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Conserving Leucaena ssp. Germplasm collection

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Executive summary

Pastures of the tropical forage tree legume leucaena (*Leucaena leucocephala*) are the most productive, profitable and sustainable pastures available to graziers in northern Australia. Graziers are adopting this pasture system with over 200,000 ha established at present. Whilst the leucaena pasture technology has been largely developed in Australia by CSIRO, QDAF and The University of Queensland (UQ), Professor James Brewbaker at the University of Hawaii (UH) is the pre-eminent plant breeder who has worked on the *Leucaena* genus of tropical forage tree legumes for over 50 years. The objective of this study was to import unique Leucaena germplasm from UH and to collect seed to provide long term benefit to the Australian Leucaena industry by providing accessions that can be used in plant breeding programs to develop novel traits like cold tolerance, psyllid resistance and sterility.

Eighty-seven (87) superior accessions of a diverse array of *Leucaena* taxa, some recently delineated by botanists, were selected from the UH collection (and other sources) based upon physical inspection of representative plants in existing arboreta, published and unpublished (theses) trial results and original plant collection notes that described the populations of trees from which the seed was collected in Central America.

In September 2008 a Leucaena germplasm orchard of 87 taxa was established at Redlands Research Station, Cleveland QLD. Controlled pollination of the taxa in this orchard was not possible for a number of reasons including, (i) the wide variation in flowering of the taxa meant that constant monitoring on a daily bases was required to capture all the opportunities to carry out the pollinations. This was not possible given the limited human resources allocated to this project, (ii) some taxa did not flower or rarely flowered. The lack of flowering of some accessions may have resulted from the absence of photo-temperature queues that stimulate flowering (iii) some taxa were outcompeted because of their small stature. Some of these taxa still have value as parents in hybrid combination to make sterile triploids e.g. L. greggii and L. retusa (iv) some taxa did not recover well from the original coppice treatment. However, these taxa have been given a better opportunity of healthy growth by removing nearby competing trees.

Over a five year (2012-2017) period seeds were collected from 61/87 taxa planted. Seeds were successfully collected from all the self-compatible tetraploid (4X=104 or 112 chromosomes) species grown in the orchard including L. leucocephala, L. pallida, L. confertiflora and L. diversifolia. Seed collection from the self-incompatible diploid (2X=52 or 56 chromosomes) species was more problematic.

All 87 taxa have been retained and the orchard can be used for future seed collection opportunities and breeding programs. To this end the trees have been thinned and coppiced to facilitate greater accessibility for those working the orchard. This orchard will now provide a core resource for the development of sterile leucaena by providing diploid and tetraploid taxa that can be inter-crossed to make sterile triploids.

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1 Background

Pastures of the tropical forage tree legume leucaena (*Leucaena leucocephala*) are the most productive, profitable and sustainable pastures available to graziers in northern Australia and currently over 200,000 ha have been established.

Whilst the leucaena pasture technology has been largely developed in Australia by CSIRO, QDAF and The University of Queensland (UQ), Professor James Brewbaker at the University of Hawaii (UH) is the pre-eminent plant breeder who has worked on the *Leucaena* genus of tropical forage tree legumes for over 50 years. Professor Brewbaker has collected a wide range of *Leucaena* species and accessions from Central and South America, and with many students has bred some of this material into new hybrids (e.g. KX2). Access to the UH collection of germplasm will be useful to further improve *Leucaena* varieties in future for Australian graziers. For example, frost resistance is known to exist in 3 different *Leucaena* species and could be transferred to the new KX2 varieties, further improving the productivity of leucaena in Qld by minimizing winter damage, and increasing the environmental adaptation of leucaena well into NSW (at least 300 km south of the border). This germplasm could also provide genetic insurance against future pest and disease threats that may challenge existing commercial leucaena varieties.

Professor Brewbaker has retired and has been scaling back his *Leucaena* breeding and evaluation activities. Unfortunately Prof. Brewbaker does not have any younger scientists following through with his *Leucaena* work (largely because of the lack of interest in leucaena pastures by the US beef industry that is so heavily biased toward grain feeding animals). Consequently, there is a real likelihood that all of his *Leucaena* breeding experience and plant material may be lost. Acquiring the UH collection will safeguard the future of this valuable material, with the only other significant collection being held by The Oxford Forestry Institute in the UK (its future looks secure at the moment). The Leucaena Network (a grazier advocacy group) feel it would be prudent for the Australian beef industry to invest in capturing Prof. Brewbaker's knowledge and conserving as much of the useful UH germplasm as possible by bringing it to Australia.

The UQ Leucaena Germplasm Collection

During 2007-2008, MLA invested in a study tour (B.STU.0223) by Dr Scott Dalzell (UQ) to examine the UH collection for superior leucaena genetic stocks that have relevance to Australian conditions and production systems. UH has a collection of some 1050 different accessions of *Leucaena* spp. that have been collected by Dr Brewbaker, his students and colleagues, and exchanged between international research institutions. All of this material has been grown and evaluated at UH Waimanalo Research Station. Eighty (80) superior accessions of a diverse array of *Leucaena* taxa, some recently delineated by botanists, were selected based upon physical inspection of representative plants in existing arboreta, published and unpublished (theses) trial results and original plant collection notes that described the populations of trees from which the seed was collected in Central America. A significant number of accessions from the Oxford Forestry Institute collection that were held by UH were also selected for import.

2 Project objectives

Leucaena germplasm has been imported from U Hawaii (Prof James Brewbaker) through AQIS into Australia and a collection comprising 62 taxa was grown at the QDAF Redlands Research Station since September 2008. The objective of this project was to seed increase the leucaena taxa grown for use in future breeding programs. Accessions selected for importation from UH were chosen using 2 criteria: (i) those taxa that were currently not represented or poorly represented in the collection held by the Australian Tropical Crops and Forages Germplasm Collection (ATCFGC); and (ii) those accessions that have potential to contribute in the short-medium term to *Leucaena* breeding programs in Australia.

3 Methodology

3.1 Establishment and Growth

Seed of 80 leucaena taxa were imported through AQIS into Australia and planted in a quarantine glasshouse at Eagle Farm Brisbane to establish seedlings that could be later transferred to the field. Because some imported seed was not viable only 62/80 taxa could be transferred. In addition to these 62 an additional 25 taxa from UQ's genetic stocks were established in the field in September 2008 at QDAF (Queensland Department of Agriculture and Fisheries) Redlands Research Station. A rhizobial strain and broad based fertilizer were applied at planting and irrigation provided as needed through the establishment phase. An 18 row orchard was established with rows 2m apart. Each taxa was planted in plots the length of which was determined by the number of seedlings available for each taxa at the time of planting. The planting arrangement of the orchard is given in Fig 1. There was excellent survival of seedlings and at least one plant was established of more than 95% of the taxa. Overall, plant growth was excellent and at the start of the current project B.BNP.0696 in April 2012 the orchard consisted of mostly mature trees in some cases 5 m tall. In spring of 2012 a coppice treatment was applied to reduce the canopy height of the orchard and to make seed collection more manageable.

3.2 Seed collection

Over a five year (2012-2017) period seeds were collected from 61/87 taxa planted. For self-compatible species pods were collected from clusters of multiple pods (>6) to increase the chance of obtaining self-pollinated seed. From self-incompatible species (the majority of taxa) pods were collected from clusters of 2-3 pods again to increase the chance of obtaining self or sib-pollinated seed. A complete list of seed collected is provided in Table 1. A list of taxa for which no seed has been collected to date is given in Table 2. Table 2 also indicates which taxa have survived, have healthy trees but have remained seedless. All harvested seed was immediately dried at 35 C for at least one week and placed in a freezer to kill any bruchid (Callosobruchus spp or Acanthoscelides spp) larvae. After freezing seeds were placed into small coin envelopes and returned to the freezer.

4 Discussion

4.1 Diploid/Tetraploid seed increase

Leucaena pasture technology has been largely developed in Australia by CSIRO, QDAF (originally QDPI) and The University of Queensland (UQ). Over the last 40 years leucaena has been used extensively in sub-tropical and tropical regions of Queensland to fatten cattle in the rainfall zone greater than 600mm. Typically, leucaena is sown in hedge rows 6-8m apart separated by swards of a perennial C4 grass such as buffel, Rhodes grass or green panic. Today leucaena/grass systems are considered to be some of the most successful in terms of fattening cattle under dryland conditions. Commercial leucaena cultivars are all tetraploid and breeding programs have focused on crossing among tetraploid species for developing new types with specific attributes. For example, UQ and MLA have recently released the psyllid resistant cultivar "Redlands" after a breeding program that involved intercrossing two tetraploid species, leucocephala and pallida. However, substantially more genetic variation in the leucaena primary gene pool exists and much of this germplasm has been accumulated over a long period of time by Professor James Brewbaker at the University of Hawaii (UH). As a result of a strong collaboration between UQ and UH leucaena germplasm developed by Prof Brewbaker and with value for future breeding programs was made available to Australian researchers by the establishment of an orchard at Redlands Research Station in 2008. The main objective of the current project was to generate seed stocks of Leucaena diploid and tetraploid taxa obtained from UH.

Controlled pollination of the taxa in this orchard was not possible for a number of reasons including, (i) the wide variation in flowering of the taxa meant that constant monitoring on a daily bases was required to capture all the opportunities to carry out the pollinations. This was not possible given the limited human resources allocated to this project, (ii) some taxa did not flower or rarely flowered (iii) some taxa were outcompeted because of their small stature (iv) some taxa did not recover well from the original coppice treatment.

Seeds were successfully collected from all the self-compatible tetraploid (4X=108 chromosomes) species grown in the orchard including L. leucocephala, L. pallida, L. confertiflora and L. diversifolia. As expected, seed collection from the self-incompatible diploid (2X=54 chromosomes) species was more problematic although seeds were successfully obtained from the following species, L. collinsii, L. cuspidata, L. esculenta, L. greggii, L. involucrata, L. lempirana, L. macrophylla, L. magnifica, L. pueblana, L. retusa, L. salvadorensis, and L. trichandra. It is expected that some of this seed will consist of half-sibs. There were no seeds collected form the diploid species L. lanceolata, L. matudae, L. multicapitula, L. trichodes primarily because they either didn't flower at all or flowering events were so short that natural sib-pollinations were not possible. For some small tree diploid species such as L. greggii and L. retusa seed set was always poor because the trees were unable to compete with other vigorous trees of surrounding species an effect made worse by their poor recovery from the initial coppice treatment.

Importantly, this orchard will now provide parental material for other breeding objectives in leucaena, for example, sterility and cold tolerance. Sterile hybrids can be developed in leucaena by inter-crossing species of different ploidy. For example tetraploid (4X) species like leucocephala, diversifolia and pallida can be crossed with diploid (2X) species like magnifica, esculenta, greggii, retusa and macrophylla to form sterile triploids. The challenge will be to obtain sterile triploid plants that have the appropriate feed quality to fatten cattle. Cold tolerance can be developed by inter crossing species such as pallida, collinsii, greggii and retusa each of which are known to have evolved in high altitude environments and individually have cold tolerance. Cold tolerant leucaena can help push the cultivated area into Northern NSW where drought tolerant, leguminous, perennial forage is desperately needed. Sterile leucaena would have great value in the Pilbara and Kimberley regions of WA where seeded varieties are not permitted on leasehold land the predominant land title on grazing land in that state.

5 Conclusions/recommendations

A leucaena orchard representing useful taxa imported from UH was successfully established and maintained at Redlands Research Station, Cleveland Queensland. Seed was successfully collected from all species except for L. lanceolata, L. matudae, L. multicapitula, L. trichodes. However, all taxa have been retained and can be used for future seed collection opportunities and breeding programs. To this end the trees have been thinned and coppiced to facilitate greater accessibility for those working the orchard. This orchard will now provide a core resource for the development of sterile and or cold tolerant leucaena by providing diploid and tetraploid taxa that can be inter-crossed to make triploid interspecific hybrids.

6 Key messages

The orchard established by UQ at Redlands Research Station should be retained to provide a core resource for developing sterile and or cold tolerant leucaena that can be directed to new areas of Australia for red meat production.