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Dr. Michael Gregoire
Biotechnology Regulatory Services
USDA-APHIS
4700 River Road, Unit 98
Riverdale, MD 20737

Re: Inquiry regarding APHIS position on null-segregant TALEN-mutagenized rice lines as non-regulated articles

Dear Dr. Gregoire,

With this letter, we are asking that Biotechnology Regulatory Services confirm our understanding that a collection of null-segregant rice lines from Transcription activator-like effector nuclease (TALEN)-mutagenized rice plants do not meet the criteria for regulated articles. We plan to plant the seedlings of these lines in an Iowa State University (ISU) research farm located in Ames, Iowa, in the summer of 2014.

Our research group has pioneered in developing and applying TALEN technology in plant genetic mutagenesis (Ting et al, 2010, 2011, 2012). TALENs, fusion proteins of Transcription activator-like (TAL) effector DNA binding domains and the DNA cleavage domain of restriction enzyme *Fok*I, are newly emerging genetic tools for targeted mutagenesis *in vivo*. This biotechnology, when applied in plants, involves several steps to produce null-segregant progeny. First, synthetic TALEN genes are transferred into embryonic cells (so-called calli); second, TALEN proteins expressed from the TALEN genes recognize with a high specificity a DNA sequence in a gene of interest and cause a double strand break that is quickly repaired by a usually error-prone DNA repair process called non-homologous end joining; third, cells that contain the TALEN-transgene with an additional linked selectable marker gene (T-DNA) and desired genetic changes are regenerated into plantlets; and finally, the plants are sexually self- or out-crossed and progeny are screened for those that only contain the desired genetic changes/traits but not the T-DNA that is sorted away through genetic segregation.

Specifically, we have used this technology to generate five rice lines (Ting-1 to -5) from the cultivar Kitaake, a photoperiod-insensitive *Japonica* rice variety that can be grown in Iowa in the summer for one life cycle. These lines contain the TALEN-mutagenized DNA sequence changes

in the promoters of two rice genes, but no T-DNA remains in the genomes. The promoter changes (deletions of a few base pairs) have converted the two disease susceptibility genes into resistance genes to bacterial blight caused by the pathogen *Xanthomonas oryzae* pv. *oryzae* (Xoo). These five lines were selectively bred from self-pollinated, transgenic rice plants mutagenized by the TALENs.

We have performed two rounds of mutagenesis to change the two genes. In the first round, the promoter of the sugar transporter gene *OsSWEET14* (or *Os11N3*) on rice chromosome 11 was mutagenized using synthetic TALEN gene constructs. This particular gene, in addition to its function of plant growth and production, is also a disease susceptibility gene to bacterial blight disease caused by Xoo. The vulnerable element is located in the promoter, which can be activated by two native TAL effectors of some Xoo field isolates. Through the mutagenesis and self-crossing/selective breeding in the laboratory, one TALEN-mutagenized line was obtained that contain the desired genotype and phenotype and that also lack the transgene, as verified by a number of different PCR-based approaches designed to detect the presence of T-DNA.

Similarly, this one line was subjected to another round of mutagenesis with a TALEN construct that targets another rice sugar transporter gene, *OsSWEET11* (or *Os8N3*) on chromosome 8, also a disease susceptibility gene hijacked by some Xoo field isolates. Amongst the progeny of TALEN-mutagenized and self-pollinated plants, five progeny individuals (Ting-1 to -5) were identified that possess the site-specific genetic changes and desired disease resistance trait. Molecular characterization using multiple PCR primers confirmed that these lines do not carry the T-DNA or the backbone of the T-DNA vector. The plants derived from the first round of mutagenesis have been described in detail in our published work (Ting et al., 2012), and we are in the process of preparing a manuscript for publication to describe the plants obtained in the second round of TALEN-mutagenesis.

We are seeking your position on whether these non-transgene containing, null-segregant rice lines are non-regulated articles. We are planning to conduct a field trial of some of these lines in order to evaluate their phenotypic and physiological characteristics. This trial will be conducted in the upcoming summer (2014) at an ISU research location in Ames, Iowa. If needed, we will be happy to provide further information and/or present our published and unpublished data in detail to you and other APHIS regulatory officials in a near future meeting.

Sincerely,

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References

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Li, T., Huang, S., Zhao, X., Wright, D., Carpenter, S., Spalding, M.H., Weeks, P.D. and Yang, B. 2011. The modularly assembled designer TAL effector nucleases for targeted gene knockout and gene replacement in eukaryotes. Nucleic Acids Research, 39, 6315-6325. (doi: 10.1093/nar/gkr188, first published online March 31, 2011).

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