INTRODUCTION

- Concerns about gene flow from sorghum to its weedy relatives (shattercane, SH; and johnsongrass, JG) have limited regulatory approval of genetically modified traits, such as herbicide resistance. This significantly limits sorghum production relative to corn (Zea mays L.), despite sorghum’s greater drought tolerance.

- ALS-resistant (ALS-R) grain sorghum ('Inzen', DuPont-Pioneer) is in the final stages of commercialization. Inzen carries a target-site mutation in the ALS gene (Trp574Leu) conferring resistance to imidazolinone and sulfonurea herbicides.

- Inzen will inevitably outcross to SH and JG; thus, its unique alleles may serve as an effective landscape-level indicator of weed evolution responses when a new nuclear crop trait is highly adopted by growers, transfers to weedy relatives, and increases their fitness in typical production systems.

- Competitive allele-specific PCR (KASP) markers are a fluorescence-based SNP genotyping technology. Molecular inversion probes (MIPs) have been used in human genetics for many years to screen populations of cancer cells at rapidly evolving loci. MIPs can be multiplexed, and each one can amplify target sequences from 1-500 bp.

- The high-throughput, low-cost nature of these markers makes them especially suitable for large-scale population genetic studies.

- Here we present the current status of KASP and MIP marker development intended to monitor ALS allele variants and SSR loci in sorghum, SH, and JG as the foundation of a regional monitoring program for crop-to-weed gene flow and weed population genetic diversity following commercial deployment of Inzen sorghum.

- Similar methods could be deployed for any ecological risk monitoring study.

MATERIALS AND METHODS

- KASP primers targeting a unique short-range haplotype of Inzen (Val560Ile + Trp574Leu) were used to screen samples of Inzen (ALS-R), a wild-type grain sorghum hybrid (87P06, DuPont-Pioneer), and SH and JG populations segregating for wild-type and ALS-R alleles (Welte et al, 2014) (Figure 1 & Table 1).

- Libraries were prepared for 7 samples previously genotyped by KASP using a KAPA HyperPlus kit and KAPA A & B adapters. Size selection was optimized for 500 bp fragment lengths using KAPA Pure Beads. Adapter-ligated libraries were amplified in 8 cycles of PCR to generate 100 ng finished libraries. Samples were normalized such that JG DNA concentration was 2x that of sorghum and SH DNA concentration.

- Whole-genome sequence was generated on an Illumina NextSeqTM 500 using 75 bp paired-end reads. 1x coverage was obtained using a gap-tolerant aligner (GSNAP) to the reference genome (v3.1). Alignments at ALS are found in Figure 2.

- 41 SSRs (Billot et al, 2013; Table 2) used to genotype over 3300 accessions of wild and domestic sorghum from the global composite germplasm collection (GCGC) covering all ten chromosomes are being converted into MIPs.

RESULTS & DISCUSSION

- The short-range Inzen ALS haplotype (Val560Ile + Trp574Leu) was detected in only one population of JG (JG-35); however, no individual was homozygous, confirming the relative rarity of the Inzen ALS-R allele.

- 27 SSR primer pairs showed 100% homology with the reference genome, whereas the remaining 14 have SNPs or are not specific to single loci, and are under further development.

- In a regional monitoring program, KASP markers may be used to preliminarily determine if new ALS-R in situ weed populations have the short-range Val560Ile + Trp574Leu Inzen haplotype. If so, plants may be genotyped with MIPs targeting a longer-range Inzen ALS haplotype. Additional MIPs targeting SSRs will simultaneously document population genetic diversity while providing supporting evidence of gene flow.

CONCLUSION & FUTURE DIRECTIONS

- MIPs targeting ALS, which we previously de novo assembled across Sorghum species (Welte et al, 2017), are being developed to identify a longer haplotype unique to Inzen to distinguish between newly-evolved alleles in SH and JG due to herbicide selective pressure, and those conferred by crop-to-weed gene flow.

- Results from ecological risk monitoring studies will assist Federal regulatory agencies (USDA-APHIS) in making science-based decisions about the introduction of genetically-modified sorghum in the U.S., plant breeding companies interested in limiting gene flow from genetically modified sorghum to its wild relatives, and other researchers developing environmental stewardship plans for the management of potential genetically modified sorghum traits.

- We will be field-testing a portable, isothermal PCR prototype using KASP genotyping chemistry in 2017. This technology will be able to determine if weedy populations contain the short-range Val560Ile + Trp574Leu Inzen haplotype in as little as 30 minutes (Figure 3).

- If an Inzen allele is preliminarily detected, individuals will be collected and sequenced in the lab using MIPs to confirm gene flow from Inzen and the frequency of ALS-R alleles in the population.