

DATE: January 22, 2011

TO: APHIS Biotechnology Regulatory Service

FROM: Ramsey S. Lewis, North Carolina State University

SUBJECT: Inquiry regarding APHIS position on novel breeding method

APHIS Regulatory Official:

My name is Dr. Ramsey S. Lewis, and I am an associate professor at North Carolina State University where our research activities are directed towards tobacco genetics as well as development of new tobacco cultivars. Our research program has historically used both conventional plant breeding approaches and those involving plant genetic engineering.

Members of our program have an important question for APHIS regarding whether or not a particular type of novel breeding methodology would result in genetic materials considered as regulatable articles according to U.S. law and APHIS guidelines. The question is, 'Is a newly developed plant variety considered a regulatable article if it does not possess genetic elements introduced through the plant transformation process, but was derived from a progenitor individual that did carry such elements?' If a transgene insertion was segregated away under greenhouse or laboratory conditions and cannot be detected, is the non-transgenic derivative considered a regulatable article according to existing laws and regulations?

Our specific example relates to the application of a novel breeding method recently outlined by Lewis and Kernodle (2009; publication attached) to tobacco cultivar development. The approach is a modification of the often-used backcrossing method and utilizes a gene designated as *FT* derived from *Arabidopsis thaliana* to reduce the time required to complete each backcross generation. The backcrossing method is typically used to transfer genes controlling one or a few traits (usually **naturally** occurring) to an existing elite line. Five to eight cycles of backcrossing are typically used to complete this task, and the time required to complete the process is largely dependent upon generation time. In tobacco, this can take greater than three years.

It the method outlined by Lewis and Kernodle (2009), the *FT* gene is introduced into a tobacco plant using transgenic methods and is expressed under the control of the CaMV 35S promoter. The *FT* gene is also linked to the selectable marker gene *nptII*. Using sexual crossing the *FT* transgene locus is first combined into a single genotype also carrying a gene or genes of interest (in our case, **non-alien** genes conferring tobacco harm reduction characteristics). An initial hybridization event is made between this genotype and an elite line into which the non-GMO trait of interest is to be introduced. Multiple backcrosses to the non-transgenic elite line (recurrent parent) are then made under laboratory conditions. In all backcross generations, the breeder would select for the trait of interest (**native** to the tobacco genome) in addition to the early flowering phenotype conferred by the *FT* transgene locus. After the final backcross is made (5 to 8 backcrosses are typically made in a plant breeding program), an early-flowering individual possessing the trait of interest would be self-pollinated. Amongst these progeny, the breeder would identify individuals possessing the non-GMO trait of interest, and that **do not** carry the transgene insertion (i.e. the transgene locus is segregated away). These null segregants are identified on the basis of a normal flowering phenotype. The absence of components of the transgene locus is also subsequently verified by a number of different PCR-based approaches designed to test for the presence of *FT*, the CaMV 35S promoter, *nos* terminator sequence, *nos* promoter, and *nptII* (selectable marker gene). This use of this methodology dramatically reduces the time required to commercialize a new tobacco cultivar.

We desire to utilize this method for transferring traits such as those conferring harm reduction characteristics to elite lines for the purpose of developing commercial tobacco cultivars, but desire some statement from APHIS regarding the regulatable status of the derived materials that **do not** carry the introduced *FT* transgene locus. Our question is, 'Is the newly-derived **non-transgenic**, tobacco line considered a regulatable article according to U.S. law and APHIS guidelines?' Your agency has likely been approached with this question previously. In concept, our proposed breeding method for tobacco is identical to the "FasTrack" breeding method currently being promoted by the USDA-ARS research group at Kerneysville, WV, to accelerate the process of fruit tree breeding (details found at www.ars.usda.gov/News/docs.htm?docid=19385). We have noticed some previous public documentation that could be relevant. When Monsanto deregulated glyphosate-resistant soybean lines derived from a plant designated as '40-3-2,' they reported in APHIS documentation (on APHIS website) and also in published information that they segregated away a fragmented GUS transgene insertion prior to the creation of plant 40-3-2. Plant 40-3-2 does not carry GUS. We see no evidence where GUS and its associated elements were subjected to the APHIS-driven degeneration process in the late 1990's.

We feel that materials derived from our proposed breeding program do not meet the definition of a regulatable article because they do not carry an integrated foreign gene locus. This can be verified by an array of different PCR strategies targeted to multiple components of the transgene locus. Furthermore, the backcrossing process can be carried out under confined conditions because of the small physical size of the early-flowering tobacco plants (see Figure 2 of the attached publication). There is therefore no 'release' of the transgene event to the environment. We are seeking some official position from APHIS on this matter as it relates to U.S. law and regulatory requirements, however, because it affects our research activities and possibly our cultivar development program. We would greatly appreciate your response on this issue. My phone number is 919-513-4802. My e-mail address is ramsey_lewis@ncsu.edu. My mailing address is:

Ramsey S. Lewis
Campus Box 7620
Crop Science Department
N.C. State University
Raleigh, NC 27695.

We thank you in advance for your response.

Respectfully yours,

A handwritten signature in black ink that reads "Ramsey S. Lewis". The signature is written in a cursive style with a large, looping initial 'R'.

Ramsey S. Lewis