Genetic resource centre for annual pasture legumes for acid soils

Summary
The Australian Trifolium Genetic Resource Centre (ATGRC), hosted by the Department of Agriculture and Food, Western Australia (DAFWA), is part of the Australian network of centres and has the mandate for *Trifolium*, *Ornithopus* and other species generally adapted to acid soils. The activities of the Centre include germplasm acquisition, characterisation, conservation and distribution. Seed is conserved at -18°C for long term conservation and 3°C for distribution to pasture researchers in Australia and around the world. Information on the origin of germplasm and plant characteristics is available on a Microsoft Access database for the purpose of selection of cohorts for field evaluation.

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Old or Archival Reports (Projects that concluded in 2007 or earlier)

The information contained in these older reports is now several years old, and may have been wholly or partially superseded or built upon in subsequent work funded by GRDC or others. Readers should be aware that more recent research may be more useful for their needs. Findings related to agricultural chemical use are also potentially out of date and are not to be taken as a recommendation for their use.

Conclusions

Over the period of this project, between 1997 and 2003, the ATGRC undertook the essential tasks associated with managing a genebank. They include germplasm acquisition, characterisation, conservation and distribution. Approximately 3,000 new accessions from 90 species of *Trifolium*, *Ornithopus* and *Biserrula* were acquired, either from collections in the wild or other existing ex situ collections. Most of the germplasm originated from Turkey, Greece, Italy and Spain, or from overseas genebanks at the International Centre for Agricultural Research in the Dry Areas (ICARDA) and New Zealand. The species of greatest potential included *T. glanduliferum* (gland clover), *T. spumosum* (bladder clover), *T. hirtum* (rose clover), *T. purpureum* (purple clover), *T. dasyurum* (eastern Star clover), *O. sativus* (French serradella) and *B. pelecinus* (biserrula). All 3,000 accessions were grown in nursery rows at the Medina Research Station, where plants were assessed for important agronomic, ecological and morphological traits. Seed was harvested, cleaned, dried at low temperature and conserved in the Centre. Seed was conserved in medium and short term storage for distribution and in long term for storage for future utilisation. Data associated with the new introductions was entered into a Microsoft Access database for easy access. This includes passport, habitat, plant characterisation and seed inventory data. Passport data includes country of origin, name of collector, date of collection, etc. Examples of collection site or habitat data are altitude, aspect, slope, soil texture, soil pH and habitat type. Plant characterisation data includes flowering time, growth vigour, growth habit, stem, leaf and flower characters and pod or fruit form. Inventory data is concerned with seed weight conserved and specific location in the store. The databases were used by ATGRC staff to select suitable germplasm for distribution, primarily to researchers to include in national field testing. Over 7,000 accessions were distributed to pasture researchers over the seven year period, with most being Australian recipients. The value of this work to the industry is difficult to quantify because of the significant time period between initial plant introduction and eventual commercial release of a new variety. New pasture varieties developed in recent times that originate from the Centre give a strong indication of the importance and relevance of genebank activities. There are many recent commercial successes including Cadiz™ French serradella, Prima gland clover, Santorini, Charano and Yelbini™ yellow serradellas, and Izmir™, Coolamon™ and Napier™ subterranean clovers. In addition, there are several releases planned within the next one to four years, including *T. dasyurum*, *T. purpureum*, *T. hirtum* and *T. spumosum*. New cohorts of species from the ATGRC are also either in progress or planned for field testing within the next year or two. There is no doubt about the importance of maintaining a genetic resource centre for plant improvement. There is also no doubt about the importance of continuing pasture improvement activities in southern Australia.

Recommendations

Coordination of germplasm collecting

Germplasm collecting missions are very often undertaken outside of this and other Genetic Resource Centre projects. Because Genetic Resource Centres are particularly concerned with germplasm acquisition, it would be wise to coordinate germplasm collecting activities through relevant centres. The lack of coordination often leads to inefficiencies and difficulties after germplasm has entered the country. For example, recording of site data could be standardised to a degree, thereby reducing data manipulation prior to computerisation. Difficulties at the quarantine barrier can better be managed if advice is sought from curators prior to departing on a collecting mission. Centres are the holders of an immensely valuable source of...
information about existing collections, yet often this information is not utilised when collecting missions are at the conception stage. There are many practical aspects to collecting that can be shared between experienced curators and prospective collectors. Historically, plant breeders have been the driving force behind germplasm collection. The recommendation is that any future germplasm collecting projects have a milestone that formalises coordination with appropriate curators.

Australian Plant Genetic Resource Information System (AusPGRIS)
The AusPGRIS database is a very important method of promoting the Australian network of Genetic Resource Centres and making available to the world valuable passport and collection site data of germplasm holdings in Australia. Unfortunately the AusPGRIS project did not achieve all its milestones, resulting in the failure of uploading data from the ATGRC on an annual basis. The recommendation is that the GRDC fund the writing of a transfer protocol to enable easy transfer of data. This may be built into the new National Genetic Resource Centre (NGRC) project.

Mandates
To avoid future duplication of effort between centres, it is recommended that the new national project facilitates a process to enable additions to be made to centre's mandates and also a review process to ensure centres are keeping to their mandates.

Other collections
DAFWA hosts the world collection of narrow leafed lupins (NLL) \((Lupinus angustifolius)\) and other related wild species. It funds the acquisition (including quarantine), characterisation, conservation and distribution activities of this collection. Lupins are mandated to the Australian Temperate Field Crops Collection in Horsham, Victoria (VIC), however, that Centre undertakes no genetic resource work with this genus. It is recommended that the GRDC consider supporting this collection, considering the importance of both the germplasm collection and the lupin industry in Australia. Other collections should also be included in the Australian network, including the grass collection in Hamilton, VIC, and the legume collection in Launceston, Tasmania (TAS).

Core collections
As collections grow in size through continuing acquisition, the need for better management and utilisation increases. The development of core collections of major species can improve the effectiveness of cohort selection for field testing, reduce the cost of germplasm conservation and better guide future collecting. The recommendation is that core collections be developed for all major species of pasture legumes held in Australian centres.

Outcomes
The principal outcome of this work is to improve the range of acid tolerant legumes available to breeders working in Australian pasture improvement programs now and into the future. This is achieved by maintaining a Genetic Resource Centre which undertakes the core functions of germplasm acquisition, characterisation, conservation and distribution. New pasture legume varieties for growers in southern Australia aimed at improved farm productivity and sustainability is the ultimate outcome of this work.

The ATGRC has the Australian mandate for pasture and forage species of legumes adapted to acid soils with a Mediterranean climate. Located at DAFWA, it provides an essential service close to the relevant pasture plant improvement activities. The Centre is involved in collection and acquisition of germplasm from both in situ (wild) and ex situ (collections) sources, characterisation of germplasm for use by breeders in the selection process, distribution of seed to both Australian and international breeders and conservation of seed for future utilisation. The ATGRC has provided these essential services to breeders since it was established in 1984. Many commercial successes have resulted over this time and are in part due to the support role played by the centre. Such successes have included Cadiz\(^{(0)}\) French serradella, Santorini, Charano and Yelbini\(^{(0)}\), yellow serradellas, NitroPlus\(^{(0)}\), Prolific and Morbulk Persian clovers, Cefalu\(^{(0)}\) arrowleaf clover, Prima gland clover, and York, Riverina\(^{(0)}\), Napier\(^{(0)}\), Coolamon\(^{(0)}\) and Izmir\(^{(0)}\) subterranean clovers. A number of species are expected to be commercialised soon including \(T. spumosum\) (bladder clover), \(T. dasyurum\) (eastern Star clover) and \(T. pupleureum\) (purple clover), all of which were a focus of Genetic Resource Centre activity and support to the breeding programs. The ATGRC has the largest world collection of a number of \(Trifolium\) species, including subterranean clover, yellow serradella \((O. compressus)\) and \(B. pelecinus\).

Information on the holdings in the Centre and details of germplasm exploration and conservation can be viewed on the DAFWA website. Passport and collection site data can be viewed on the AusPGRIS website. More detailed query of all genetic resource centre data can be made on the local database.
Germplasm (seed), collection site data and plant data are maintained by the ATGRC for current plant research activities and also conserved for research well into the future. Plant improvement depends heavily on a well organised and well managed genetic resource centre. The outcomes of this project have made this possible now and into the future.

**Achievements/Benefits**

Background importance of the issues that this project was designed to address

Plant improvement depends heavily on access to a large genetic diversity of currently important and potentially important species. Genetic Resource Centres are the best establishments from which to source germplasm for such plant improvement activities. Australia has a network of Centres that serve plant improvement across the country. The ATGRC, located at DAFWA, serves pasture legume improvement predominantly for acid soils in southern Australia. The official mandate for the ATCRC includes *Trifolium* (clover), *Orrithopus* (serradella) and other species generally adapted to acid soils. Unofficial mandates for the Centre include *Biserriula, Dorycium, Galega, Securigera, Coronilla, Sutherlandia and Cytisus*. During the early stages of the operation of the Centre, soon after it was established in 1984, attempts were made to acquire all germplasm of mandated species held in other Australian collections. Collections from Brisbane in Queensland (QLD), Grafton in New South Wales (NSW), Hamilton in VIC, Launceston in TAS, and Adelaide in South Australia (SA) were acquired. Unfortunately, germplasm collections of *Trifolium* have continued to grow in the Australian Medicago Genetic Resource Centre at the South Australian Research and Development Institute (SARDI) and the legume collection at the Department of Agriculture and Fisheries in Launceston, TAS. A failure to honour existing mandates can undermine national plant improvement efforts, but it is hoped that the new National Plant Genetic Resource Centre project will address this issue.

Core genetic resource centre activities include acquisition, characterisation, conservation and distribution of germplasm. It is these core activities that the ATGRC has been concerned with during the seven years of this project. The activities of the Centre have been guided to a large extent by pasture breeders and researchers operating within the National Annual Pasture Legumes Improvement Program (NAPLIP). Recommendations from the annual meetings have been sought for activities to be undertaken each year. This process has been crucial in maintaining relevance of the Centre to national pasture legume priorities. Other national programs, including in particular perennial pasture improvement managed through the Cooperative Research Centre (CRC) for Plant Based Management of Dryland Salinity (Salinity CRC), influence the activities of the ATGRC. However, genetic resource work with perennial species is managed through a different GRDC funded project. International interests in the activities of the Centre extend to knowledge of germplasm holdings, access to passport and collection site data and availability of seed for distribution. International researchers rarely influence annual nursery activities of the Centre and do so if there are species of common interest, or if old germplasm needing regeneration is in demand.

The relevance of the core activities listed above is best seen through an understanding of the priorities set out by associated plant improvement and plant breeding projects underway in Australia. Improved varieties of existing species to tackle known shortcomings is likely to be a continuing priority. However, development of new species to complement existing ones and to suit often more challenging niches not currently well provided for is an emerging priority. In addition, new species with quite different characteristics are required for new systems of pasture production compared with the traditional ley system, including phase pastures, companions with subtropical grasses and specialist hay use. During this project, there has been a continuing need for the services provided by the Centre and the following section will detail those achievements.

**Major achievements and how they will benefit the industry**

**Germplasm Acquisition**

Table 1 (Attachment 1) shows the acquired germplasm that was grown during the project between 1997 and 2003. Germplasm was acquired from 39 separate collections from 13 countries over a period of 16 years from 1986 to 2002. These collections were undertaken either by Australian researchers, or had the involvement of an Australian researcher. In terms of numbers of accessions, the important collections were from Turkey, Greece, Italy and Spain. Most of the accessions from collections undertaken earlier than 1996 were grown in nursery rows at the Medina Research Station before this project commenced.

Germplasm was also sourced from seven genetic resource centres, including ICARDA, which has the most comprehensive collection of pasture legume species from Mediterranean environments. The Polish and New Zealand genebanks were significant sources of germplasm of *O. sativus* in the later part of this project.

Plant species acquired or collected can be seen in Table 2 (Attachment 2) and amount to 80 species of *Trifolium*, eight...
species of *Ornithopus*, *B. pelecinus*, *Hymenocarpos cirsinatus* and *Lotus ornithopodioides*. In total, over 3,000 accessions were acquired and grown over the period of the project. This translated to approximately 3,900 individual field plots as a result of the particular planting methodology and the need to grow accessions in more than one year.

Tables 3 and 4 (Attachments 3 and 4) summarise the origin of the species collected from the wild and acquired from other genebanks. Passport data and collection site, or site habitat data, have been entered into a Microsoft Access database at the centre. This information is important in assisting in prioritising germplasm for growing and characterising and also assists in identifying priorities for future collection and acquisition. Standardisation of such data would make data entry more efficient. However, to achieve this among all collectors and genebanks is unrealistic.

Germlasm Characterisation
Table 5 (Attachment 5) shows the number of lines of each species generated from the accessions planted in nursery rows. Over 6,000 lines were generated from 3,000 accessions. Cross pollinating species are generally maintained as a single diverse line, with little or no separation, unless there is a specific need to isolate plants with important characters. Mostly self pollinating species, such as *O. compressus* and *Biserrula pelecinus*, are grown as single spaced plants and separated into their diverse genotypes. Species grown each year was a reflection of priority settings and availability of germplasm. Some species of high priority were well represented in existing collections, for example *T. hirtum*, *T. resupinatum* and *T. spumosum*. Other high priority species were not well represented, for example *T. diffusum*, *T. glanduliferum* and *T. palaeatinum*. Other species of high priority were actively sought from other Genetic Resource Centres and were the target of more detailed characterisation, for example *T. dasyurum* and *T. purpureum*. This ‘targeted characterisation’ was undertaken and funded through NAPLIP and will be briefly summarised later. Routine characterisation was undertaken on all lines and included both characters of agronomic or ecological importance and morphological characters of diagnostic value. Flowering time, growth vigour in winter and spring, growth habit, seed yield and leaf marks were all assessed. Other leaf, stem, flower and seed pod characters of significance were sometimes assessed. Table 6 (Attachment 6) illustrates the range in flowering time of each species.

Characterisation data has been entered into a Microsoft Access database at the Centre. Researchers outside of the Centre can query the database, or they can request Centre staff to undertake their queries. This information is particularly important in the selection of lines to include in cohorts of species for field evaluation trials.

Germlasm Conservation
All lines grown and characterised in nursery plots over the period of this project have been conserved or are in the drying process prior to conservation in the Centre. Seed is conserved in short and medium term storage for distribution and in long term storage for security well into the future. Small duplicate samples have also been sent to CSIRO, in Canberra, as insurance against damage or loss of seed from the Centre. A small subset of conserved lines is sampled for germination testing in order to allow the monitoring of seed viability in the centre over time. Approximately 10% of all lines are sampled for testing prior to conservation and up to 50% of lines in species that are likely to have poor longevity due to inherent soft seed. Unfortunately, germination tests have not been undertaken for lines grown during this project. Pressure on the budget from increasing operating costs has meant that costly, low priority activities, such as germination testing, have ceased.

Germlasm Distribution
Seed dispatch is managed through a computerised seed inventory system for greater efficiency. Table 7 (Attachment 7) shows a summary of the history of seed distribution during the period of this project. Requests are divided into state, national and international and also show the proportion of germplasm requests used for NAPLIP. Numbers of accessions distributed each year varied enormously depending on demand. Australian field testing programs tend to follow a cyclic pattern from initial cohort screening to final commercialisation and can explain some of the variation in demand. International demand continues to be firm and is given a high priority. This is because international relations are very important in ensuring future access to exotic germplasm. Records of germplasm distribution prior to 2003 are kept in paper form on file, while requests from 2003 onwards are recorded on the new seed inventory database. This new Access database was funded by internal CLIMA monies and replaced the existing Omnis system for Macintosh, which has been unsupported for many years. The new database is linked to the existing database for passport and characterisation data.

Targeted Characterisation and Plant Selection
Targeted characterisation involves the measurement of additional plant characters in species that are considered to be
particularly high priority. This work is funded through the WA component of NAPLIP and has been reported in detail in reports presented to the annual collaborators meetings. Below is a brief summary of these activities:

1999-2001 T. purpureum and T. dasyurum
1999 Trifolium, Ornithopus and Biserrula from the East Aegean Island of Greece
2001-2003 O. isthmocarpus
2002 T. cherleri
2002-2003 O. sativus

The ATGRC has undertaken single plant selection in a number of species additional to its core activities. Details of these activities have also been presented to NAPLIP. Below is a brief summary including the selection criteria:

T. purpureum - early flowering, improved winter vigour, improved seed threshability, increased seed size.
T. michelianum - early flowering, increased hard seed level.
O. isthmocarpus - pod characters that suit commercial seed production
O. sativus (naturalised population) - increased hard seed
T. vesiculosum and T. isthmocarpum - leaf marks for floriculture industry.

Benefit to industry

Often 10 or more years can elapse between the initial plant introduction and commercial release of a new variety. Consequently, putting a value on recent plant introduction is difficult, if not impossible. To best gain an understanding of the value of the activities of the ATGRC to the industry, it is recommended to view workshop proceedings of annual meetings of collaborators of NAPLIP. Table 8 (Attachment 8) details commercialisation of pasture legume species by either NAPLIP or DAFWA and CLIMA. This table also details likely future commercial releases and planned cohorts of germplasm for field testing.

Commercial success over the past eight years is impressive by any standards. Improvement over existing varieties of established species can be seen in T. subterraneum, T. resupinatum, T. michelianum and O. compressus. There has also been commercial release of O. sativus, a new species to Australia and without doubt one of the most successful examples of adoption of a pasture variety in WA. Development of a completely new species to agriculture is a challenge to most researchers. Pasture researchers at DAFWA and CLIMA have succeeded in doing this by commercialising T. glanduliferum, a new species from the eastern Mediterranean. It is significant to note that the commercialisation of T. spumosum and T. dasyurum, also new species to agriculture, is planned within the next three years and L. ornithopodioides, a new species to Australia and most of the Mediterranean, is also planned within this time frame.

Plant introduction through the ATGRC over the period of this project has provided the germplasm to feed into cohorts for national field testing. T. purpureum and O. sativus are examples of this. It is important to note also that recent overseas plant collecting continues to provide more priority germplasm for genetic resource characterisation and inclusion into future cohorts. The ATGRC will continue to provide essential and relevant support to plant improvement in southern Australia.

Other research

Other research opportunities were conceived and realised midway through this project and were subsequently funded by the GRDC. Specific reference is made to UWA00040 (Germplasm collection of Trifolium and other pasture legume species from short season, low latitude regions in the Mediterranean) and UWA00005 (Improving the utilisation of pasture germplasm by the development of a core collection using ecogeographical and molecular techniques). These two projects are progressing extremely well and should continue to be supported after their completion dates in order to gain the most benefit from the research. For example, core collections should be developed for other species held in Australian germplasm collections once the best methodology is determined through the findings of the current project. A new project focusing in a similar way on lupin collections and funded by the GRDC supports the view that this is valuable research. The recent mission to Eritrea has created the opportunity to access crop landraces of wheat, barley, chickpeas and faba beans that have not previously been accessible. The GRDC should consider providing financial support for projects in Eritrea to build their capacity to do agricultural research in exchange for making available their crop landraces. Finding suitable pasture legumes, in particular perennial species, for the harsh conditions of the southern Australian wheat belts is proving especially difficult. More support should be provided to search other regions of the world for adapted species. However, there should be serious, long term support for the domestication of Australian native legume species which clearly are well adapted to the local environment. Recent collections in the WA rangelands by Richard Bennett and Megan Ryan, of the University of Western Australia (UWA) give a strong indication of the potential of native pasture legumes. The effort required in domesticating wild species clearly...
requires serious investment. Genetic erosion in many parts of the world makes reliance on exotic germplasm risky, hence the long term strategy is to invest in the domestication and conservation of our own native plants.

The GRDC has supported a new National Genetic Resource Centre (NGRC) project for the next three years. With additional support from other Rural Industries Research Centres (RIRCS), the future of Genetic Resource Centres in Australia looks bright.

**Intellectual property summary**

This project does not produce any intellectual property (IP).

**Additional information**

See Table 9 in the attached document for list of publications.