94-227-01P

DEPARTMENT OF AGRICULTURE

Animal and Plant Health Inspection Service

[Docket No. 94-096-1]

Addition of One Genetically Engineered Tomato Line 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 one genetically engineered tomato line to those subject to its October 19, 1992, interpretive ruling for FLAVR SAVR™ tomatoes, that the subject FLAVR SAVR™ lines need no longer be regulated. The effect of this action is that one additional delayed-softening tomato line, which has been modified by the addition of genetic material identical to that added to other tomato lines covered by the initial determination and is designated by Calgene as FLAVR SAVR™ line N73 1436-111, will also no longer be subject to regulation under 7 CFR part 340.

FOR FURTHER INFORMATION CONTACT: Dr. Michael G. Schechtman, Senior Microbiologist, Biotechnology Coordination and Technical Assistance, BBEP, APHIS, USDA, room 850, Federal Building, 6505 Belcrest Road, Hyattsville, MD 20782, (301) 436-7601.

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. 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 the action was that previously field-tested lines of the FLAVR SAVR™ tomato and their descendants 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 was 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 permit.

The new line that is the subject of this notice was a new transformant produced using one of the same plasmid vectors that were previously reviewed in our October 19, 1992, determination. Line N73 1436-111 has been field tested in accordance with APHIS regulations in 7 CFR part 340. Data provided to APHIS indicates that the new transformant line, produced in a manner identical to the earlier transformant lines, behaves similarly to those earlier FLAVR SAVR™ tomato lines to which the determination initially applied. Reports from field trials and other data indicate that tomato line N73 1436-111 grows normally, exhibits the expected morphological, reproductive, and physiological properties, and does not have unexpected pest or disease susceptibility or symptoms. Therefore, APHIS' determination of nonregulated status of October 19, 1992, is considered to apply to this new transformant line as well.

Done in Washington, DC, this 27th day of September 1994.

Terry L. Medley,

Acting Administrator, Animal and Plant Health Inspection Service.

[FR Doc. 94-24374 Filed 9-30-94; 8:45 am]

BILLING CODE 3410-34-P

94-227-01P

December 22, 1994

CALGENE

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

Request to Add Additional FLAVR SAVR™ Tomato Lines to USDA Determination on Nonregulated Status

Dear Dr. Payne:

Please find attached two field trial reports for 94-195-01N and 94-118-01N. We request that you add the new pCGN1436 and pCGN4109 FLAVR SAVR tomato lines to the existing FLAVR SAVR Determination. The field trials showed that these additional FLAVR SAVR tomato lines had the same intended effect of already deregulated lines and none had any enhanced plant pest or weediness characteristics. The field performance of these lines was comparable to the deregulated lines and there were no characteristics or concerns to warrant additional testing. As with all FLAVR SAVR tomato lines commercialized, we will conduct appropriate food safety assessment as per our consultations with the FDA.

The following lines are requested for addition:

Variety	Construct	Event	Variety	Construct	Event
105F	1436	2018	105F	1436	2049
105F	1436	2035			
35F	4109a	3013	519A	4109a	4505
35F	4109a	3023	519A	4109a	4527
42F	4109a	4050	519A	4109a	4621
42F	4109a	4101	519A	4109a	4676
84F	4109a	148	531A	4109a	2105
88F	4109a	2738	532A	4109a	2270
121F	4109a	333	532A	4109a	5097
121F	4109a	1071	585A	4109a	3604
121F	4109a	1120	585A	4109A	3530
137F	4109a	71	540A	4109a	1739
138F	4109a	164			

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

Sincerely,

Keith Redenbaugh, Ph.D.
Manager, Regulatory Affairs

Enclosures

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#3-cal
1/10/94

3/12

CALGENE
Field Trial Report 94-195-01N
12/12/94

Field Trial Under USDA Notification

Locations	Gene(s)	Date of Notification	Notification Number
Fresno, King Counties	asPG, SPS, asPG-asACC synthase	July 12, 1994	94-195-01N

Contains No Confidential Business Information

Introduction

The objective of the trial was to evaluate genetically engineered fresh market tomato plants. The genes and related construct numbers evaluated are antisense polygalacturonase (FLAVR SAVR)(pCGN4109a and pCGN1436), sucrose phosphate synthase (SPS) (pCGN3812); and antisense ACC synthase-antisense polygalacturonase (asPG-asACC synthase)(pCGN3312).

Chronology

Site	Seeded in greenhouse	Planted	Harvested	Inspections by Calgene	Destruction
Huron, CA	July 1, 1994	August 16, 1994	November 15 and 16, 1994	September 20, October 26 and November 1, 1994	November 21-23, 1994

Trial Entries

Variety	Construct	Event
UC82B	3812	9
105F	1436	2018
105F	1436	2035
105F	1436	2049
35F	4109a	3013
35F	4109a	3023
42F	4109a	4050
42F	4109a	4101
88F	4109a	2738
115F	4109a	201
115F	4109a	309
121F	4109a	333
121F	4109a	1120
124F	4109a	120a
137F	4109a	71
137F	4109a	265
138F	4109a	164
141F	4109a	25a
141F	4109a	25c
141F	4109a	81
519A	4109a	4505
519A	4109a	4527
519A	4109a	4621
519A	4109a	4676
531A	4109a	2105
532A	4109a	2270
532A	4109a	5097
585A	4109a	3604
585A	4109A	3530
540A	4109a	1739
105F	3312	8396
115F	3312	2028
115F	3312	2075
115F	3312	2214
138F	3312	4010
138F	3312	4154
138F	3312	4365
42F	3312	7169
519A	3312	1133
519A	3312	1439
519A	3312	1500
532A	3312	6133
532A	3312	6411
CR5	3312	3032
CR5	3312	3150
CR5	3312	3166
SOLARSET	3312	107

Maintenance of Transgenic Plant materials

To prevent the dissemination of propagules, the following precautions were taken. All transplants were grown in Calgene greenhouses. Seed was maintained and handled under the control of Calgene personnel within Calgene facilities. Transport of transplants to the field was conducted within enclosed trailers.

Field Operations

Planting of the transplants was done by mechanical transplanter under the supervision of Calgene personnel. Any leftover transplants were destroyed and left in the field. Standard tomato cultural practices were conducted by the farmer during the seedling and vegetative stages. Plants were grown on poles. Pesticides and herbicides were applied either through the drip lines or by ground and aerial application. Harvesting was conducted by Calgene personnel. Fruit was placed in sealed buckets and transported within in an enclosed and locked van and trailer to Calgene greenhouse facilities for seed harvest.

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 cultivations and herbicide applications. Neighboring tomatoes and weed species were checked during the three field inspections by Calgene personnel and no evidence of horizontal movement into other organisms by 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: Stink bug, army worm, pin worm, tomato fruit worm and horn worm, aphids, russet mites, thrips, white flies, leaf miners and flea beetle.

DISEASES: Phytophthora, fusarium, crown gall, stem canker, powdery mildew, tobacco mosaic, early and late blight, tomato spec., and leaf spot.

Specifically, each trial was monitored weekly by a Pest Control Advisor (PCA) or 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 their respective non-transgenic controls on 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 September 20, October 26 and November 1, 1994 whereas the field was walked through and observed on a plant by plant, line by line basis, comparing the transgenic plants to their non-transgenic controls. On these dates no crown gall disease or cauliflower mosaic virus (CaMV) was observed. Aphids, pinworms, tomato fruit worms and fruit flies were observed in both transgenic and non-transgenic plants. Plant pests were equally dispersed, with no significant population differences between plant lines. Plants in each portion of the trial were examined for any abnormalities arising from the transformation process utilizing the controls included for each transgenic line to aid the observation process. These

CALGENE
Field Trial Report 94-118-01N
11/29/94

Field Trial Under USDA Notification

Locations	Gene(s)	Date of Notification	Notification Number
San Joaquin County, Tracy, CA.	asPG, asACC synthase, asPG-asACC synthase, SPS, ACCD, Samase	May 28. 1994	94-118-01N

Contains No Confidential Business Information

Introduction

The objective of the trial was to evaluate genes in fresh market tomatoes. The genes and related construct numbers evaluated are

- asPG (FLAVR SAVR) = antisense polygalacturonase (pCGN4109a)
- asACC synthase = antisense ACC synthase (pCGN3304a)
- asPG-asACC synthase = antisense ACC synthase-antisense polygalacturonase pCGN3312)
- SPS = sucrose phosphate synthase (pCGN3812)
- ACCD = ACC deaminase (4116 and 4140)
- Samase (pGA - SESKN and pCGN4199)

The field trial was divided into two plantings; Manteca I was planted June 7-8, 1994 and Manteca II on July 6-7, 1994.

Chronology

Site	Seeded in greenhouse	Planted	Harvested	Inspections by Calgene	Destruction
Tracy, CA	April 20 and June 2, 1994	June 6-8, 1994 and July 6-7, 1994	Weekly from August 29 through November 10, 1994	August 11, September 8, 20 and 22, October 10, 11 and 25 and November 2, 1994	November 21 and 22, 1994

observations were conducted throughout the entire growing cycle. The advanced lines grown in the trials did not exhibit abnormal appearance or characteristics.

In addition to Calgene personnel walking the field, the field was also walked by Bob Gilbertson of University of California, Davis, Department of Virology and Ed Beckman, Manager of the California Tomato Board, to make observations on diseases present in the Huron fields. Cucumber Mosaic Virus, Tobacco Etch Virus and Potato Virus Y were a major problem for tomato fields in the Huron area this year. The Calgene field trial was badly affected by Cucumber Mosaic virus. All of the plants in the field trial were effected by the virus, which showed no preference for either transgenic or non-transgenic plants. Commercial varieties in neighboring fields also were effected by this virus which was vectored by aphids. Although the plants growth was stunted by the virus, they produced normal fruit.

The only observed difference between transgenic plants and non-transgenic plants in this field trial was between plants containing the asACCS gene (pCGN3312 constructs), and their respective controls. Some plant lines transformed with these constructs exhibited a delayed ripening phenotype resulting in an absence of red fruit in those lines. All other plant characteristics; flowering, maturity, and yield were within normal range. Plants transformed with the asPG and SPS constructs appeared normal with respect to plant morphology and fruit development when compared to their non-transgenic parents.

Harvest and Gene Function

Fruit from the Huron trial was harvested November 15 and 16, 1994. All events from this trial were harvested for seed. Fruit were picked into plastic buckets which were then sealed with lids. The buckets were transported to the Calgene - Galt greenhouse facility in an enclosed trailer or enclosed van for seed extraction. Fruit parts other than seed were destroyed.

Trial Destruction

Upon completion of the field trial harvest, the poles and string were then pulled and a flail mower was used to chop the plants. The field was then disked three times using the cooperating farmer's equipment. To prevent seed dissemination, field equipment was scraped and water tanks equipped with high pressure pumps were used to clean both the tractor and implement to remove all reproductive plant parts. This procedure was performed within the field trial boundaries.

The field was disked November 21 through 23, 1994 in preparation for the planting of the next crop. The field is scheduled to planted as a pistachio orchard.

Trial Entries

Manteca 1

Variety	Construct	Event
UC82B	Control	
UC82B	3812	9
84F		
84F	4109a	148
114F	Control	
114F	4109a	26
115F	Control	201
115F	4109a	201
115F	4109a	309
121F	Control	
121F	4109a	333
121F	4109a	1071
121F	4109a	1120
124F	Control	
124F	4109a	120a
137F	Control	
137F	4109a	265
138F	Control	
138F	4109a	164
141F		
141F	4109a	25a
141F	4109a	25c
141F	4109a	81
105F	Control	
105F	3312	8010
105F	3312	8034
105F	3312	8065
105F	3312	8217
105F	3312	8218
105F	3312	8396
115F	3312	2028
115F	3312	2075
115F	3312	2111
115F	3312	2131
115F	3312	2148
115F	3312	2214
138F	3312	4010
138F	3312	4102
138F	3312	4112
138F	3312	4114
138F	3312	4142
138F	3312	4228
138F	3312	4392
138F	3312	4412
138F	3312	4423
138F	3312	4460

Variety	Construct	Event
42F	3312	7002
42F	3312	7003
42F	3312	7018
42F	3312	7027
42F	3312	7032
42F	3312	7066
42F	3312	7169
42F	3312	7181
519A	3312	1369
519A	3312	1439
519A	3312	1480
519A	3312	1500
519A	3312	1523
519A	3312	1547
519A	3312	1602
519A	3312	1631
519A	3312	1635
519A	3312	1571a
532A	3312	6051
532A	3312	6137
532A	3312	6201
532A	3312	6329
532A	3312	6342
532A	3312	6347
532A	3312	6411
84F	3312	5010
84F	3312	5013
84F	3312	5062
84F	3312	5068
84F	3312	5129
84F	3312	5331
CR5	3312	3032
CR5	3312	3079
CR5	3312	3093
CR5	3312	3142
CR5	3312	3148
CR5	3312	3150
CR5	3312	3166
SOLARSET	3312	10
SOLARSET	3312	50
SOLARSET	3312	51
SOLARSET	3312	92
SOLARSET	3312	100
SOLARSET	3312	105
SOLARSET	3312	107
SOLARSET	3312	40a

Manteca 2

Variety	Construct	Event
84F	Control	
84F	3304a	8a
84F	3304a	23
84F	3304a	43
84F	3304a	53a
84F	3304a	72
84F	3304a	93
84F	3304a	110
84F	3304a	117
84F	3304a	121
84F	3304a	125B
84F	3304a	142
84F	3304a	144
84F	3304a	155
121F	Control	
121F	3304a	4C
121F	3304a	10C
121F	3304a	62b
121F	3304a	64a
121F	3304a	72a
121F	3304a	75b
121F	3304a	85a
121F	3304a	208a
115F	Control	
115F	3312	2075
115F	3312	2214
115F	3312	2345
115F	3312	2128
138F	Control	
138F	3312	4010
CR5	Control	
CR5	3312	3150
CR5	3312	3142
CR5	3312	3071
CR3	Control	
CR3	4116	111
CR3	4116	133
CR3	4116	183
CR3	4116	189
519A	Control	
519A	4109a	4505
532A	Control	
532A	4109a	5097
585A	Control	
585A	4109a	3604
CR3	Control	
CR3	4199	114
CR3	4199	267
UC82B	Control	
UC82B	3812	9

Maintenance of Transgenic Plant materials

To prevent the dissemination of propagules, the following precautions were taken. All transplants were grown in Calgene greenhouses. Seed was maintained and handled under the control of Calgene personnel within Calgene facilities. Transport of transplants to the field was conducted within an enclosed trailer.

Field Operations

Planting of the transplants was done by hand for the Manteca 1 portion of the trial and by a mechanical transplanter for Manteca 2. Planting was supervised by Calgene personnel. Any leftover transplants were destroyed and left in the field. Standard tomato cultural practices were conducted by the farmer during the seedling and vegetative stages. Plants were grown on poles. Pesticides and herbicides were applied either through the drip lines or by ground and aerial application. Harvesting and subsequent transport of the transgenic fruit was conducted by Calgene personnel within sealed buckets in enclosed vehicles to Calgene facilities.

Containment and Safety

Bare earth buffers of 30 feet each were maintained on all sides of each trial to enhance the prevention of pollen transfer and seed dissemination. This zone was kept weed free by periodic cultivations and herbicide applications. No evidence of horizontal movement into other organisms by the transgenic tomatoes was observed during the growing season.

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: Stink bug, army worm, pin worm, tomato fruit worm and horn worm, aphids, russet mites, thrips, white flies, leaf miners and flea beetle.

DISEASES: Phytophthora, fusarium, crown gall, stem canker, powdery mildew, tobacco mosaic, early and late blight, tomato spec., and leaf spot.

Specifically, each trial was monitored weekly by a Pest Control Advisor (PCA) or cooperator hired by Calgene. As the plants approached maturity, the frequency of monitoring was increased; generally 2 to 3 times per week.

The field was walked and observations were made comparing the transgenic plants to their respective non-transgenic controls on seven separate occasions including the dates of harvest, by Calgene research staff. Plant observations were made regarding plant morphology, flowering, fruit set, fruit ripening, and general plant health with respect to disease and insects. Inspections were made on (8/11, 9/8 9/20, 9/22, 10/10, 10/11, 10/25 and 11/2/94) whereas the field was walked through and observed on a plant by plant, line by line basis, comparing the transgenic plants to their non-transgenic siblings. On these dates no crown gall disease or cauliflower mosaic virus (CaMV) was observed. Aphids, russet mites and pinworms were observed in both transgenic and non-transgenic plants. Plant pests were equally dispersed, with no significant population differences between plant lines. Plants in each portion of the trial were examined for any abnormalities arising from the transformation process utilizing the controls included for each transgenic line to aid the observation process. These observations were conducted throughout the entire growing

cycle. The advanced lines grown in the trials did not exhibit abnormal appearance or characteristics.

The only observed difference between transgenic plants and non-transgenic plants in this field trial was between plants containing the asACCS gene (pCGN3304a and pCGN3312 constructs), or the Samase genes (pCGN4199 and pGA-SESKN) and their respective controls. Some plant lines transformed with these constructs exhibited a delayed ripening phenotype resulting in an absence of red fruit in those lines. All other plant characteristics; flowering, maturity, and yield were within normal range. Plants transformed with the asPG and SPS constructs appeared normal with respect to plant morphology and fruit development when compared to their non-transgenic parents.

Harvest and Gene Function

Fruit from the Manteca I portion of this trial was harvested on a weekly basis starting August 29, 1994 and ending September 28, 1994. Fruit from the Manteca II portion of the trial were harvested on a weekly basis starting October 6, 1994 and ending November 10, 1994.

Fruit from the pCGN3812 lines (SPS) were harvested from Manteca I on 8/29, 9/14, and 9/28/94 for laboratory analysis, and 9/19/94 for yield analysis in the field. Fruit from Manteca II were harvested on 10/19/94 for laboratory analysis, and 10/26/94 for yield analysis in the field. Fruit were harvested at stage 6 (red) and were placed into plastic zip lock bags, and the bags containing fruit were placed into a cardboard tomato packing box. Fruit were transported to Calgene laboratory facilities in an enclosed van for evaluation of alteration in carbohydrate partitioning and utilization as measured by R/I (refractive index).

Fruit from the pCGN3304a (asACCS), pCGN3312 (asPG/asACCS), pCGN4116 and pCGN4140 (ACC deaminase) and pGA-SESKN and pCGN4199 (Samase) lines were harvested at stages 2, 3 and 4 (breaker, turning and pink) and shipped using simulated industry conditions to Davis, CA facility for evaluation of delayed ripening phenotypes, and shippability. Fruit were picked on a weekly basis from August 29 to November 10, 1994 and transported to Calgene laboratory facilities in an enclosed van.

Fruit from the pCGN4109a (asPG) lines were harvested at stage 6 (red) and were placed into plastic zip lock bags, and the bags containing fruit were placed into a cardboard tomato packing box. Fruit were transported to Calgene laboratory facilities in an enclosed van. The fruit were assayed for Vitamin A and C levels as well as levels of reduction of the enzyme polygalacturonase.

At completion of all evaluations, fruit were either destroyed (autoclaved) in Davis or shipped to Galt, CA for seed collection and destruction of remaining fruit parts.

Trial Destruction

Upon completion of the field trial harvest, the poles and string were then pulled and a flail mower was used to chop the plants. The field was then disked three times using the cooperating farmer's equipment. To prevent seed dissemination, field equipment was scraped and water tanks equipped with high pressure pumps were used to clean both the tractor and implement to remove all reproductive plant parts. This procedure was performed within the field trial boundaries.

The field was disked on 11/21&22/94 and will lay fallow during the winter 1994/95.