

December 20, 2011

Michael Gregoire
Deputy Administrator, Biotechnology Regulatory Services
4700 River Rd, Unit 98
Riverdale, MD 20737

Re: Confirmation of regulatory status

Dear Dr. Gregoire,

I am writing to seek confirmation from Biotechnology Regulatory Services that a new agricultural technology developed by my laboratory does not meet the definition of a regulated procedure.

My laboratory implements a procedure to down-regulate expression of a native plant gene, *MUTS HOMOLOG 1 (MSH1)*, by RNA interference. The process involves introduction of an RNAi transgene to a crop of interest (*Sorghum bicolor* L), via *Agrobacterium*-mediated transformation, followed by removal of the transgene by natural genetic segregation. Derived plant lines, confirmed to lack the transgene but displaying a novel phenotype, are then crossed to wildtype germplasm to induce an epigenetic effect in subsequent generations. As a plant breeding strategy, a subsequent (non-transgenic) selection that is deployed for field testing would be 6-10 generations removed from the transgenic progenitor. Consequently, the transgene is used in a single-generation process to induce an epigenomic effect, followed by removal of the transgene and subsequent plant selection using traditional plant breeding strategies. We submit that this procedure, which deploys only non-transgenic materials for testing, would present no risk of creating a plant pest, according to the Plant Protection Act.

The method utilizes a disarmed *Agrobacterium* strain for introduction of the transgene. The RNAi construction includes:

- *Zea Mays* Ubiquitin 1 promoter
- Segment from *Sorghum bicolor* MSH1 endonuclease domain cDNA
- Intron sequence from *Arabidopsis thaliana* Chromosome IV
- Inverted segment from *Sorghum bicolor* MSH1 endonuclease domain cDNA
- CaMV 35S terminator
- Standard binary vector pPZP212 used for cloning

The phenotype that is created by MSH1-RNAi suppression involves developmental reprogramming, including reduced growth rate, enhanced branching, delayed flowering, and delayed transition to maturity. The crossing of these reprogrammed plants to wildtype plant lines results in markedly enhanced agronomic variation for plant biomass, growth rate, yield, height and several other agronomic parameters. These traits respond to selection in subsequent generations and can, therefore, be integrated to a plant breeding regimen.

Because the derived materials, using our procedure, contain no foreign DNA sequences and are modified in no way genetically other than, perhaps, by endogenous transposable element activity and/or epigenetic DNA modifications, we submit that these modified materials do not comprise APHIS regulated articles.

Sincerely

A handwritten signature in cursive script that reads "Smackenzie".

Sally Mackenzie
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