Notices

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This section of the FEDERAL REGISTER contains documents other than rules or proposed rules that are applicable to the public. Notices of hearings and investigations, committee meetings, agency decisions and rulings, delegations of authority, filing of petitions and applications and agency statements of organization and functions are examples of documents appearing in this section.

DEPARTMENT OF AGRICULTURE

Animal and Plant Health Inspection Service

[Docket No. 95-015-1]

Determination of Nonregulated Status for Additional Calgene, Inc., Genetically Engineered FLAVR SAVR™ Tomato Lines

AGENCY: Animal and Plant Health Inspection Service, USDA.

ACTION: Notice.

SUMMARY: The Animal and Plant Health Inspection Service is announcing that it has added 20 additional genetically engineered tomato lines to those subject to its October 19, 1992, interpretive ruling that the subject FLAVR SAVR™ lines need no longer be regulated. The effect of this action is that 20 additional delayed softening tomato lines, which have been modified by the incorporation of genetic material described by Calgene, Inc., in its initial request for an interpretive ruling, will no longer be subject to regulation under 7 CFR part 340.

FOR FURTHER INFORMATION CONTACT: Dr. Keith Reding, Biotechnologist, Animal and Plant Health Inspection Service, Biotechnology, Biologics, and Environmental Protection, Biotechnology Permits, 4700 River Road Unit 147, Riverdale, MD 20737-1228; (301) 734-7612.

SUPPLEMENTARY INFORMATION: On October 19, 1992, the Animal and Plant Health Inspection Service (APHIS) published in the **Federal Register** (57 FR 47608-47616, Docket No. 92-087-2) a notice announcing the issuance of an interpretive ruling that the Calgene, Inc., FLAVR SAVR™ tomato does not present a plant pest risk and is not a regulated article under the regulations contained in 7 CFR part 340. This action was in response to a petition submitted by Calgene seeking a determination from APHIS that its FLAVR SAVR™ tomato no longer be deemed a regulated article, based on an absence of plant pest risk. The effect of the action was that previously field tested lines of the FLAVR SAVR™ tomato and their progeny would no longer be regulated under these regulations.

FLAVR SAVR™ tomatoes were defined by Calgene in its initial petition to include any tomatoes transformed with one of seven identified plasmid vectors that all carry an antisense copy of the tomato polygalacturonase gene and a bacterial neomycin phosphotransferase gene with associated regulatory sequences. Calgene's initial request to APHIS in 1992 was for a determination pertaining to all FLAVR SAVR™ transformants produced in tomatoes using any one of the seven plasmid vectors. Calgene indicated in its petition that data provided to the Agency were representative of the data gathered for all lines tested up to that time. The initial determination announced by APHIS on October 19, 1992, only applied to those lines that had already been field tested. However, APHIS indicated that new lines were likely to exhibit properties similar to those of lines already field tested under permit. The determination also allowed for cross-breeding of the identified FLAVR SAVR™ tomato lines with any other lines or cultivars of tomato without a permit. One additional FLAVR SAVR™ tomato line was added to the original determination on October 3, 1994 (59 FR 50220, Docket No. 94-096-1), and nine additional FLAVR SAVR™ tomato lines were added to the original determination on November 18, 1994 (59 FR 59746, Docket No. 94-125-1).

Seventeen of the 20 additional FLAVR SAVR™—tomato lines that are the subject of this notice were constructed using the plasmid vector pCGN4109, and the remaining three lines were constructed using the plasmid vector pCGN1436. These two vectors were among the seven included in Calgene's initial petition to APHIS. In our determination of October 19, 1992, the lines using these vectors were not deregulated because they had not been field tested. These lines have since been field tested in accordance with APHIS regulations at 7 CFR part 340, and data provided to APHIS indicate that the new transformants, produced in a manner identical to the earlier transformant lines, behave similarly to those earlier FLAVR SAVR™ tomato lines to which the determination initially applied. Reports from field trials and other data indicate that the new tomato lines grow normally, exhibit the expected morphological, reproductive, and physiological properties, and do not have unexpected pest or disease susceptibility or symptoms. Therefore, the APHIS determination of nonregulated status of October 19, 1992, applies as well to the new transformed lines.

Done in Washington, DC, this 16th day of March 1995.

Terry L. Medley,

Acting Administrator, Animal and Plant Health Inspection Service.

[FR Doc. 95-7132 Filed 3-22-95; 8:45 am]

BILLING CODE 3410-34-P

CALGENE

January 24, 1995

Dr. Keith Reding
Biotechnology, Biologics, and
Environmental Protection
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
6505 Belcrest Road
Hyattsville, MD 20782

Additional FLAVR SAVR™ Tomato Lines: December 22, 1994 and January 12, 1995 Requests

Dear Dr. Reding:

This letter is to clarify our two requests of December 22, 1994 and January 12, 1995.

The purpose of creating new FLAVR SAVR tomato lines is to develop and select new varieties for each of the critical growing regions for tomatoes in the United States and Mexico. This in an ongoing process that will continue indefinitely (as for any good tomato variety development program). We do this using two methods: 1) hybridization and 2) transformation. Both methods have advantages and disadvantages, of course, and we use each depending on the specific breeding material we are working with. However, in either case, the inserted DNA is identical, based on either the pCGN1436 or pCGN4109 constructs; there are no differences in the inserted sequences. As you know, FLAVR SAVR tomato varieties produced using these two constructs have been deregulated by USDA APHIS. This request is for adding additional lines developed using these same constructs.

For both hybridization and transformation, we measure the level of polygalacturonase. For the additional lines identified in our Dec. 22 and Jan. 12 letters, transformation was used. For these lines, the PG levels were reduced as compared to nontransgenic controls. This is the predicted, intended effect.

For transformation, only, we do Southern analysis to ensure that there is no transfer beyond the border. This procedure, then, ensures that there is no new inserted DNA in the new lines. All the additional FLAVR SAVR tomato lines were produced using either the pCGN1436 or pCGN4109 constructs. All were determined by Southern analysis not to have DNA transfer outside the borders. This means that these additional lines do not

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contain any new inserted DNA sequences that are not in the already deregulated FLAVR SAVR tomato lines.

For your information, we already submitted data on one of these new additional FLAVR SAVR tomato lines. On October 14, 1994 we submitted PG and Southern data on line 532A-4109a-5097, but then indicated in our October 27, 1994 letter that this line was still in the field and the final data were not available. This line is one of those in our Dec. 22 request.

I believe the above information is sufficient to ensure that the additional FLAVR SAVR tomato lines do not contain any new inserted DNA sequences, that the lines do not differ in the intended effect (lower PG), and that they can be added to our existing Determination without additional data.

Please give me a call if you have any questions about this request. Thank you very much.

Sincerely,

Dr. Keith Redenbaugh / kw

Keith Redenbaugh, Ph.D.
Regulatory Affairs

CALGENE

January 26, 1995

Dr. Keith Reding
Biotechnology, Biologics, and
Environmental Protection
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
6505 Belcrest Road
Hyattsville, MD 20782

FLAVR SAVR™ Tomato Lines for Addition to Determination

Dear Dr. Reding:

Following are the FLAVR SAVR tomato transformation events (lines) we wish added to our existing Determination of Nonregulated Status. Since submitting our letters of December 22, 1994 and January 12, 1995, we have dropped several lines from consideration; hence, this list is somewhat shorter. All these lines have been field tested under Notification, and field trial reports were submitted to USDA APHIS BBEP.

FLAVR SAVR tomato transformation events (lines) to add to Determination:

105F	1436	2018				
105F	1436	2035				
105F	1436	2049				
35F	4109a	3023		519A	4109a	4621
84F	4109a	148		519A	4109a	4676
88F	4109a	2797		531A	4109a	2105
121F	4109a	333		531A	4109a	2270
121F	4109a	1071		532A	4109a	5097
121F	4109a	1120		540A	4109a	1739
137F	4109a	71		585A	4109a	3604
138F	4109a	164		585A	4109a	3530
519A	4109a	4527				

Attached are example data on Southern analysis showing there was no transfer beyond the left and right borders. All lines were selected on medium containing kanamycin, demonstrating the presence of the *kan*^{r} gene. All were tested for polygalacturonase and shown to have lowered activity. PG

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To Dr. Keith Reding	From K. Redenbaugh
Co.	Co.
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activity level for some of these lines are included: 84F 4109a-148, 88F 4109a-2797, 121F 4109a-333, 532a 4109a-5097 and 585a 4109a-3604. The inserted genes were stable as demonstrated by Mendelian inheritance.

Transformation event 519a 4109a-4505 has been withdrawn from commercial consideration and is not requested for addition to our Determination.

The enclosed Southerns also contain analyses for lines that were previously added to the Determination and are included only because they were present on the Southerns: 114F 4109a-26, 115F 4109a-309, 124F 4109a-120a, 141F 4109a-25C, 141F 4109a-81.

Southern data and PG activity for line 540a 4109a-1823 are also enclosed; however, this line is still being tested for the first time in the field and has not been subject to a field trial report yet.

If you have additional questions, please give me a call. Thank you.

Sincerely,

Keith Redenbaugh, Ph.D.
Manager, Regulatory Affairs

Example PG Enzyme Activity

Generation	Genotype	PG Activity*
	84F Control	1.28 ± 0.32
T_1	84F 4109a - 148	0.38 ⊥ 0.26
	88F Control	1.06 ± 0.08
T_1	88F 4109a - 2797	0.09 ± 0.12
	121F Control	1.05 ± 0.21
T_1	121F 4109a-333	0.13 ± 0.11
	532a Control	1.04 ± 0.12
T_1	532a 4109a - 5097	0.12 ± 0.03
	585a Control	1.06 ± 0.10
T_1	585a 4109a - 3604	0.19
	540a Control	0.91 ± 0.17
T_1	540a 4109a - 1823	0.17 ± 0.06

*Modification of Sheehy, et al. (1988. PNAS 85:8805) PG assay.

Southerns analysis for DNA transfer beyond T-DNA borders

Figures 1 and 2 are example Southern hybridization results for a blot containing restriction digested DNA of some lines we wish to commercialize. DNA on this blot was digested with restriction enzymes BamHI and BglII, and probed with a *kan*^{r} probe (data not shown), PG and 1532 probes. The blot was stripped between hybridizations with each probe.

DNA on the blot was hybridized to a PG probe (Sanders et al. 1992) and exposed to film for one day (Figure 1). The lane containing one copy of pCGN3312 plasmid DNA contains the inserted antisense PG gene fragment expected when a BamHI and BglII restriction digest is utilized and the DNA is hybridized to the PG probe. The lane containing 141F control DNA represents the endogenous PG gene fragment present in tomatoes when this same analysis is used. No antisense PG fragment is present in this lane. The lanes containing restriction digested DNA from lines transformed with pCGN4109a all contain the expected lower band representing the introduced antisense PG gene fragment in addition to the endogenous PG gene fragment (upper band) present in both transgenic and non-transgenic tomato lines. In addition to providing a means of counting antisense PG gene number, this blot also demonstrates that there is DNA present on the blot.

This blot was then stripped and reprobed with pCGN1532. The blot was exposed to film for 2 days (Figure 2). The lane containing one copy of pCGN3312 plasmid DNA (containing the same backbone structure as pCGN4109a), had the 5 bands expected when cut with restriction enzymes BamHI and BglII. Transgenic plant lines 84F 4109a-148, 88F 4109a-2797, 114F

4109a-26, 115F 4109a-309, 121F 4109a-333, 124F 4109a-120a, 141F 4109a-25C, 141F 4109a-81, 519a 4109a-4505, 532a 4109a-5097, 540a 4109a-1823 and 585a 4109a-3604. did not show any hybridization to the 1532 probe. These results confirm previous Southern results of the same plants utilizing the 1532 probe which were done as an initial screen of all transformants.

References

Sanders, R., R. Sheehy and B. Martineau. 1992. An efficient and reliable method for determining the number of transgenes inserted into transgenic plants. Plant Molec. Biol. Rep. 10:164-172.

Sheehy, R., M. Kramer and W. Hiatt. 1988. Reduction of polygalacturonase activity in tomato fruit by antisense RNA. Proc. Natl. Acad. Sci. USA. 85:8805-8809.

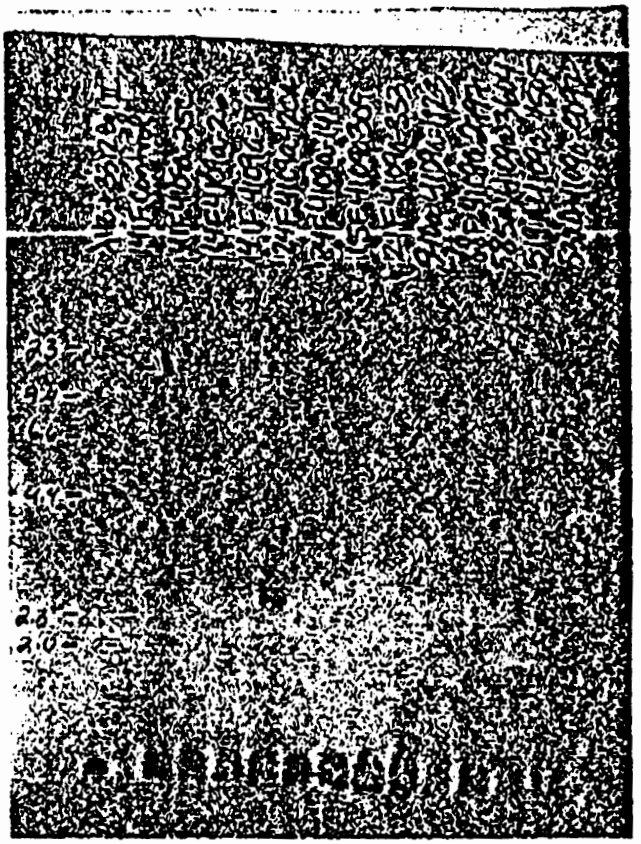

Figure 1. Southern of tomato lines transformed with 4109a (asPG). Plant DNA was digested with the restriction enzymes BamHI and BglII. One copy of BglII restriction digested pCGN3312 plasmid DNA (containing the same backbone structure as pCGN4109a) spiked into the lane containing the molecular weight markers was utilized as a positive control. This blot was previously probed with a *kan*^{r} probe and exposed (data not shown) and then stripped of probe. The blot was then hybridized a second time with a PG probe. The blot was exposed to X-Ray film for 1 day. Lane assignments are as follows:

(1) Molecular markers spiked with one copy of pCGN3312, (2) 114F non-transgenic control, (3) 141F 4109a-25C, (4) 114F 4109a-26 (5) 141F 4109a-81 (6) 124F 4109a-120a, (7) 84F 4109a-148, (8) 115F 4109a 309, (9) 121F 4109a-333, (10) 540a 4109a-1823, (11) 88F 4109a-2797, (12) 585a 4109a-3604, (13) 519a 4109a-4505, (14) 532a 4109a-5097.

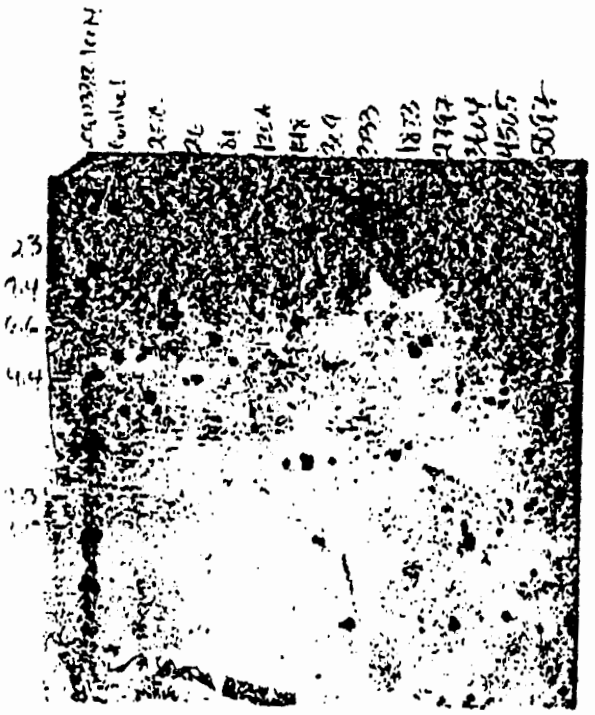

Figure 2 Southern of tomato lines transformed with 4109a (asPG). Plant DNA was digested with the restriction enzymes BamHI and BglII. One copy of BglII restriction digested pCGN3312 plasmid DNA (containing the same backbone structure as pCGN4109a) spiked into the lane containing the molecular weight markers was utilized as a positive control. This blot was previously probed with a *kan*^{r} probe and a PG probe. The blot was stripped a second time and probed with pCGN1532 which was linearized with the restriction enzymes BamHI and EcoRI and exposed to X-Ray film for 2 days. Lane assignments are as follows:

(1) Molecular markers spiked with one copy of pCGN3312, (2) 114F non-transgenic control, (3) 141F 4109a-25C, (4) 114F 4109a-26 (5) 141F 4109a-81 (6) 124F 4109a-120a, (7) 84F 4109a-148, (8) 115F 4109a-309, (9) 121F 4109a-333, (10) 540a 4109a-1823, (11) 88F 4109a-2797, (12) 585a 4109a-3604, (13) 519a 4109a-4505, (14) 532a 4109a-5097.