

# Notices

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Friday, July 28, 1995

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## Animal and Plant Health Inspection Service

[Docket No. 95-056-1]

### Addition of Two Genetically Engineered Tomato Lines to Determination of Nonregulated Status for Calgene, Inc.

**AGENCY:** Animal and Plant Health Inspection Service, USDA.

**ACTION:** Notice.

**SUMMARY:** The Animal and Plant Health Inspection Service is announcing that it has added two genetically engineered tomato lines to those subject to its October 19, 1992, interpretive ruling that certain FLAVR SAVR™ lines need no longer be regulated. The effect of this action is that two 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. Subhash Gupta, Biotechnologist, Biotechnology Permits, BBEP, APHIS, 4700 River Road Unit 147, Riverdale, MD 20737-1237; (301) 734-8761.

**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 previously field

tested lines of the Calgene, Inc., FLAVR SAVR™ tomato do not present a plant pest risk and are not regulated articles under the regulations contained in 7 CFR part 340. That 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 that action was that previously field tested lines of the FLAVR SAVR™ tomato and their progeny would no longer be regulated under the regulations in 7 CFR part 340.

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 tomato using any one of the seven plasmid vectors. Calgene indicated in its petition that data provided to APHIS 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. Since the publication of the October 19, 1992, determination, a total of 30 FLAVR SAVR™ tomato lines have been added to the original determination; those additions were announced in notices published in the *Federal Register* on October 3, 1994 (59 FR 50220, Docket No. 94-096-1); November 18, 1994 (59 FR 59746, Docket No. 94-125-1); and March 23, 1995 (60 FR 15284, Docket No. 95-015-1).

The FLAVR SAVR™ tomato lines that are the subject of this notice, designated 519a 4109a-4645 and 540a 4109a-1823, were constructed using the plasmid pCGN4109, which contains the promoter/terminator from either pCGN1557 or pCGN1578. These latter

two vectors were among the seven included in Calgene's initial petition to APHIS. FLAVR SAVR™ tomato lines constructed using these vectors were not included in our October 19, 1992, determination because they had not yet been field tested. These lines have since been field tested in accordance with APHIS' regulations in 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 original determination 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 October 19, 1992, of nonregulated status of previously tested FLAVR SAVR™ tomato lines applies as well to the new transformed lines.

Done in Washington, DC, this 18th day of July 1995.

Terry L. Medley,

*Acting Administrator, Animal and Plant Health Inspection Service.*

[FR Doc. 95-18572 Filed 7-27-95; 8:45 am]

BILLING CODE 3410-34-P



**CALGENE, Inc.**

1920 Fifth Street  
Davis, CA 95616  
USA

Telephone: (916) 753-6313  
Fax: (916) 753-1510

**DATE:** June 27, 1995

**TO:** Dr. John Payne  
USDA APHIS BBEP

**URGENT**

**FAX:** 301-734-8724

**FROM:** Lori Malyj Manager, Regulatory Affairs

**PAGES:** 9 (including this page)

**Message:**

**Subject:** Additional FLAVR SAVR lines

Dear John,

Enclosed is a request to add 2 new FLAVR SAVR lines to the Determination, as per our phone conversation today. We appreciate your assistance in expediting this offshoot from our continuing discussions.

The original will follow by Fed X.

Thank you.

6/27  
SG

2/6-2  
R



# CALGENE

June 26, 1995

Dr. John Payne  
Acting Director,  
USDA APHIS BBEP  
4700 River Road, Unit 147  
Riverdale, MD 20737  
phone 301-734-7602

Subject: Request to amend the Determination of Non-regulated Status to add two FLAVR SAVR™ tomato lines

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Dear Dr. Payne,

Enclosed is a request to add two additional FLAVR SAVR tomato lines to the existing Determination of Non-regulated Status for this product. This request is separate from Calgene's May 1, 1995 pending request to add new lines through self-certification.

Calgene asks that the review be expedited in a similar manner to addition of previous FLAVR SAVR tomato lines. The enclosure contains the same type of data and information as the most recent addition of FLAVR SAVR lines [January 26, 1995 request, finalized in 60FR 15284 (March 23, 1995)].

This request contains no confidential business information.

The ability to grow these lines commercially in late July is extremely important to Calgene and our subsidiary Calgene Fresh, and we appreciate your prompt assistance. Please call either me or Keith Redenbaugh at 916-753-6313 should you have questions or require additional information.

Sincerely,

Lori Malyj  
Manager, Regulatory Affairs

Enclosure

1920 Fifth Street  
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**FLAVR SAVR™ Tomato Lines for Addition to the Determination**

Calgene requests that the following FLAVR SAVR tomato transformation events (lines) be added to the existing Determination of Nonregulated Status:

519a 4109a-4645  
540a 4109a-1823

derived from either pCG 1557 or pCG 1578

Tomato lines produced using the identical construct (pCGN4109) and methods have been previously deregulated by APHIS. The safety of FLAVR SAVR tomatoes and the lack of potential for weediness have been previously addressed. The genetic sequences in pCGN4109 and their gene products have been extensively characterized. The tomato lines which are the subject of this current request also meet the definition of FLAVR SAVR tomato and demonstrate the same characteristics as previous lines.

The data supplied in this attachment was produced using the same protocols as the data in support of the previous addition of FLAVR SAVR lines [e.g. January 26, 1995 request, finalized in 60FR 15284 (March 23, 1995)]. Presence of the antisense PG gene is demonstrated by reduced polygalacturonase enzyme activity (Section A, below). The presence of the *kan<sup>r</sup>* gene is confirmed by the transformation process used for all FLAVR SAVR lines: selection on media containing kanamycin. Results of Southern analysis show there was no transfer beyond the left and right borders for these FLAVR SAVR lines (Section B, below). The two lines have been field tested under Notification, and a field trial report has been submitted previously to USDA APHIS BBEP (copy provided in Section C, below).

**Section A. Polygalacturonase (PG) Enzyme Activity**

Line 519A 4109a - 4645 shows markedly reduced PG enzyme activity as compared to the non-transgenic control. This is the predicted, intended effect of the FLAVR SAVR gene.

Generation	Genotype	PG Activity*
	519A Control (non-transgenic)	0.99 ± 0.08
T <sub>1</sub>	519A 4109a - 4645	0.19 ± 0.13
T <sub>2</sub>	519A 4109a - 4645	0.01 ± 0.02

540a 4109a - 1823  
T<sub>1</sub> → PG = 0.17 ± 0.06

\*Assay procedure previously provided. The procedure is based on Sheehy, et al. (1988. PNAS 85:8805).

checked  
JK

✓ Data showing similar reduction in PG levels for line 540a 4109a-1823 were provided with the January 26, 1995 Request for Addition to Determination.

## Section B. Southern analysis for DNA transfer beyond T-DNA borders

The data supplied in this attachment was produced using the same protocols as the data in support of the previous additions of FLAVR SAVR lines. The results of analysis confirm that there was no transfer beyond the left and right borders in the two tomato lines.

The protocol used to analyze these lines has been extensively used by Calgene to test FLAVR SAVR lines for the presence of backbone sequences. Data generated using this protocol has been previously reviewed by APHIS in regard to the deregulation of FLAVR SAVR lines, as follows:

60 FR: 15284, March 23, 1995

59 FR: 59746, November 18, 1994

In addition, data generated using this protocol were reviewed by FDA in connection with the concluded consultation on food safety of FLAVR SAVR tomato (FDA. 1994 (May 23). Calgene, Inc.; Availability of Letter Concluding Consultation (Docket No. 91A-0330). Federal Register. 59:26647-26648).

This method is a very sensitive analytical technique to detect whether backbone sequences have been integrated into the transformed plant. Plasmid pCGN1532 contains identical backbone sequences to the constructs used to produce FLAVR SAVR tomatoes (pCGN1436; pCGN4109), as it is the common backbone to Calgene's 7 binaries. The pCGN1532 DNA is used as the probe template to generate a nested set of uniformly labeled probes (via random primed DNA labeling) that cover all backbone sequences in the constructs used to produce the transgenic plants.

OK ✓ Southern analysis showing lack of transfer beyond the T-DNA borders for line 540a 4109a-1823 was provided with the January 26, 1995 Request for Addition to Determination [finalized 60 FR: 15284 (March 23, 1995)].

Figure 1 (following page) provides Southern hybridization data for line 519a 4109a-4645. This figure also contains analyses for other lines not under consideration for this request. The filter was exposed to film for 3 days to produce the autoradiogram.

Lane 15, containing one copy of pCGN1436 plasmid DNA (with the same backbone structure as pCGN4109a) was used for a positive control and showed the 5 bands expected when cut with the restriction enzyme BglII and hybridized to the pCGN1532 probe. Lane 9, containing DNA from line 540A 4109a-1650, a plant known to contain transfer beyond the T-DNA borders based on previous analyses, was loaded with about half the DNA used in the other lanes. It also shows hybridization to the pCGN1532 probe, as expected. Lane 8 contains DNA from a non-transformed control plant and does not show hybridization to the pCGN1532 probe. Transgenic plant line 519a 4109a-4645 in Lane 1 did not show any hybridization to the pCGN1532 probe.

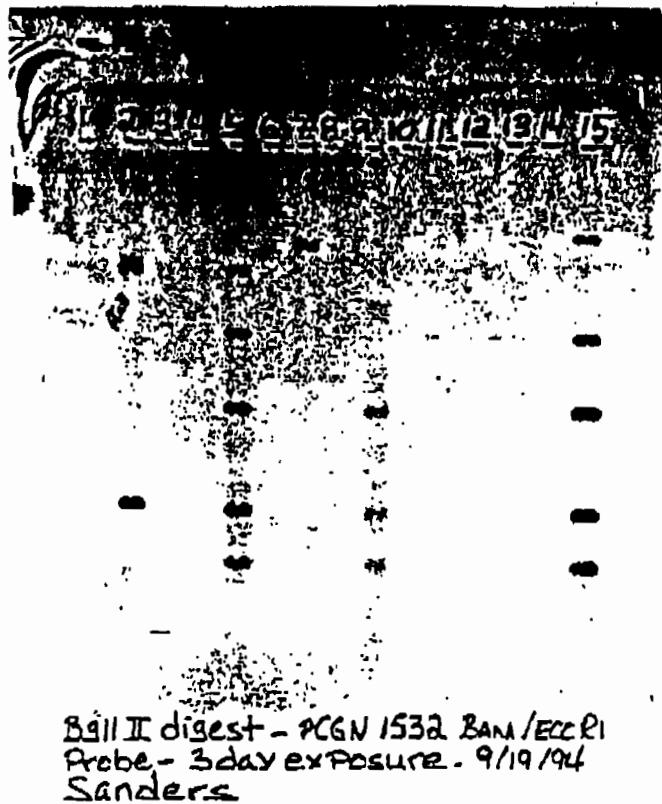


Figure 1. Southern of tomato lines transformed with 4109a (asPG). Plant DNA was digested with the restriction enzyme BglII. One copy of BglII restriction enzyme-digested pCGN1436 plasmid DNA (containing the same backbone structure as pCGN4109a) was utilized as a positive control. The blot was probed with random-primed labelled pCGN1532 (linearized with the restriction enzymes BamHI and EcoRI before labelling) and exposed to X-ray film for 3 days. Lane assignments are as follows:

- (1) 519A 4109a-4645, (2) 519A 4109a-4509, (3) 540A 4109a-1819,
- (4) 540A 4109a-1776 (5) 540A 4109a-1772 (6) 540A 4109a-1763,
- (7) 540A 4109a-1759, (8) 540A non-transformed control,
- (9) 540A 4109a-1650, (10) 540A 4109a- 1555, (11) 540A 4109a-1526,
- (12) 137F 4109a-143B, (13) 137F 4109a-30, (14) molecular weight markers, (15) pCGN1436.

**Section C: Field Trial Report**

A copy of the previously filed report on field trial 94-230-07N is provided. This trial included the tomato lines which are the subject of this request, and demonstrates that the characteristics are as expected for tomatoes.





**CALGENE**

Field Trial Report 94-230-07N

4/6/95

Rick Sanders and Cate McGuire

**Field Trial Under USDA Notification**

Location	Transgenic plant	Notification Number
Galt, San Joaquin Co., CA	FLAVR SAVR tomato (antisense polygalacturonase)	94-230-07N

Contains No Confidential Business Information

**Introduction**

The objective of the trial was to evaluate new FLAVR SAVR tomato lines. The plants were produced using construct pCGN4109a, and meet the definition of the USDA Determination of Non-regulated Status for FLAVR SAVR tomato, except that they had not been previously field tested. The results demonstrate that these lines exhibit characteristics as expected for FLAVR SAVR tomato plants, and that plant characteristics and resistance to pests and diseases are normal for tomatoes.

**Chronology**

Site	Seeded in greenhouse	Planted	Harvested	Inspections by Calgene	Destruction
Galt, San Joaquin Co, CA	July 1, 1994 & August 1, 1994	September 20, 1994	December 1, 1994, January 26 and March 26, 1995	November 13, 1994, December 28, 1994, January 20, 1995 and March 23, 1995	March 27, 1995

**Trial Entries**

519A 4109a-4645

540A 4109a-1823

These lines represent additional transformation events of FLAVR SAVR tomato.

## **Maintenance of Transgenic Plant materials**

To prevent the dissemination of propagules, the following precautions were taken. All transplants were grown in Calgene Galt greenhouses, Galt, California. The germination frequency was above 85% for both transgenic entries. Seed was maintained and handled under the supervision of Calgene personnel. Transplants were planted in a field adjacent to the greenhouses.

## **Field Operations**

Planting of the transplants was done by hand under the supervision of Calgene personnel. Any leftover transplants were destroyed. Standard tomato cultural practices were conducted by the farmer during the seedling and vegetative stages. Plants were grown in wire tomato cages. In late November, plants were enclosed in a plastic tunnel to prevent damage by frost. Pesticides and herbicides were applied by ground application. Harvesting and subsequent transport of the transgenic fruit was conducted by Calgene personnel. Fruit were placed into bags and bags were placed into plastic 5 gallon buckets. Fruit were transported in an enclosed van to the Calgene lab facilities for analysis.

## **Containment and Safety**

Bare earth buffers of 30 feet each were maintained on all sides of the trial to enhance the prevention of pollen transfer and seed dissemination. This zone was kept weed free by periodic herbicide applications and cultivation; there were four inspections by Calgene personnel. No evidence of gene movement into other organisms from the transgenic tomatoes was observed.

## **Plant Observations**

During the growing season, the field trial was monitored regularly to check for potential problems with insects, diseases, stresses, and abnormal phenotypes:

**INSECTS:** army worm, pin worm, tomato fruit worm and , aphids, russet mites, thrips, white flies, leaf miners and flea beetle.

**DISEASES:** Phytophthora, Fusarium, crown gall, stem canker, tobacco mosaic virus, early and late blight, tomato mottle virus, tomato spotted wilt, bacterial wilt, bacterial spot and bacterial speck.

Specifically, each trial was monitored weekly by a cooperator hired by Calgene as well as the field checks by Calgene personnel.

The field was walked and observations were made comparing the transgenic plants to non-transgenic controls on at least four separate occasions including the dates of harvest, by Calgene research staff. Plant observations were made regarding plant morphology, including leaf type, growth habit, flowering, fruit set, fruit ripening, and general plant health with respect to disease and insects. Inspections were made on November 13, December 28, 1994 and January 20 and March 23, 1995 whereas the field was walked through and observed on a plant by plant, line by line basis. On these dates no crown gall disease or cauliflower mosaic virus (CaMV) was observed. White flies, aphids, mites and thrips were observed at this trial. Botrytis and TMV were the only plant diseases observed in this trial. Plant diseases and pests were distributed equally amongst control and transgenic plants.

Plans for the trial were announced by the U.S. Department of Justice in a report by the U.S. Attorney General, Robert F. Kennedy, in a letter to the President, dated June 1, 1964. The report stated that the trial would be held in New Orleans, Louisiana, and that the trial would be held in the city of New Orleans, Louisiana, and that the trial would be held in the city of New Orleans, Louisiana.

Plants in the trial were examined for any abnormalities arising from the transformation process by the Calgene breeder. Observations by Calgene personnel were conducted throughout the entire growing cycle on a weekly basis. The advanced lines grown in this trial did not exhibit abnormal appearance and were selected for seed harvest.

There were no observed differences between transgenic plants and their respective non-transgenic controls in this field trial. Both lines were normal with regard to plant characteristics; flowering, maturity, and yield.

#### **Harvest and Fruit Evaluation**

Fruit from the Galt trial was harvested December 1, 1994 for analysis of PG enzyme activity. Fruit were picked into bags which were then placed into a 5 gallon plastic bucket and transported to the Calgene Davis lab in an enclosed van. Fruit tested from this trial exhibited significantly reduced PG enzyme activity as expected. Fruit were harvested for seed on March 27, 1995. Seed was processed at the adjacent Galt greenhouse facilities. Fruit parts other than seed were destroyed at the Galt greenhouse facility.

#### **Trial Destruction**

Upon completion of the field trial harvest tomato plants were pulled out of the ground by hand and destroyed. The field was disked using the cooperating farmer's equipment. To prevent seed dissemination, field equipment was washed within the field trial boundaries to remove all reproductive plant parts. The field was disked March 27, 1995 and will not be replanted to tomatoes for at least 6 months; with the possible exception of new transgenic planting under future notification. The field will be monitored during this time for volunteers monthly by the grower and Calgene personnel. If volunteers occur they will be either mechanically or chemically destroyed.