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Australian genetic resources of Trifolium and Ornithopus species

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The introduction of improved pasture legumes into the cereal growing areas of southern Australia changed the face of our agricultural systems. Subterranean clover was the first pasture legume to have a major impact, and the first varieties were available commercially in the 1930s. Since then, many varieties have been released, leading to the most recent release of Denmark, Goulburn and Leura in 1992.

The development of burr, barrel and murex medics also had an impact on the pasture production of less acid heavy soils. Yellow serradella is still being developed for the more acid sandy soils.

This development of improved pasture legumes would not have been possible without breeders observing the distribution of plants and collecting seed of these exotic pasture legumes from Mediterranean countries and also those naturalised within Australia.

This germplasm is used in preliminary evaluation programs and is now stored in the safety of a genetic resource centre. Some of the germplasm is selected for use in breeding and field evaluation.

The Department of Agriculture is home to one of eight genetic resource centres in the nation. Its South Perth site houses the Australian Trifolium Genetic Resource Centre (ATGRC) to conserve germplasm, in the form of seed, of Trifolium and Ornithopus species. Much of this research is funded by the Wool Research and Development Corporation. The ATGRC is part of an international network of plant genetic resource centres to conserve germplasm of important agricultural plant species.
Russia led the way

The most famous collector of plant germplasm was the Russian geneticist Nikolai Ivanovich Vavilov (1887-1943). He was the first scientist who recognised the importance of the centres of origin in providing the greatest concentration of genetic diversity of cultivated plants, and he personally collected about 250,000 plant accessions. Surviving germplasm from his collections can be found in the Vavilov Institute, St Petersburg.

Unfortunately, Vavilov’s theories were questioned by other scientists of the day and he lost favour with the authorities. He was sent to prison, where it is believed he died.

Today, there is an increasing urgency to collect and maintain germplasm which is rapidly being lost from the major centres of diversity.

This germplasm is under threat from over-grazing, intensification of cultivation, desertification and displacement by improved varieties developed by industrial nations. Poor storage conditions also have caused some loss of existing germplasm. The germplasm of some nations has also been difficult to access for political reasons.

The Mediterranean region boasts the greatest genetic diversity of temperate pasture plants, including species of *Trifolium* (especially subterranean clover), annual *Medicago* and *Ornithopus* (serradella). Australian scientists have been involved in many seed collecting missions to this region.

While the justification for collecting new germplasm has initially been the need to improve existing varieties and to extend their distribution, the need to conserve this material in a gene bank for future use has become increasingly more important.

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**What’s in the collection**

The resource centre holds just over 12,000 accessions, 58 per cent being subterranean clover, 37 per cent other *Trifolium* species and the remaining 5 per cent *Ornithopus* species.

Most of the serradella accessions are *O. compressus* (83 per cent) while the other four species (*O. pinnatus, O. ishmocarpus, O. satisus* and *O. perpusillus*) account for the remainder. About 80 per cent of the serradella accessions were collected in Spain, Portugal, Morocco and the Greek Islands.

Subterranean clover accessions originated from 20 nations in the Mediterranean region, but most were collected in Sardinia and the Greek Islands. The biggest subspecies group is *subterraneum*, followed by *brachycalyctum*.

A total of 136 accessions belong to the important, waterlogging tolerant subspecies *yanninicum*.

Species in the genus *Trifolium* are grouped into eight taxonomic sections (Zohary and Heller, 1984). Most accessions (not including subterranean clover) belong in the sections *Trifolium, Vesicaria* and *Lotoidea*. Over 100 species are represented in the collection. The most common species include *T. cherleri* (cupped clover), *T. resupinatum* (persian clover), *T. glomeratum* (cluster clover), *T. hirtum* (rose clover), *T. purpureum* (purple clover), *T. fragiferum* (strawberry clover) and *T. spumosum* (bladder clover).

**Characterisation and preliminary evaluation**

Growing and measuring the performance of plant accessions from new collections provides valuable information to pasture research scientists for use in selection or breeding programs.

Collections from overseas are placed in quarantine. Clover seed is fumigated with phosphine gas if insect damage is evident, and is hand cleaned if fungi (ergots or sclerotia) are present. Serradella seed is treated only for insect damage.
There are just over 12,000 accessions in the South Perth site of the Australian Trifolium Genetic Resource Centre to conserve germplasm, in the form of seed, of Trifolium and Ornithopus species.

**Species**
- compressus 83.5%
- subterraneum 68.5%
- yanninicum 1.9%
- brachycalyicum 29.5%
- perennial 0.6%
- sativus 1.5%
- isothamnus 4.1%

**Sub species**
- subterraneum 68.5%
- yanninicum 1.9%
- brachycalyicum 29.5%

**Collections**
- Old collections from Israel (Katzmeher), CSIRO (Bailey) and the W.A. Department of Agriculture 38.7%
- South Australian collection 23.7%
- Recently requested accessions from other resource centres of high priority species including Persian, balansa, arrowleaf, Moroccan (T. isthmocarpum), and strawberry clovers 11.0%
- Other Trifolium species (444) 38.8%
- T. subterraneum (693) 57.5%
- Ornithopus species (655) 5.4%

**An accession**
An accession is a discrete or distinct entity in the form of a quantity of seed packaged in a glass or aluminium container.

The seed of any one accession is described as a genotype, strain or variety, or in the case of outcrossing species, a population of these.

Leigh Sonnemann sealing hermetic foil envelopes of seed for controlled storage.
Morphological characteristics are especially valuable for descriptive and diagnostic purposes. Large and comprehensive lists have been developed for important species including subterranean and white clovers. Characters that may have some agronomic significance are measured in most species and include growth habit, stem thickness, leaf size, peduncle length, strength of burial (in subterranean clover), and legume strength (in yellow serradella). Morphological characters of particular value for diagnostic purposes in subterranean clover include leaf markers and calyx and stipule pigmentation.

Preliminary agronomic evaluation involves the measurement of growth vigour (visual assessment of herbage production), flowering time (days from planting to appearance of first flower), seed production, disease and insect resistance, and hard seed and isoflavone content.

Individual plants of self-pollinating species that display variation in any single character are separated. It is common to find several genotypes collected from a single site where there is a high level of genetic diversity. Genotypes are regrown in the following year to confirm the basis for their separation, and to provide additional seed, which if derived from a single plant is often insufficient in the first year of planting.

Seed is then separated from the seed head and pod. At least one seed is taken from each clover seed head and serradella pod. For cross-pollinating clover species, a larger number of seeds is taken to ensure that most, if not all, the genetic variability is present in the initial growing. Seed is scarified using sand paper before planting to ensure imbibition and germination.

New collections normally have a small amount of seed and usually of doubtful quality. For these reasons, seed is planted in small pots in a glasshouse. Soon after seedlings have emerged appropriate rhizobium is applied. Four or five weeks after emergence seedlings are transplanted to the field.

Characterisation and preliminary evaluation consists of species identification, morphological characterisation and preliminary agronomic evaluation. Over 100 species of *Trifolium* (including many subspecies) and five species of *Ornithopus* have been documented. Every newly collected accession requires identification, often involving the inspection of plants through their entire growth stage, particularly when identifying subspecies.

**Some Australian varieties of temperate pasture legumes registered since 1975**

<table>
<thead>
<tr>
<th>Species</th>
<th>Variety name</th>
<th>Year of registration</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. subterraneum</em> spp. <em>subterraneum</em></td>
<td>Nungarin</td>
<td>1976</td>
<td>Bred</td>
</tr>
<tr>
<td></td>
<td>Esperance</td>
<td>1978</td>
<td>Bred</td>
</tr>
<tr>
<td></td>
<td>Enfield</td>
<td>1982</td>
<td>Naturalised in Australia</td>
</tr>
<tr>
<td></td>
<td>Dalkeith</td>
<td>1983</td>
<td>Naturalised in Australia</td>
</tr>
<tr>
<td></td>
<td>Junee</td>
<td>1985</td>
<td>Bred</td>
</tr>
<tr>
<td></td>
<td>Karriale</td>
<td>1985</td>
<td>Bred</td>
</tr>
<tr>
<td></td>
<td>Greenorange</td>
<td>1985</td>
<td>Bred</td>
</tr>
<tr>
<td></td>
<td>Denmark</td>
<td>1991</td>
<td>Sardinia</td>
</tr>
<tr>
<td></td>
<td>Goulburn</td>
<td>1991</td>
<td>Sardinia</td>
</tr>
<tr>
<td></td>
<td>Leura</td>
<td>1991</td>
<td>Sardinia</td>
</tr>
<tr>
<td><em>T. subterraneum</em> spp. <em>yanninicum</em></td>
<td>Trikkala</td>
<td>1975</td>
<td>Bred</td>
</tr>
<tr>
<td></td>
<td>Larisa</td>
<td>1975</td>
<td>Greece</td>
</tr>
<tr>
<td></td>
<td>Meteora</td>
<td>1981</td>
<td>Greece</td>
</tr>
<tr>
<td></td>
<td>Gosse</td>
<td>1992</td>
<td>Bred</td>
</tr>
<tr>
<td><em>T. subterraneum</em> spp. <em>brachycalyceum</em></td>
<td>Rosedale</td>
<td>1988</td>
<td>Turkey</td>
</tr>
<tr>
<td><em>T. michelianum</em></td>
<td>Paradana</td>
<td>1984</td>
<td>Turkey</td>
</tr>
<tr>
<td><em>T. resupinatum</em></td>
<td>Kyambro</td>
<td>1988</td>
<td>Turkey</td>
</tr>
<tr>
<td><em>T. pratense</em></td>
<td>Redquin</td>
<td>1979</td>
<td>Bred</td>
</tr>
<tr>
<td></td>
<td>Astred</td>
<td>1992</td>
<td>Portugal</td>
</tr>
<tr>
<td><em>T. repens</em></td>
<td>Siral</td>
<td>1976</td>
<td>Algeria</td>
</tr>
<tr>
<td><em>T. ambiguum</em></td>
<td>Prairie</td>
<td>1977</td>
<td>USSR</td>
</tr>
<tr>
<td></td>
<td>Forest</td>
<td>1977</td>
<td>USSR</td>
</tr>
<tr>
<td></td>
<td>Alpine</td>
<td>1983</td>
<td>USSR</td>
</tr>
<tr>
<td></td>
<td>Monara</td>
<td>1983</td>
<td>USSR</td>
</tr>
<tr>
<td><em>O. compressus</em></td>
<td>Tauro</td>
<td>1986</td>
<td>Italy</td>
</tr>
<tr>
<td></td>
<td>Avila</td>
<td>1987</td>
<td>Spain</td>
</tr>
<tr>
<td></td>
<td>Eneabba</td>
<td>1988</td>
<td>Bred</td>
</tr>
<tr>
<td></td>
<td>Madeira</td>
<td>1988</td>
<td>Madeira</td>
</tr>
<tr>
<td></td>
<td>Eliga</td>
<td>1988</td>
<td>Morocco</td>
</tr>
<tr>
<td></td>
<td>Paros</td>
<td>1990</td>
<td>Greece</td>
</tr>
<tr>
<td><em>O. pinnatus</em></td>
<td>Jebala</td>
<td>1988</td>
<td>Morocco</td>
</tr>
</tbody>
</table>
Species that are moderately or highly cross-pollinating exist as a population of several distinguishable, often very variable types. Attempts can be made to separate plant types with particular agronomic significance, however, as a rule these mixed accessions or populations are maintained as they are found in the original collection. Attempts are made to reduce cross-pollination between accessions, by physical separation and by use of cereal barriers to localise bee activity.

New accessions of *T. repens* (white clover), a highly cross-pollinating species, are sent to the white clover breeding centre in New South Wales. These plants are grown in cages with small nuclear bee hives to enhance seed production and to eliminate cross-pollination between accessions.

When plants have matured they are taken from the field by hand and placed in a small, stationary threshing machine to harvest the seed. The seed is cleaned and weighed ready for storage in the genetic resource centre.

**Conservation**

Three levels of temperature storage exist in the resource centre; long term storage (base collection, -18°C), medium term storage (active collection: seed for distribution and regeneration, 4°C), and short term storage (active collection: overflow seed, 10°C).

Seed for long and medium term storage is dried slowly to reduce its moisture content to about 5 per cent and then sealed in hermetic foil envelopes and placed in the appropriate temperature-controlled storage. An additional seed sample is processed for storage in the genetic resource centre for indigenous wild relatives located in Canberra. Excess seed is not dried but placed directly into airtight glass jars in short term storage.

The amount of seed stored in the base collection is important. At least 4000 seeds for genetically uniform material and 12,000 seeds for heterogeneous material should be kept. About 15 g of seed of *Trifolium* species and 20 g of pods of *Omithopus* species are stored in the base collection. Between 20 and 30 g of seed or pods are placed in the medium term store.

All information on each accession is stored in a computerised data base.

**Distribution**

Seed is available for use by recognised agricultural research organisations throughout the world. Free exchange of germplasm is fundamental to the existence of a global network of genetic resource centres. Only small amounts are sent: 2 g of large seeded clovers such as *T. subterraneum*; 0.5 to 1 g of small seeded clovers such as Persian or strawberry clovers; 4 g of pod of serradella.

When the weight of seed of any accession in the active collection (medium term store) reaches 2 g, no further orders can be met until the accession has been regenerated and fresh seed stored in the centre.

Much of the seed distributed is used in field evaluation.

**New varieties for Australia**

The National Subterranean Clover Improvement Program (NSCIP) continues to draw on newly acquired germplasm for field testing.
The Western Australian Department of Agriculture funds the preliminary evaluation and conservation of *T. subterraneum*.

**Acknowledgements**

I thank the Wool Research and Development Corporation for financing work with *Omithopus* species and *Trifolium* species other than *subterranean clover*.

**Further reading**


Maintaining a viable collection of germplasm of *Trifolium* and *Omithopus* species is vital for the future improvement of legume pastures in southern Australia.

Serradellas have been evaluated nationally since 1980, resulting in the registration of seven varieties, the most recent being Paros. A new group of early maturing accessions of serradella is being evaluated in the Western Australian wheatbelt. Several other *Trifolium* species are being evaluated in parts of southern Australia. These include white, red, Persian and balansa clovers. The table lists some varieties registered in Australia since 1975.

Three new varieties Denmark, Goulburn and Leura, originating from collections in Sardinia, were released in 1992.

A view of the test plantings, showing how each pasture plant is separated from its neighbour.

The short term (or active collection) seed store. Here Helen Williams is processing a request for seed.