Assessment of population genetic variation and structure of *Acacia woodmaniorum*, and its phylogenetic relationship to other *Acacia* species

Six month report to Karara Mining Ltd by the Department of Environment and Conservation.

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Our environment, our future

Summary

This report describes the progress made in the first six months of the project on *Acacia woodmaniorum* undertaken by the Department of Environment and Conservation for Karara Mining Ltd. A Research Scientist position commenced for the project in September 2008, and initial site visits and germplasm collections were conducted in October 2008. Both large and small scales as well as high throughput DeoxyriboNucleic Acid (DNA) extraction protocols have been optimised for the species and four microsatellite libraries have been produced by Genetic Identification Systems (GIS). Currently microsatellite primers are being screened and Polymerase Chain Reaction (PCR) amplification conditions optimised. Field work entailing the bulk of the germplasm collections required to commence soon.

Progress to Date

Field Work

An initial visit to the site at Karara station was conducted in October 2008. Greg Woodman assisted with taxa identification for both *Acacia woodmaniorum* and *Acacia karina*. Ten samples of *A. woodmaniorum* were collected from across the Mungada Ridge and Terapod sites. A second field trip was conducted in December 2008 and all plants at the Terapod and Old Western Mining Pit sites were tagged, sampled and recorded by differential Global Positioning System (dGPS). One population at Jasper Hill was tagged, sampled and dGPS'd.

DNA Extraction

Large scale DNA extraction protocols for fresh material and small scale extraction protocols for lyophilised material have been optimised for *A. woodmaniourm* phyllode material. A high throughput semi-automated protocol for 96 lyophilised samples has also been optimised for use over the bulk of the project. The protocols produce high concentrations of high quality DNA.

Development of Microsatellite library and optimisation of suitable primers

Genomic DNA was sent to GIS in October 2008 and two di- and two tri-nucleotide microsatellite libraries produced for *A. woodmaniorum*. Information on libraries and designed microsatellite primers was received from GIS in late January 2009. A total of 103 unique microsatellite containing clones were identified from the four libraries and PCR primer pairs designed for 80 microsatellite containing clones. 36 primer combinations for 36 unique microsatellite loci have been synthesised and tested for suitable amplification of loci using an M13 universal primer tagged three primer system. Primer combinations have been tested over 6 individual DNA samples covering each 'site' (Mungada Ridge, Terapod, Jasper Hill and the Old Western Mining Pit). PCR amplification and locus suitability has been tested by electrophoresis on polyacrylamide gels. About twelve of these primer pairs are

ready for further testing via fragment analysis on a sequencer. An additional 24 primer combinations have been ordered for testing and optimisation.

Challenges

Field work has been hampered by the environmental conditions over summer which have been unsuitable for germplasm collections. Access to the Jasper Hill site is hampered by rehabilitated access tracks. Early field work was also hampered by long travel times to and from sites each morning and afternoon as accommodation was available only in the town of Perenjori. Accommodation is now available for us on site and this should make field work for germplasm collections more productive.

Only small amounts of seed pods were observed on plants from October to December 2008. A lack of pod production and seed set will make it difficult to produce estimates of gene flow via progeny analysis.