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Agriculture and Food Development Authority

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Dr. Michael J. Firko
APHIS Deputy Administrator,
Biotechnology Regulatory Services
4700 River Rd, Unit 98
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October 7th, 2015

Re: Confirmation of Regulatory Status for Ensifer Mediated Transformation (EMT)

Dear Dr. Firko,
We have recently developed a novel transformation platform for modifying the genetic content of crop plants. The platform relies on the naturally occurring gene transfer capabilities of the soil bacterium *Ensifer adhaerens* in a transformation method termed 'Ensifer Mediated Transformation' (EMT). Because *E. adhaerens* itself is not a plant pest, no endogenous plant pest components are involved in the transformation. In addition, the native plant genomes that will be used are fully classified, hence we understand there is no basis to conclude that crop plants engineered with this vector agent are regulated articles within the meaning of 7 C.F.R § 340.1, as they do not satisfy the regulatory criteria that would subject it to the Animal Plant Health Inspection Service's (APHIS) oversight. However, before proceeding further we request that Biotechnology Regulatory Services confirm our understanding that crop plants modified via EMT for the purposes of transgenesis/cisgenesis/genome editing (e.g. ZFN, TALEN, CRISPR/Cas) are not regulated articles within the meaning of the current regulatory framework.

I. The novel vector agent *Ensifer adhaerens*

To facilitate understanding of the organism in question we wish to outline the following summary information:

A. General Biology/Behaviour

E. adhaerens is a non-spore forming, gram-negative soil bacterium (Willems et al. 2003) that demonstrates non-nodulating behaviour toward its symbionts (Rogel et al, 2001). Investigations of *E. adhaerens'* behaviour indicate a non-obligate predatory behaviour toward other bacterium that is only advantageous to the host in poor and low-quality soil and growth mediums (Casida, 1982). A plant beneficial bacterium (Zhou et al. 2013), the non-pathogenic *E. adhaerens* (strain Ov14), which underpins EMT, was originally isolated from agricultural soil and has been morphologically assessed (Rathore et al. 2015).

B. Phylogenetic classification of *E. adhaerens* and its genetic distinction from *A. tumefaciens*

E. adhaerens strain Ov14 is housed within the Family Rhizobiaceae, belonging to the Genus Ensifer/Sinorhizobium. A comparative genomic assessment between the genome of *E. adhaerens* strain Ov14, *A. tumefaciens* and an additional plant transforming bacterium *Sinorhizobium meliloti* confirms the synteny between *E. adhaerens* strain Ov14 and *S. meliloti*, in contrast to that seen between *E. adhaerens* and *A. tumefaciens* (see Fig. 1, Rudder et al. 2014); with *E. adhaerens* strain Ov14 lacking several of the vir genes necessary for genetic transformation by *Agrobacterium* spp. Furthermore, the genetic separation of *E. adhaerens* strain Ov14 and *A. tumefaciens* is further confirmed based on a

phylogenetic assessment of 19 conserved genes found across both genomes (see Fig. 3, Rudder et al. 2014).

C. Transformation Platform

The genetic transformation platform that is EMT is based on the use of *E. adhaerens* strain Ov14, which when equipped with an exogenous Ti plasmid (in a binary or unitary plasmid arrangement) employs its endogenous genetic systems to successfully engineer the targeted plant genome. With a continuously expanding host range, (to date, safflower, barley, wheat, canola, potato, cassava, tobacco, rice, maize, soybean have been confirmed as amenable to EMT via stable and/or transient transformation assays), EMT is applicable to both monocot (Zuniga et al. 2015) and dicot (Wendt et al. 2012) recipient organisms.

II. The novel vector agent, *Ensifer adhaerens* and its position within the definition of a regulated article

Under APHIS regulations, we understand that a genetically engineered organism is considered a "regulated article" if:

- the donor organism, recipient organism, or vector agent belongs to a genera or taxa designated in 7 C.F.R. § 340.2, and
- the organism meets the definition of a plant pest.

Acknowledging that specific donor and recipient organism(s) may require review on a case-by-case basis as EMT-generated varieties are developed, the central point remains that the vector agent itself, *E. adhaerens* is contained within the genus Ensifer/Sinorhizobium and as such does not belong to any taxa explicitly identified in § 340.2. Consequently, we understand that *E. adhaerens* itself does not satisfy the first criterion for a 'regulated article'. Since the first criterion is not satisfied, we understand that it is not necessary to consider the second criterion. Nevertheless, in regard to the second criterion it is important to point out that *E. adhaerens* does not meet the definition of a plant pest as set forth in § 340.1 either, based on its published biology as a classified rhizobial symbiont. In addition, genetic transformation mediated by the vector agent *E. adhaerens* does not change the plants' basic biological characteristics nor does it produce a plant that would directly feed on, infect, parasitize, or contaminate plants, or adversely affect other organisms that are beneficial to plants. Separately, we understand that an additional definition of a regulated article includes engineered organisms that are unclassified or whose classification is unknown and any plant product that contains such organisms. *E. adhaerens*, as described above, is a well-characterized plant symbiont. Therefore, we comprehend that crop plants genetically engineered via EMT with the vector agent *E. adhaerens* cannot be considered regulated articles because *E. adhaerens* is either unclassified or because its classification is unknown.

III. Finding that engineered crops developed with the vector agent *E. adhaerens* are not regulated articles is consistent with previous APHIS determinations.

Finding that engineered crops developed using EMT with *E. adhaerens* as the vector agent are not regulated articles is consistent with other APHIS regulatory status determinations. For example, APHIS has previously determined that engineered crops developed via biolistic (gene gun) methods that do not introduce genes from plant pest species into plant pest recipients were not regulated articles. Relevant examples include: Petunias engineered to produce altered vegetative pigmentation, glyphosate tolerant Kentucky Bluegrass and St. Augustine grass, high yielding switchgrass, and grapes genetically engineered with increased anthocyanin production. The above examples were produced through biolistic methods and were determined to be non-regulated articles as neither donor/recipient organism were plant pests nor was the vector/vector agent used to mediate the genetic transformation.

APHIS has additionally determined that genome editing achieved through oligonucleotide-mediated mutagenesis does not, on its own, produce plants that meet the definition of a regulated article. Relevant examples include: corn with reduced phytate biosynthesis achieved through Zinc-Finger mediated gene knockouts, production of double haploid plants via Centromere-Mediated Chromosome Elimination, targeted deletions achieved through meganuclease (I-Crel) activity and more recently the finding that FAD3KO soybean developed via TALEN editing was not a regulated article. Combined these examples demonstrate instances during which a novel strategy for modifying the genetic content of a plant (or

plants) was recognized to not fall within the regulatory scope of the USDA set forth under 7 C.F.R. Part 340.

IV. Conclusion

APHIS's interpretation of its Part 340 regulations dictates a finding that engineered crops developed with non-plant pest vector agents are not regulated articles. EMT is an enabling technology for the genetic engineering of crop genomes and as such is compatible for the purposes of transgenesis, cisgensis and genome editing. Genetically engineered plants produced with the novel vector agent *E. adhaerens* will not incorporate gene sequences native to plant pests into other known plant pests. Likewise, *E. adhaerens* is not itself a microorganism known to be a plant pest. Therefore, we believe there is no scientific basis to determine that plants developed via EMT are, or will become, plant pests within the meaning of the Plant Protection Act.

We look forward to receiving your response on this Letter of Inquiry and if needed we will be happy to provide further information and/or explanation on our published data via email or through a future meeting if you request.

Yours sincerely,

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