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SUBJECT: Confirming non-regulated status of tobacco varieties grown using novel breeding method

APHIS / BRS Biotech Query Team:

I am writing to seek confirmation that varieties of tobacco which are null-segregant (NS also known as negative-segregant) lines derived from genetically-engineered (GE) tobacco plants using an accelerated tobacco breeding method are not considered to be regulated articles when it has been confirmed using phenotypic and molecular analyses that the NS lines derived using this novel breeding approach do not contain inserted genetic material and do not contain sequences from a plant pest.

In response to correspondence submitted by various researchers, APHIS has evaluated novel approaches to accelerating the introduction of desired traits in plants (including genetic engineering techniques) when used in combination with conventional back-crossing techniques. Of particular relevance to this inquiry is APHIS's October 27, 2011 response to a January 2011 inquiry submitted by Dr. Ramsey S. Lewis, a professor at North Carolina State University, concerning his efforts in developing new tobacco cultivars. *See* attached copies of the pertinent correspondence.

Through this inquiry I am seeking:

1. Confirmation that APHIS's 2011 interpretation (of non-regulated article status) would be applicable to any variety of tobacco cultivar derived using the same methods described in Dr. Lewis' correspondence when the NS tobacco variety is shown through phenotypic and molecular analyses to be indistinguishable from tobacco plants of the same variety when developed in a non-GE based breeding program because they do not contain inserted, transgenic material and do not contain sequences from a plant pest.

2. Confirmation that the phenotypic and molecular analyses referenced in item 1., above, of the NS tobacco variety lines derived using this novel breeding approach would only need to be performed by the investigator/developer of the cultivar and only prior to the initial introduction of the tobacco variety grown from seeds for use in field trials or other non-contained growing environments, including exercises that might lead to use of the crop for commercial purposes.

Background

I have been consulted by an investigator who is familiar with and intends to make use of the methods described by Dr. Lewis in his 2011 correspondence to APHIS, as has been detailed more fully in the 2009 publication by Drs. Lewis and Kernodle which appeared in the journal, *Theoretical Applications of Genetics* (2009; 118(8): 1499-508). *See* attached copy of publication. The investigator wishes to develop for potential commercial use multiple tobacco varieties which, if the research efforts are successful, will exhibit desired traits which reduce harmful elements in a variety of tobacco cultivars. To hasten the development of the reduced harm trait in tobacco varieties in which it occurs naturally, the investigator will rely on enhancement of conventional back-crossing methods. To achieve these enhancements, the investigator will, using transgenic methods, introduce in certain tobacco cultivars which already exhibit the desired reduced harm characteristic (the elite lines), the gene known as *FT (Flowering Locus T)* as derived from *Arabidopsis thaliana.* Because the introduction of the *FT* gene (which is expressed under the control of the CaMV 35S promoter) has been shown to significantly hasten flowering in tobacco cultivars, it is anticipated that a variety of elite tobacco cultivars can be developed using cultivar lines which already exhibit the desired reduced harm traits in materially fewer growing cycles than would be required if the investigators used only standard cross-breeding methods. The *FT* gene also is linked to the selectable marker gene *npt*II; thus, its presence can be readily detected and eventually selected out using back-crossing to be implemented in final back-crossing to be performed using the progeny identified by the investigator which exhibit the non-transgenic trait of interest, and selected plants that also do not carry the transgene insertion. In the final backcross, an early-flowering individual possessing the reduced-harm trait of interest in the elite line would be self-pollinated. From its progeny, the investigator would identify individuals possessing the reduce harm trait of interest, that do not also carry the transgene insertion (because the transgene locus has been segregated away). These null segregants will be identified on the basis of a normal flowering phenotype, and because the absence of components of the transgene locus can be demonstrated using PCR-based methods to test for the presence of *FT*, the CaMV 35S promoter, *nos* terminator sequence, *nos* promoter, and *npt*II (selectable marker gene).

Questions Presented

As enumerated above, through this correspondence we seek your concurrence that NS tobacco varieties derived using the methods described in this correspondence and in the attached documents are not considered by APHIS to be regulated articles because the NS tobacco plants are indistinguishable from plants developed in a non-GE based breeding program, and have never contained inserted, transgenic material.

We also seek your concurrence that investigators who obtain seeds from the NS tobacco plant varieties derived using these methods described in this correspondence and third parties to whom an investigator might provide such NS tobacco plants and/or their seeds may consider the plants and plants grown from such seeds (and the seed progeny those plants subsequently produce) to be non-regulated articles if the investigator has, through phenotypic and molecular analyses, confirmed prior to introducing such plants or seeds in field trials or other non-contained growing exercises that the NS tobacco variety lines derived do not contain inserted, transgenic material and do not contain sequences from a plant pest.

In the event APHIS cannot concur with us on this second point, we seek your guidance concerning when such phenotypic and molecular analyses should be conducted and the NS tobacco plants or seeds upon which such analysis should be performed and the records that APHIS would expect to be maintained of such testing.

We are aware that the inadvertent release of tobacco plants that do contain inserted transgenic material might (under certain circumstances) constitute a violation of APHIS's regulations, and that the NS tobacco variety lines in question might be subject to regulations administered by other federal agencies.

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Please contact me if you require additional information or have questions concerning the foregoing. My phone number is 202-942-5477; my e-mail address is lawrence.culleen@aporter.com. My mailing address is:

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I thank you for your timely consideration of this inquiry.

Sincerely,

Lawrence E. Culleen

Enclosures