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A molecular catalog of independent mutations that inactivate Argonaute5 encoded by the epistatic k1 alleles resulting in release of gene silencing and the saddle seed trait

*Lila Vodkin**, Department of Crop Sciences, University of Illinois, Illinois, USA
Sarah Jones, Department of Crop Sciences, University of Illinois, Illinois, USA
Young Cho, Institute for Genomic Biology, University of Illinois, Illinois, USA

The “saddle” pattern of mixed black and yellow colors on the same soybean seed results from interactions of silencing alleles at the *I* (inhibitor) locus with recessive alleles of the *K1* locus, recently identified to encode an Argonaute5 (*AGO5*) protein of the RNAi pathway (Cho et al., Plant Cell 2017). Recessive *k1*alleles overcome the effect of the dominant *I* and I^{i} alleles that normally inhibit seed color by producing short-interfering RNAs (siRNAs) targeting chalcone synthase (*CHS*) mRNAs. In a Clark mutant (PI547439) with homozygous I^{i} *k1* alleles, we found a 129 bp deletion in the *AGO5* gene that would lead to an inactive protein. We then used NGS amplicon sequencing of the 6.2 kb *AGO5* gene (Glyma.11G190900) from a number of additional spontaneous saddle pattern mutant lines from the USDA germplasm collection and determined that five more of them had different lesions in the *AGO5* gene relative to each parent variety. Generally, these were small deletions or insertions in one of the 20 exons that would cause altered protein structure and premature termination. The letter K appears to have been chosen as the gene symbol from Kurakake, the Japanese variety that was used as the source of the saddle trait in the first reported inheritance studies in 1929. We examined two varieties named Kurakake and they both contained the same 4 bp deletion within Exon 6 of the *AGO5* gene that would cause premature termination. However, there are other genes that produce a saddle phenotype as does the I^{i} *k1* combination, including the I^{k} allele of the *I*locus and the *k3* allele. We are currently genotyping more saddle pattern variants in the USDA germplasm collection to determine whether other genes in the silencing pathway, in addition to AGO5, can be identified at the molecular level.