Bayer CropScience

December 14, 2015

Dr. Michael J. Firko
Deputy Administrator
Biotechnology Regulatory Services
Animal and Plant Health Inspection Service
United States Department of Agriculture
4700 River Rd, Unit 98
Riverdale, MD 20737

Re: Tobacco modified for increased biomass production is not a regulated article

Dear Dr. Firko,

Bayer CropScience LP
2 T.W. Alexander Drive
Research Triangle Park, NC 27709

Bayer CropScience (BCS) hereby requests confirmation that tobacco (*Nicotiana tabacum*) lines transformed for enhanced photosynthetic efficiency and increased biomass production are not regulated articles as defined at 7 C.F.R. § 340.1. In accordance with the regulations at 7 C.F.R § 340.1, an organism altered or produced through genetic engineering may be deemed a regulated article if (1) either the donor organism, the recipient organism, or the vector or vector agent is listed in 7 C.F.R § 340.2 and meets the definition of plant pest, and (2) the genetically engineered organism meets the definition of plant pest or is a genetically engineered organism that the Administrator determines is a plant pest or has reason to believe is a plant pest.[1] The BCS tobacco lines do not contain any genetic sequences derived from plant pests listed at 7 C.F.R. § 340.2. Moreover, no genetic sequences from plant pests were involved in any transformation step for the tobacco lines. The means of transformation of the tobacco lines was microprojectile bombardment, which was utilized to vector sequences into the recipient organism. Finally, as BCS discusses in greater detail below, there is no reason to believe that the transformed tobacco lines are plant pests.

[1] "Plant pest" is defined as "[a]ny living stage, including active and dormant forms) of insects, mites, nematodes, slugs, snails, protozoa, or other living invertebrate animals, bacteria, fungi, other parasitic plants, or reproductive parts thereof; viruses; or any organisms similar to or allied with any of the foregoing; or any infectious agents or substances, which can directly or indirectly injure or cause disease or damage in or to any plants or parts thereof, or any processed, manufactured, or other products of plants.

The increased biomass phenotype of the transformed tobacco lines results from enhanced photosynthetic efficiency achieved by a modification to the amino acid sequence of the large subunit of the Rubisco enzyme. The development of these lines requires sequential chloroplast DNA transformations. The first transformation generates a 'master line' with decreased Rubisco efficiency, which can only survive in enhanced CO_2 conditions. This master line is then subjected to chloroplast retransformation for higher efficiency Rubisco compared to the native enzyme. This technology is described in the following published literature:

Whitney, S.M., and R.E. Sharwood. 2008. Construction of a tobacco master line to improve Rubisco engineering in chloroplasts. Journal of Experimental Botany. Vol. 59, No. 7, pp. 1909-1921.

The chloroplast transformations for each sequential step are accomplished utilizing microprojectile bombardment. The generation of the tobacco master line is thoroughly described in Whitney and Sharwood (2008). The construct for generating the master line contains two gene cassettes. The first gene cassette consists of a promoter derived from *N. tabacum*, a bacterial Rubisco gene from *Rhodospirillum rubrum*, and a terminator derived from *N. tabacum*. The second gene cassette contains a selectable marker gene from *Shigella flexneri* resulting in resistance to spectinomycin and streptomycin, and flanked by loxP sites. The sequence containing both gene cassettes replaces the endogenous gene encoding the large subunit of Rubisco. The master line is then generated by removal of the selectable marker cassette from the initial chloroplast transformant.

The construct utilized for the chloroplast retransformation step, resulting in the replacement of the bacterial Rubisco gene with the mutant tobacco Rubisco large subunit gene, and ultimately in the increased biomass phenotype, consists of two gene cassettes. The first gene cassette is for the modified Rubisco large subunit for increased efficiency, consisting of three elements. First, a promoter (P*rbc*L), the promoter region of the large subunit of Rubisco transcript derived from *N. tabacum*. The second element is the coding sequence (*rbc*L*) for the large subunit of Rubisco transcript containing one, two, or three amino acid changes resulting in the increased efficiency of the enzyme. The terminator (T*rbc*L) is the 3' untranslated region of the large subunit of Rubisco transcript derived from *N. tabacum*. The second gene cassette is for a selectable marker resulting in resistance to spectinomycin and streptomycin. This cassette is essentially inserted as a promoter-less gene, except 18 bp of the 5' region from *rbc*L derived from *N. tabacum*. The spectinomycin and streptomycin resistance gene (*aadA)* is derived from *S. flexneri*. The terminator (T*rps16*) consists of 148 bp of the 3' untranslated region of the rps16 transcript derived from *N. tabacum.*

All transformations in the development of the genetically engineered tobacco lines were vectored using microprojectile bombardment.

As noted above, the regulations at 7 C.F.R. § 340 provide that a genetically engineered plant is deemed a regulated article if (1) either the donor organism or the recipient organism is designated at 7 C.F.R. § 340.2, or a vector or vector agent are designated at 7 C.F.R § 340.2, and (2) the genetically engineered organism meets the definition of plant pest, or if APHIS determines it to be a plant pest. The BCS transformed tobacco lines do not meet any of these criteria. The recipient organism, *Nicotiana tabacum*, is not a plant pest; nor are the donor organisms from which the transformation sequences are derived, *N. tabacum*, *Rhodospirillum rubrum*, and *Shigella flexneri*, plant pests. The means of transforming the recipient plant, microprojectile bombardment, has been recognized numerous times by BRS as not involving plant pests. Finally, there is no reason to conjecture or believe that a tobacco line with increased biomass production would be a plant pest.

Thus, given that the transformed tobacco lines described above meet all of the criteria that APHIS has consistently set forth for determining that a genetically engineered plant is not a regulated article, we hereby request confirmation that tobacco lines genetically engineered as described in this letter are not regulated articles. BCS thanks BRS in advance for prompt consideration and confirmation of the non-regulated status of the BCS tobacco lines. Should you have any questions regarding this inquiry, please contact me at michael.weeks@bayer.com or (919) 549-2119.

Sincerely,

Michael Weeks, M.Sc.
US Regulatory Manager