

2008

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Frances M. Shapter
Southern Cross University

Sylvia Malory
Southern Cross University

Ian Chivers
Southern Cross University

Robert J. Henry
Southern Cross University

Publication details

Shapter, FM, Malory, S, Chivers, I & Henry, RJ 2008, 'Accelerated domestication of Australian grasses as new sustainable food and fodder crops', paper presented to 58th Australian Cereal Chemistry Conference, Gold Coast, Qld., 31 August - 4 September.

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ACCELERATED DOMESTICATION OF AUSTRALIAN GRASSES AS NEW SUSTAINABLE FOOD AND FODDER CROPS

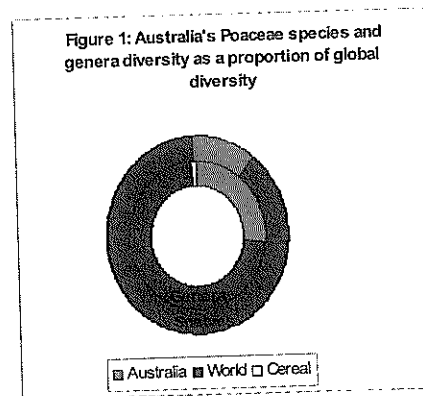
F.M. Shapter¹, S. Malory¹, I. Chivers² and R.J. Henry¹

¹Centre for Plant Conservation Genetics, Southern Cross University, Lismore NSW, Australia

²Native Seeds Pty Ltd, Sandringham VIC, Australia

INTRODUCTION

Australia is home to 10% of the world's 10,000 grass species (Figure 1) and yet none of the currently cultivated species are native to this continent. The potential of several of Australia's native species to be domesticated as new cereal crops was recorded by botanists as early as 1895 (Turner 1895). Native cereals would have enormous environmental advantages over currently cultivated crops and potentially allow niche cereal production on what has previously been characterised as unsuitable or marginal land. Niche native cereals have the added benefit of being highly marketable as ingredients or stand alone products. The model species selected for accelerated domestication in the current study is *Microlaena stipoides*, a distant relative of rice, which can be utilised as a dual purpose fodder and grain crop concurrently.



Previous work undertaken at the Centre for Plant Conservation Genetics has confirmed the expected synteny between the *Microlaena* genome and other cereals, particularly rice (Shapter et al 2008). Based on this genomic similarity domestication genes are now being characterised in *Microlaena* to develop a molecular toolkit for screening mutant breeding populations for key domestication traits, such as non-shattering grains, as well as other grain quality traits. The abundance of publically available cereal genomic data is a key resource and can be utilised in conjunction with large scale, high throughput SNP analyses such as TILLING (Slade and Knauf 2005), EcoTILLING (Cordeiro et al 2005) and endonucleolytic mutation analysis by internal labelling (EMAIL) (Cross et al 2008) for selection of individuals for breeding programs. Successful accelerated domestication of native species will provide new cereals which are better adapted to Australia's challenging climate.

AUSTRALIAN NATIVE GRASSES

Wild crop relatives

Over the past 60 years the use of wild crop relatives to develop commercially available cultivars from plant breeding programs has increased, with these hybrid derived varieties being released for most staple food plants (Hajjar and Hodgkin 2007). Plant breeders can utilise wild crop relatives as a source of novel germplasm by using conventional plant breeding or transgenic approaches to maintain high rates of crop improvement (Rao et al 2003). To date most of wild species have been targeted to improve agronomic traits such as growth habit and disease resistance. Recent advances in molecular techniques have shifted this focus to include manipulating individual genes or alleles that encode desirable functional or nutritional characteristics. Australia has four species of wild *Oryza*, 17 wild *Sorghum* species, and potentially a wild *Saccharum* (Dillon et al 2007). These species represent a unique genetic resource as due to Australia's geographic isolation and short agricultural history Australia's wild species have evolved independently from cultivated species.

A sustainable resource

Australian native grasses are better adapted to Australia's variable rainfall and marginal soils than introduced grasses and as such are already recognised for their potential as rehabilitation, turf, landscaping and pasture plants (Chivers and Aldous 2005; Davies et al 2005). Environmental adaptations such as drought, frost and shade tolerances, increased water use efficiencies, high recruitment, and an ability to tolerate acidic, saline or low nutrient soils make Australian native grasses adapted to some of the marginal areas of Australia's environment not currently used for cereal production (Waters et al 2005; Whalley et al 2005). The most limiting factor for the use of native grasses, as either pasture or grain crops, is their reduced yields when compared to domesticated species. In the case of grain yield addressing the issue of shattering at maturity greatly improves its potential to be harvested and hence commercially exploited.

Microlaena stipoides as a model species

Microlaena stipoides has been selected as the primary target species for accelerated domestication because of its favourable agronomic and genetic traits. *Microlaena*'s perennial growth cycle increases its water use efficiency while reducing tillage, and hence soil erosion, and reducing the input costs associated with annual sowing. Its predominantly cleistogamous (selfing) breeding system allows the development of stable breeding lines and additionally this species also exhibits opportunistic chasmogamous (outcrossing) breeding cycles (Huxtable 1990). This will allow for cross breeding to be utilised to introgress useful traits from mutant individuals. Broad natural variation within the species occurs within and between populations across a wide range of environmental conditions (Davies et al 2005) and most accessions collected show a tolerance to drought, acid soils, salinity, shade, frost and low fertiliser compared to current cereal crops (Whalley and Huxtable 1993; Chivers and Aldous 2005). *Microlaena* is a dual purpose crop which can be grazed for about 6-8 months of the year and then locked up to set seed for grain harvest after rainfall events in the summer months. Its high fodder value is due to a combination of good palatability, high digestibility (up to 79%), substantial biomass production (4-12t/ha) depending on water and nitrogen availability (Archer and Robinson 1988). With respect to grain production *Microlaena* already has a good plant architecture, reasonable grain yield (130-1137kg/ha), seed size comparable with rice in some accessions (Chivers and Aldous 2005) and a similar endosperm morphology to rice (Shapter et al 2008).

From a genetic perspective *Microlaena*'s close relationship to rice, the only cereal genome to have been sequenced, assembled and annotated, is very beneficial. Unlike rice however *Microlaena* is a tetraploid species with a base chromosomes number of 24 (double rice). While the polyploidy complicates both the genomics and the breeding systems required to domesticate the species it also enhances *Microlaena*'s intrinsic environmental adaptability and is beneficial for mutation breeding as it can reduce the lethality of higher rates of mutation.

DOMESTICATING WILD SPECIES

Advances in molecular genetics and genomics

The vast array of proteomic and particularly genomic data that has become available in the past two decades has led to a rapid increase in our understanding of the genetic basis for domestication. Quantitative trait loci and/or in many cases both the gene and protein sequences for many of the key domestication traits have been established. Rice has become the primary species for a large proportion of this work because of its importance to world food supply, relatively small genome and the completion of the genome project (Sweeney and McCouch 2007). Due to the high genomic synteny across the grasses, there is an increasing understanding of the genetic basis of much of the 'domestication syndrome' which includes traits such as shattering, pericarp colour, dwarfism, earliness, seed dormancy, awn length, grain size, grain number and panicle shape (Sweeney and McCouch 2007; Vaughn et al 2007). Many of the genes related to domestication are due to loss of function mutations. For example, seed shattering, the shedding of seeds from the plant when they are ripe, requires a cascade of events controlled by many genes. Loss of seed shattering is a key domestication trait as the seed must stay on the plant to be harvested. The non-shattering phenotype is induced by the loss of function of either of two genes, *sh4* and *qsh1* (Konishi et al 2006; Li et al 2006). Loss of seed shattering may be identified by targeted screening of the homologs of these genes in *Microlaena* for an induced or naturally occurring non-shattering allele. Once the desirable genotypic variations have been identified from within either natural or mutated populations, these individuals can be used to develop domesticated breeding lines.

Natural variation and mutation breeding

The naturally occurring variation between and with accessions of *Microlaena* are well documented (Davies et al 2005). Some of these naturally occurring mutants have been observed to have beneficial phenotypes such as non-shattering (*data not shown*). Inducement of novel mutation by chemical or physical treatments has allowed the development of over 2570 varieties of plants in the past 80 years with over 434 varieties in rice alone (Mutant Variety Database - <http://www-mvd.iaea.org>). In 2004 this had had an estimated value of over US\$20 billion total in the US, China, Thailand, India and Japan (Ahloowalia et al 2004). Currently there are 1151 mutant grass varieties listed of which the majority are of rice, barley and wheat. As little as single nucleotide polymorphism induced by mutation can cause a loss of function of a gene (Konishi et al 2006). Within a mutated population it is expected that across the individuals a wide variety of both beneficial and deleterious mutation will be observed. Selecting the beneficial genotypes for inclusion in breeding programs using genetic markers and targeted screening of the genes of interest allows for accelerated selection of individuals for breeding programs.

High-throughput screening

Natural and mutant populations of *Microlaena* will be mined for beneficial alleles of domestication and grain quality genes by a variety of high-throughput molecular screening techniques including TILLING (Slade and Knauf 2005), EcoTILLING (Cordeiro et al 2005) and EMAIL (Cross et al 2008). Preliminary sample pooling in conjunction with the high throughput extraction will allow for cost effective, rapid SNP detection in genes of interest across large populations. Once the individuals carrying these alleles are identified they will become the basis for new 'domesticated' breeding lines of *Microlaena stipoides*.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the Australian Research Council for funding this project under the ARC Linkage program.

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