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Research Category: **GDER** **Duration of Award: 1 Year**

Project Title: Down with DON: Stable Expression of RNAi Contructs in a Marker-free Plant.

PROJECT 2 ABSTRACT

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The goal is to reduce FHB and DON in *F. graminearum* (*Fg*)-infected transgenic barley grain via expression of double-stranded (ds) RNA that has homology to key *Fg* genes for mycotoxin synthesis and/or pathogenicity. Expression of targeted genes will be suppressed via RNA interference (RNAi). Although the intended phenotype is FHB resistance, the focus of this proposal is on the development and demonstration of two improved methods of introducing transgenes: direct transposition-mediated (*Ds*) delivery and recombinase-mediated cassette exchange (RMCE). Transgenes will be delivered first via *Agrobacterium* followed by secondary delivery via transposition. Transposition is enabled by flanking transgenes with short, maize-derived *Dissociation* (*Ds*) terminal sequences which interact with *Ac transposase* (*AcT*). *AcT* will be introduced via hybridization with *AcT*-expressing plants. Transposition will deliver single-copy, *Ds*-flanked transgenes to favorable locations, and de-link them from vector backbone and selectable markers, enabling production of transgenic plants without vector backbone or marker genes. Transgenes will be either a *Ds*-flanked dsRNA-producing cassette or *Ds*-flanked TAG site. The TAG site includes sequences that enable RMCE. RMCE requires an extra step: after transposition of TAG sites, EXCH vectors carrying dsRNA transgenes will be introduced and incorporated into TAG sites via site-specific recombination.

Important elements of this proposal include: 1) delivery to useful cultivars (Conlon, Pinnacle); and 2) testing RNAi vectors in *F. graminearum* (*Fg*), prior to their introduction into plants, to enable rapid screening and optimization of potential RNAi vectors. We have secured ARS funding of ~90% of the salary of a post-doctoral researcher to support this aspect of our proposed research. Our objectives are to:

1. Construct a) *Ds*, b) RMCE, and c) EXCH barley backbone vectors (a and b completed; c in progress).
2. Construct fungal RNAi vectors targeting *TRI5, TRI6, & LAEA,* and test them in *Fg* (in progress).
3. Introduce dsRNA sequences effective against *Fg* into barley *Ds* and EXCH vectors (in progress).
4. Produce transgenic Conlon plants with *Ds*-bordered *Ds*-vectors or TAG sites (in progress).
5. Initiate transposition of *Ds*-bordered sequences by crossing to *AcT* plants (in progress).
6. Select plants with *Ds*-vectors or TAG sites segregated from *AcT* and the original insertion site.
7. For RMCE only: Introduce EXCH vectors carrying antifungal transgenes that will be incorporated into TAG sites via site-specific recombination.
8. Characterize transgene expression, FHB severity/DON, plant performance, and develop resistant lines.

With FY16-17 funding, we expect to complete Objectives 1−5 and begin addressing 6−8.