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Screening for male-sterile mutants that act before meiosis to disrupt normal cell fate specification in maize anthers

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To learn more about cell fate acquisition in the maize anther, we are focusing on mutants disrupted after organ specification in the tassel, laying accessory vegetative structures and the stamens. Mutants of interest have a normal filament, but one or more anther locule cell types mis-differentiate prior to the start of meiosis. During this approximately 7-10 day period of anther development of interest here, mitotic divisions establish populations of L1 (epidermal) and L2 (all other cell types) cells within anther locule primordia. In a transverse section a normal anther locule will contain just 5 cell types organized in rings. Starting from the outside, these layers are epidermis, endothecium, middle layer, tapetum, and microsporocytes. This developmental window is currently bracketed by *msca1* (formerly *ms22*) in which no anther locule cell types are normal and *am1* in which the centrally located presumptive meiotic cells conduct mitosis instead. Anthers in which one or more cell types fail to achieve normal function, placement, or numbers are of interest. In the mutants studied thus far, lack of normal somatic cell types seems to result in meiotic arrest, suggesting that the anther is a "system" and if any component is missing or defective, development arrests. We will be evaluating the transcriptional profiles in defined cell layers collected with laser microdissection to fine tune our understanding of early anther development.

Targeted *Mu* tagging is being used as a prelude to cloning and analysis of key genes. Highly active *Mu* lines heterozygous for a mutable *bz2* reporter allele are crossed to multiple *ms-reference//ms-reference* individuals with colorless or *bz2* seed for construction of tagging populations and to *bz2* tester (validation test performed on the same pollen collection). Seed resulting from validated pollen donors yielding ~50% spotted progeny kernels in the *bz2* test cross are pooled to organize families for the screening field. This screen is conducted at Cal Poly-SLO and is organized to provide an undergraduate summer training program. The tagging population should consist mainly of male fertile plants (*ms-reference//+*) with rare sterile individuals (*ms-reference//ms-Mu*). Fields of up to 100,000 plants are screened daily, and all tassels shedding pollen are clipped off. Newly identified mutants are photographed, shoot bagged, and ultimately crossed by *a1 Bz2 Mu killer* stocks to promote epigenetic silencing of the Mutator transposon family. In this cross, progeny seed are purple (*A1//a1 Bz2//bz2* or *bz2-mu*), while contaminating pollen from unclipped fertile tassels should result in colorless, bronze or spotted kernels. Validation of individual "puton" male-sterile plants is done with two molecular tests: PCR assay for the *bz2-mu* allele (expected in 50% of authentic mutants) and/or RT-PCR assay for a high level of the *mudrA* transposase transcript. In a number of cases, multiple sterile plants are identified within large families, consistent with pre-meiotic *Mu* insertion. Segregation of the *bz2-mu* reporter allele is tested on the group. Lessons learned from an initial tagging effort with *mac1* in 2005 have nearly eliminated false positive "contaminating" *ms-reference* carriers in the tagging populations: detassel all sources of *ms-ref* allele fertile plants and construct populations in the field, not the greenhouse. Table 1 summarizes the current screening results.

Table 1. Tagging populations and yield of putons and confirmed *Mu*-induced mutants.

		Tagging population		Male-sterile individuals		
Locus	Year(s)	No. of families	No. of kernels	Putons	*Mu* parent confirmed	Frequency
mac1	2005	210	31,237[c]	10	3	1.4×10^{-4}
	2007	34	23,083[c]	13	12	6.9×10^{-4}
ms25	2006	140	35,665	6	6	1.7×10^{-4}
msca1	2006	120	40,265	4	4	1.0×10^{-4}
ms8	2007	22	23,083	6	5	2.2×10^{-4}
ms23	2005	79	19,981	0	0	0
	2007	27	26,565	8	8	3.0×10^{-4}

The second strategy involves assessing already reported *ms* mutants of maize, primarily from phenotypic screens in the Maize Gene Discovery Project (*RescueMu* tagging), UniformMu, MTM, etc. Under the milder growing conditions of California, compared to the initial screening sites in the Midwest, about ½ of the reported ms mutants fail to exert anthers. Among these, a smaller percentage show arrested anther development (2-3 mm) consistent with problems in cell fate acquisition or maintenance. Mutants with abnormal anther length, shape, or integrity are recovered for more detailed cytological analysis to identify mutants with the characteristics important to this screen.

A third strategy involves using transcriptome profiling data for maize anthers generated on 22K and 44K Agilent arrays and the database of sequenced *RescueMu* insertion sites. The tagging grids utilized in the *RescueMu* strategy yielded plasmid DNA sequencing templates from both the Rows and the Columns of the grid, however, only the Row *RescueMu* plasmids were sequenced to reasonable depth; approximately 2-4 columns per grid were sequenced as a check that some *RescueMu* insertion sites were found in both a Row and a Column, thus specifying a particular plant within the original grid. Several hundred Row + Col sequence matches were identified for anther expressed genes and thousands are available for Row only matches; the corresponding column(s) for each insertion site are being identified by PCR of library plates containing the rescued plasmids for each row and each column of a grid. The grid plants were self-pollinated, hence the expectation is that if a *RescueMu* insertion disrupts a gene required for normal anther development prior to meiosis, ¼ of the progeny will be male-sterile and these individuals should be homozygous for the *RescueMu* insertion at the target anther expressed gene.