# Diversification of Australian sorghum using wild relatives

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# Abstract

The genetic diversity of Australian sorghum breeding populations has declined partially as a result of selection for sorghum midge (*Stenodiplosis sorghicolar* (Coquillett)) resistance and stay-green (a drought resistance trait). Wild relatives of sorghum represent a potentially valuable source of novel alleles to diversify Australian sorghum breeding populations, enhance yield advance and reduce genetic vulnerability. Diversification using wild sorghum via conventional pedigree breeding is likely to be inefficient due to the disruption of favourable linkage blocks. Instead, we have used a limited backcrossing strategy to produce a population of 255 BC1F4 lines derived from a cross between a wild sorghum *(Sorghum arundinaceum)* and an elite parent (31945-2-2). These lines were top-crossed to an elite female and the resulting F1 hybrids tested for grain yield at four trials. Significant numbers of progeny with grain yields similar to or greater than the recurrent parent were recovered. The lines were then screened with molecular markers and the regions associated with enhanced grain yield from the *S. arundinaceum* parent identified.

# Media summary

Wild sorghum species prove to be a source of useful genes for improving grain yield in hybrid grain sorghum.

#### Key words

#### Introduction

Selection for adaptation, productivity and quality attributes in pedigree breeding programs will result in the development of populations of breeding lines that are enriched with favourable linkage blocks and epistatic complexes. In Australian sorghum breeding programs, strong selection for resistance to the sorghum midge (Stenodiplosis sorghicolar (Coquillett)), stay-green (a drought resistance trait) and other agronomic traits has been associated with a decline in genetic diversity as measured by molecular markers. This decline was due to the effects of selection per se, genetic drift and linkage drag (Jordan et al. 1998, Jordan et al. 2002 and Jordan unpublished data). Low levels of genetic diversity in a hybrid breeding program can lead to increased vulnerability to unexpected pest or disease outbreaks, reduced genetic progress via plant breeding and limited expression of heterosis in F1 hybrids. In hybrid crops such as grain sorghum, there is a requirement to maintain genetic diversity within male and female breeding populations, but also to exploit heterosis by maximising the genetic distance between male and female breeding populations. Diversification of breeding populations via pedigree breeding can be inefficient because the lines used to diversify breeding populations are generally not well adapted to the target environment of the breeding program. Crossing adapted and exotic germplasm results in the break up of favourable linkage blocks and epistatic combinations. This is particularly the case with wild species because of the high frequency of unfavourable alleles. Backcross breeding is the method of choice for maintaining favourable gene combinations and linkage blocks, while introgressing valuable genes. This method has been widely used for disease resistance genes that have high heritability. Due to linkage drag and low heritability backcross breeding has been less successful for complex traits (such as yield) controlled by many genes. The advanced backcross QTL analysis technique (Tanksley and Nelson 1996) has been suggested as a method by which genes for quantitative traits present in exotic germplasm can be detected and exploited. This paper reports on the use of the advanced backcross technique to diversify Department of Primary Industries and Fisheries (DPI&F) grain sorghum germplasm using a wild relative.

#### Materials and methods

Sorghum arundinaceum is a wild relative of grain sorghum that is endemic to Australia. It has a tall (about 2m) grassy plant type with many tillers, open panicles and small grain that shatter from the head. The species is similar in appearance to Johnson Grass (S. halepense) but without rhizomes. S. arundinaceum is sexually compatible with grain sorghum and has the same chromosome number. We produced a population of BC1F4 progeny by crossing a single plant of S. arundinaceum with an elite restorer line 31945-2-2. This line was developed by the DPI&F sorghum breeding program and is adapted to the local environment. The resulting F1 plant was backcrossed to 31945-2-2 to produce a population of 1000 BC1F1 progeny. Selection was carried out over the following three generations of self-pollination for plants which did not shatter and which had appropriate height and maturity for yield testing. 230 BC1F4 progeny were top crossed to A23171, an elite female (male sterile) parent from the QDPI program, and the resulting hybrids were tested in four replicated trials at Biloela and Warwick in Queensland. The trial at Warwick was planted on the 28/11/02 while the trials at Biloela were designated Biloela 2002, Biloela 2003-1 and Biloela 2003-2 and were planted on the 16/1/02, 20/1/03 an the 5/2/03 respectively. Each trial consisted of four row plots that were hand thinned to a consistent population density. Only the middle two rows of each plot were harvested to control for any potential competitive effects. The Biloela 2002 trial was subjected to significant post-anthesis water stress while the other three trials were well watered.

The population was screened with 150 AFLP markers previously mapped in an F2 population derived by selfing the F1 plant used to generate the BC1F1 population (Janneke Drenth, unpublished data). Single marker regression was carried out using the AFLP data to detect putative QTLs associated with yield in each environment. The allele effect and the coefficient of determination (R<sup>2</sup>) were calculated for each marker. Markers that showed statistically significant associations with yield at P<0.05 were identified. For linked markers, only data relating to the most significant marker are presented in this paper.

## Results

The trial mean and broad sense heritability of grain yield are presented in Table 1. This table also shows the genetic correlations among the four environments.

Site	Mean yield kg/ha	Broad sense Heritability	Genetic Correlations		
			Warwick	Biloela 2	Biloela 1
Warwick 2002	7.57	0.57	1.00	0.69	0.67
Biloela 2003-2	5.77	0.56	0.69	1.00	0.90
Biloela 2003-1	6.31	0.52	0.67	0.90	1.00
Biloela 2002	3.68	0.13	0.30	0.40	0.40

# Table 1. Mean, broad sense heritability and genetic correlations for grain yield for three trials containing 230 Bc1F4 testcross hybrids

The histograms presented in Figure 1 show the distribution of hybrid grain yields from the population in each environment expressed as a percentage of the recurrent parent hybrid (*i.e* 23171/31945-2-2). The distributions all approximated a normal distribution. In three trials (Biloela 2003-1, Biloela 2003-2 and Warwick) the check hybrid was close to the most frequent yield while at the Biloela 2002 trial, the check

hybrid was towards the low end of the distribution. With the exception of Warwick 2002, BC1F4 testcrosses were identified that yielded significantly (p<0.050) more than the recurrent parent hybrid.

Genomic region	Source of positive allele	Trials where QTL detected	Most significant marker trait association	Largest R <sup>2</sup>	Largest effect size kg/ha
D1	31945-2-2	B2003-1, B2003- 2	0.0005	6.3%	1060
D2	S. arundinaceum	B2003-2	0.005	4.2%	520
E	31945-2-2	B2003-1, B2003- 2, W2002	0.00001	9.1%	1080
F1	31945-2-2	B2003-1, B2003- 2, W2002	0.002	4.6%	470
F2	S. arundinaceum	B2002	0.004	3.8%	330
G	S. arundinaceum	W2002	0.02	2.6%	470
н	31945-2-2	W2002	0.02	2.6%	430
J	31945-2-2	B2003-1, B2002, W2002	0.0017	4.9%	800

Table 2. Source and effect of putative QTL detected in four yield trials

Seven regions of the genome were found to be associated with genetic variation for yield in these environments (Table 2). Two regions were identified where the favourable allele was derived from *S. arundinaceum* with the remaining five regions coming from the cultivated parent.



Figure 1. Frequency Distributions of grain yields from a population 230 Bc1F4 testcross hybrids grown in four environments expressed as a % of the recurrent parent hybrid.

#### Discussion

The advanced backcross procedure advocated by Tanksley and Nelson (1996) involves QTL analysis using progeny derived from two or three cycles of backcrossing and selection. While backcrossing and selection can enhance QTL detection in diverse crosses by removing genes with major deleterious effects on adaptation, it also results in a reduction in genetic variance and an increasing unbalance of the number of individuals contributing to the non-recurrent parent marker class. The latter effects have the potential to reduce the power of mapping experiments to detect QTL (Kaeppler 1997). Our decision to use a single backcross was based on the high frequency of relatively adapted lines in the BC1F2 generation and our desire to retain as much of the *S. arundinaceum* genome as possible to maximise the power of our experiment. In addition, the assessment of yields of testcross hybrids rather than that of the line per se meant that deleterious recessive genes from the wild parent would be masked by the favourable alleles from the elite female.

Despite the single backcross and selection for appropriate height and maturity, considerable levels of genetic variance for yield were observed in the population. This was reflected in the moderate to high estimates of broad sense heritability calculated for the well watered trials indicating relatively high levels of genetic variance relative to phenotypic variance. Low heritability is commonly observed in water stressed trials and the low heritability of yield of the Biloela 2002 trial (Table 1) is most likely a result of the high levels of stress experienced by this trial. The genetic correlations between the three well watered trials are high indicating that crossover GxE interactions were not substantial and that the relative rankings of the lines were relatively consistent across environments. The low correlations between Biloela 2002 and the other trials indicate substantial changes in genotype rankings most probably due to differential response of the hybrids to water stress.

Given the low yielding phenotype of *S. arundinaceum* and the likely frequency of deleterious alleles at many loci, it was surprising to recover significant numbers of progeny with similar yield to the recurrent parent, and more surprising still to identify progeny that may have significantly higher yields. As expected

a greater number of favourable QTLs for yield were detected in the cultivated parent than the wild parent, and these QTL were detected more consistently than those from the wild parent. The consistency of detection may be in part due to the relatively small effect sizes associated with the wild QTLs. The results of the Biloela 2002 trial are tantalising, and provide an indication that alleles from *S. arundinaceum* may be of greater value in water stress environments. Numerous studies have shown that heterosis is able to make a greater contribution to yield in stress environments than high yielding environments (Axtell et al 1999). However, the results from the Biloela 2002 trial need to be considered with care due to the low heritability of yield from this trial.

## Conclusion

These results are only preliminary as the genome coverage provided by the current set of markers is poor which could result in QTLs not being detected. Also there is scope for the application of composite interval mapping and or some other covariate approach to remove the influence of large effect QTLs. The surprisingly high performance of the BCF4 progeny indicates that the introgressed regions from *S. arundinaceaum* can contribute to diversity in the cultivated background without yield penalty, and probably contain alleles that contribute to yield. Analysis of populations derived by crossing selected BC1F4 individuals to 31945-2-2 will be required to confirm the value of the *S. arundinaceum* alleles in cultivated genetic backgrounds, particularly in stress environments. While the effect sizes are relatively small, the results of this research indicate that *S. arundinaceum* has the potential to contribute valuable genes for yield improvement. Since the *Sorghum* genus has a wealth of sexually compatible species and landraces, we believe that this breeding methodology could be successfully utilised to increase genetic diversity and improve sorghum grain yield.

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