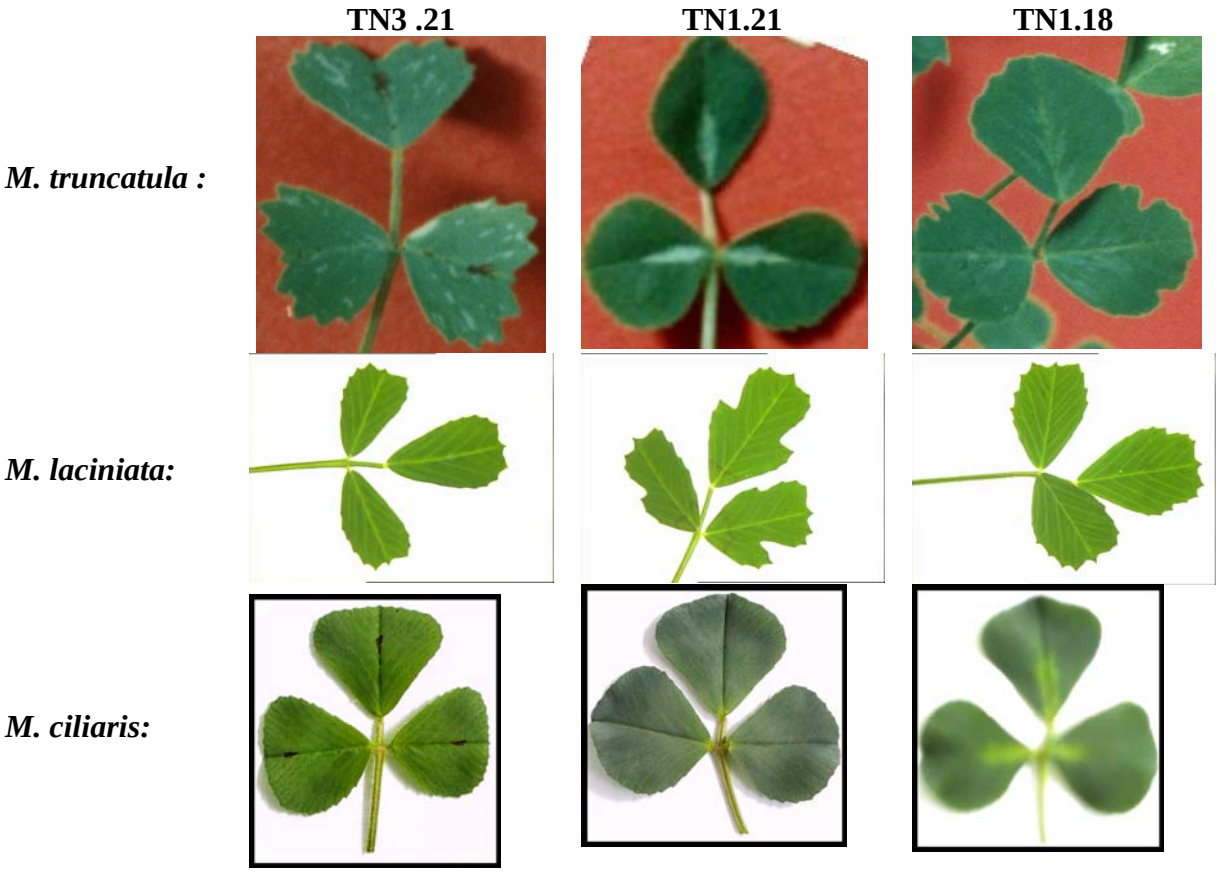
2.4 Laboratoire Interactions Légumineuses Microorganismes (LILM), Centre de Biotechnologie, technopole de Borj (CBBC), Tunisia

The LILM/CBBC *Medicago truncatula* collection has 184 pure lines created from 14 wild populations and covering the eco geographic Tunisian variability. This collection contains also 7 reference lines of diverse origins from Huguet's collection. These 184 lines were described using quantitative traits and micro satellite markers (submitted).
The LILM/CBBC collection contains also 100 *M.laciniata* pure lines and 48 from *M. ciliaris*.
Contrasting lines for agronomical traits of interest (symbiotic specificity, plant growth and development, tolerance to salinity and drought, resistance to pathogenic fungi) of *M. truncatula* and *M. laciniata* have been targeted and crossed. Recombinant inbred lines (RILs) and genetic maps are underway (under collaboration with Dr Thierry Huguet, ENSAT, France).

Example of leaflet marker variability available in tunisian populations of *Medicago truncatula*, *M. laciniata* and *M. ciliaris :*



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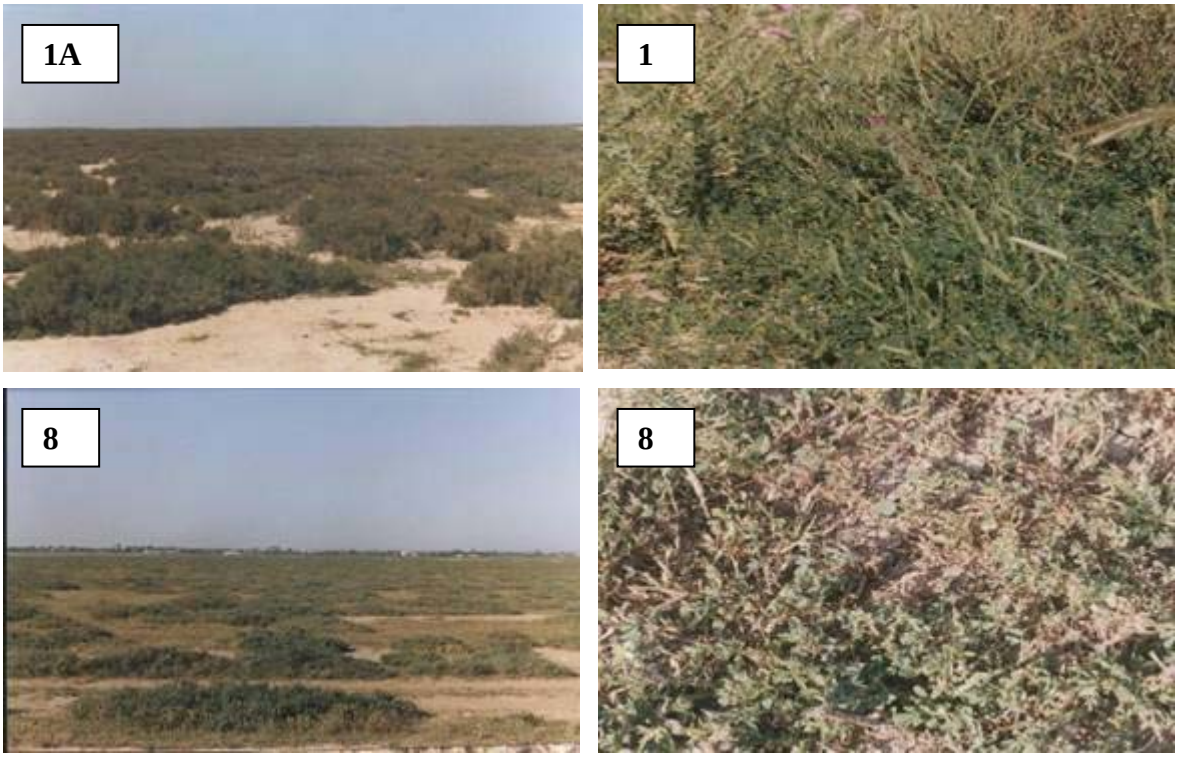
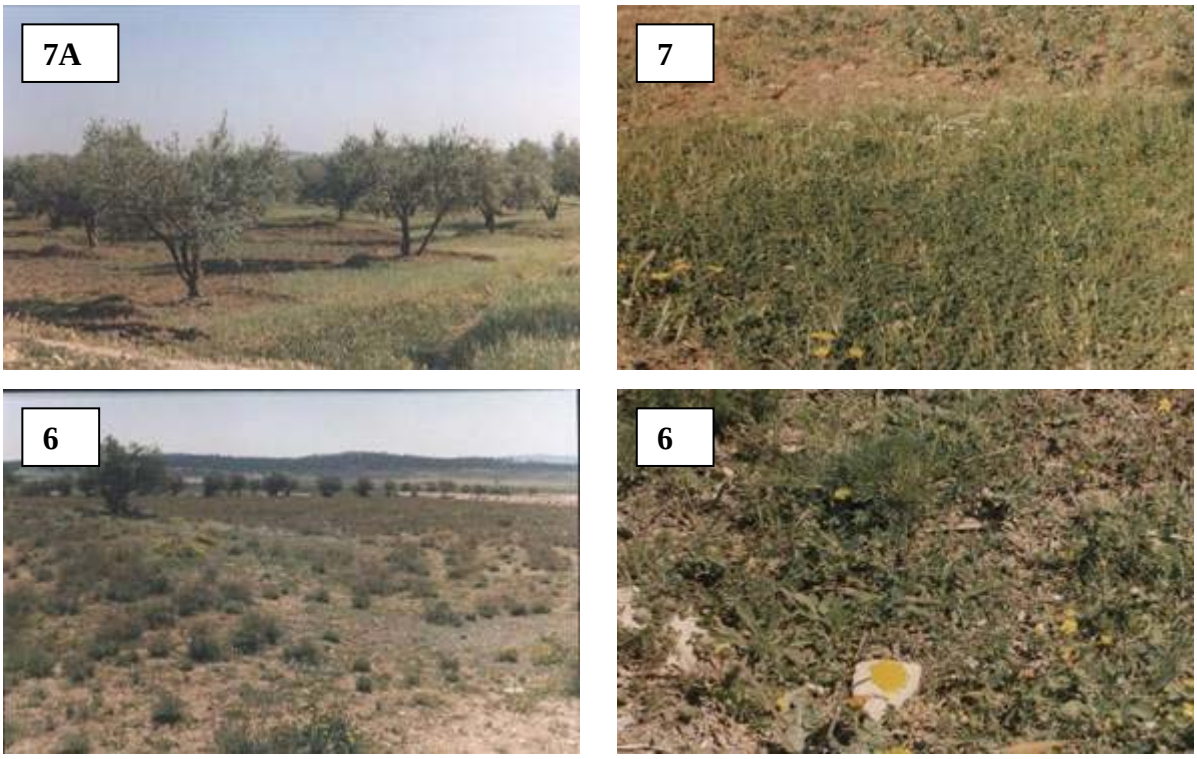
APPENDIX
Overviews of some sites of collection in Tunisia



<u>Board 1:</u> Salted zones in the semi-arid superior (8: Soliman) and inferior (1:Enfidha) Overview of the landscape (A) and non halophyte vegetation including *M. truncatula* (B)



<u>Board 2:</u> Mountainous zone in the semi-arid superior (7: ElKef) and inferior (6: Thala) Overview of the landscape (A) and herbaceous vegetation including *M. truncatula* (B)

Deguache Saharan Chott, Djérid

Bulla Regia – Sub humid zone

Rhayet , Sub humid zone

<u>Board 3:</u> Sub humid and Saharan site in Tunisia