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Targeted mutagenesis in sorghum using an improved high-throughput screening platform – a reverse genetics strategy to complement the sorghum genomics effort

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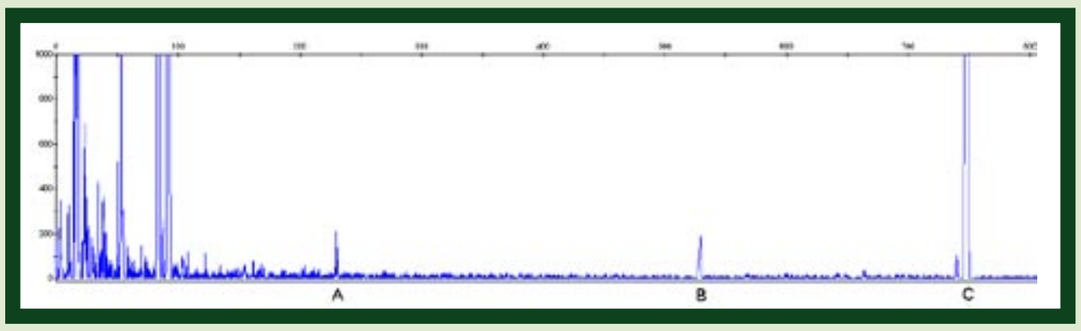
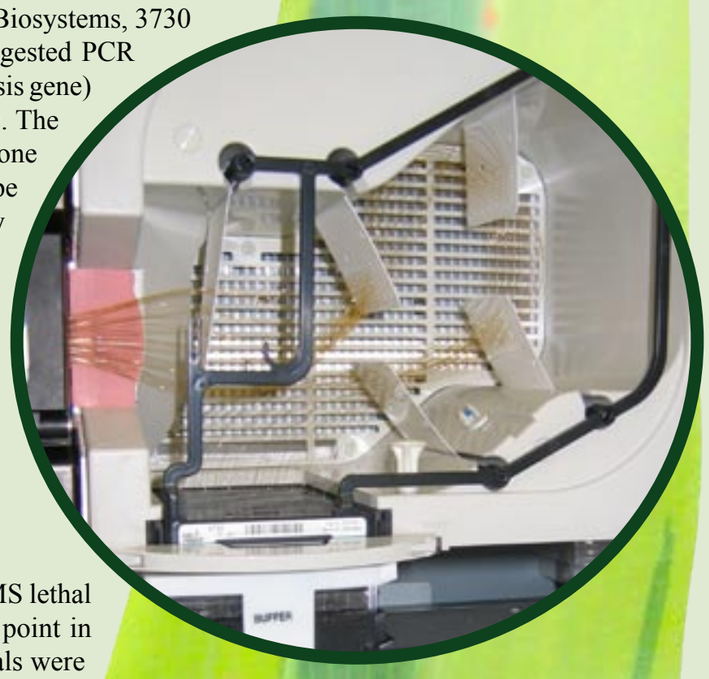
As a natural complement to the Sorghum Genomics effort, our group has undertaken a reverse genetics strategy involving point-mutagenesis, followed by CEL I mismatch screening. EMS-mutated populations have been generated in four white Sorghum lines. Our research has further developed the mutation screening technique of CEL I mismatch detection. Our modified technique is a significant improvement on the standard protocols (originally optimized for slab gel systems). Our protocol benefits from the increased sensitivity offered by a capillary electrophoresis platform, enabling a larger degree of sample pooling. Throughput has also been improved via a significant reduction in the amount of background detected. In addition, our protocol uses a reduced number of steps, once again improving the efficiency of the mutation screening process. Dosimetry trials were performed and eight treatments were chosen for application across all four lines. Treatments were chosen to represent a range of interactions between concentration (of EMS) and time (of imbibition). The resulting M2 populations will be initially mined for mutations in the genes responsible for starch synthesis and grain quality. Previous work with Maize, a model organism for Sorghum, has suggested that the EMS induced mutation density, will be approximately 2 transitions per megabase. Based on this, a population size of 5000 individuals will be sufficient to deliver a large allelic series in any gene of interest (assuming extant sequence information is available). M2 plants will be grown to maturity and M3 seed collected for phenotypic analysis of the lines of interest. In addition to our starch analysis, the populations may be made available as a resource for the Sorghum research community.



Electropherogram (Applied Biosystems, 3730 DNA Analyzer) of CEL I-digested PCR amplicons (of a starch synthesis gene) from eight different varieties. The digested pool consisted of one mutant and seven wild-type amplicons (as confirmed by sequence analysis). PCR products were labeled at both ends with 6-FAM, thus enabling both fragments from the CEL I digest to be detected. The two digested fragments are positively identified as being true cuts at a point of heteroduplex mismatch, since their sizes (peak 'A' -225bp and peak 'B' -525bp) sum to give the full amplicon length (peak 'C'-750bp). **The strong signal, relative to background, indicates that higher degrees of pooling will be possible using the capillary electrophoresis platform.**



The initial aim of our sorghum mutagenesis experiments was an EMS lethal dosage of 60%. This figure was assumed to be a good starting point in the determination of the optimal mutagen dose. Dosimetry trials were performed on a single inbred sorghum cultivar, in an attempt to model the probability of lethality as a function of concentration (of EMS) and time (of imbibition). Eight broadly ranging treatments were chosen, which approximated the required lethality. These treatments were then applied to four distinct inbred cultivars.

Cultivar 1

Treatment	EMS conc. (mM)	Imbibition time (h)	Seeds treated	Surviving plants	Lethality	M1 plants with pigment defects	M1s with pure white sectors
81	50	19.3	-	-	-	-	-
98	100	4.1	-	-	-	-	-
102	30	17.8	-	-	-	-	-
127	100	5.4	-	-	-	-	-
137	65	11.0	-	-	-	-	-
138	75	12.3	-	-	-	-	-
139	40	18.6	-	-	-	-	-
140	100	4.7	-	-	-	-	-
		Total	-	-	-	-	-

Cultivar 2

Treatment	EMS conc. (mM)	Imbibition time (h)	Seeds treated	Surviving plants	Lethality	M1 plants with pigment defects	M1s with pure white sectors
81	50	19.3	417	89	79%	48%	9%
98	100	4.1	400	209	48%	33%	8%
102	30	17.8	508	288	43%	31%	14%
127	100	5.4	500	290	42%	27%	6%
137	65	11.0	-	-	-	-	-
138	75	12.3	-	-	-	-	-
139	40	18.6	418	104	75%	70%	41%
140	100	4.7	400	262	35%	22%	2%
		Total	2643	1242	mean 53%	mean 33%	mean 10%

Cultivar 3

Treatment	EMS conc. (mM)	Imbibition time (h)	Seeds treated	Surviving plants	Lethality	M1 plants with pigment defects	M1s with pure white sectors
81	50	19.3	440	3	99%	33%	0%
98	100	4.1	400	109	73%	28%	4%
102	30	17.8	528	193	63%	23%	2%
127	100	5.4	472	137	71%	26%	5%
137	65	11.0	-	-	-	-	-
138	75	12.3	-	-	-	-	-
139	40	18.6	440	42	90%	21%	2%
140	100	4.7	390	124	68%	19%	7%
		Total	2670	608	mean 77%	mean 24%	mean 4%

Cultivar 4

Treatment	EMS conc. (mM)	Imbibition time (h)	Seeds treated	Surviving plants	Lethality	M1 plants with pigment defects	M1s with pure white sectors
81	50	19.3	375	54	86%	30%	2%
98	100	4.1	400	129	68%	36%	1%
102	30	17.8	470	229	51%	44%	3%
127	100	5.4	495	162	67%	38%	2%
137	65	11.0	-	-	-	-	-
138	75	12.3	-	-	-	-	-
139	40	18.6	383	104	73%	38%	7%
140	100	4.7	400	145	64%	37%	6%
		Total	2523	823	mean 67%	mean 39%	mean 4%

EMS treated seeds were germinated in the glasshouse, and transplanted to the field, one to two months after planting. Of those samples counted, 2673 M1 plants survived from 7836 treated seeds (66% lethality). Of the surviving M1 plants, 33% had pigment defects, and of these, 7% were plants with pure white sectors.

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