94-290-01p

ZENECA

ZENECA Plant Science
1800 Concord Pike
P.O. Box 751
Wilmington, Delaware 19897

Telephone (302) 886-5000
Telephone (800) 456-3669
Telefax (302) 886-1610

February 8, 1995

Mr. Michael A. Lidsky
Deputy Director, BBEP, APHIS, USDA
6505 Belcrest Road
Federal Building
Hyattsville, MD 20782

Subject: Petition for Release from Regulation for modified T7 processing tomato inbred lines (B, Da and F) and hybrids developed from them.

Petition # 94-290-01

Dear Mr. Lidsky:

Zeneca Plant Science, a business unit of Zeneca Inc., and Petoseed Company Inc. are requesting a determination from USDA that modified lines, B, Da, and F, and hybrids derived from them, no longer be considered regulated articles.

We are enclosing a replacement Volume I to our earlier submission under Petition # 94-290-01. We have included the additional information requested by the Agency, as well as notes and clarification as discussed with USDA scientists. We have certified that we are unaware of any adverse information that would be unfavorable to the petition.

Please return in the postage paid Federal Express pack the copies of our original submission, dated October 14.

There are no changes to Volume II, part of the original petition, which includes our bibliographies and references. Neither Volume I or Volume II contain confidential business information.

Please feel free to contact either Dr. Ed Green - Petoseed Company Inc. at (916-666-0931) or me (302-886-1224) if you need any additional information.

Sincerely,

Anne Mueller

Anne Mueller
Manager, Regulatory & Public Affairs
Zeneca Plant Science

#1-Cols 2/10/95

94-290-01p

Petition for Release from Regulation for Modified Lines B, Da and F Derived from T7 Varieties of Processing Tomatoes

Submitted by:

Zeneca Plant Sciences
United Kingdom

and

Petoseed Co., Inc.
United States of America

Summary

Tomato is one of the worlds most versatile and important food crops. We have developed inbred lines of tomato containing genes that improve the processing characteristics of these lines. Hybrids produced using these inbreds also show improved processing characteristics. The gene constructs were introduced into the parental inbred line by transformation and several hundred tomato plants were regenerated from selection experiments. After a series of tests in the laboratory, glasshouse and field, three inbred lines were identified that are suitable for commercial release and use in further breeding.

The gene constructs used for transformation were fully characterized in terms of sequence and origin. Two regions from the vector are known to be transcribed in the plant. In addition a sequence computer analysis revealed a number of potential open reading frames. None of these have the characteristics of the transcribed genes.

The candidate lines for commercialization were analyzed at the biochemical, genetic and DNA level over several seed-derived generations. Inbred lines: B, Da and F and hybrids made from them all had improved processing characteristics. The agronomic characteristics of these lines were indistinguishable from the parental inbred line and were not altered in their weediness characteristics.

Compositional analysis of the modified lines showed no significant difference in content of vitamins A and C, content of fiber, ash, titratable acidity, pH or color as compared to the parental variety. The lines were specifically improved in their processing characteristics due to the reduced degradation of pectins by the enzyme polygalacturonase.

We conclude that these tomato lines are suitable for release and commercialization and request U.S.D.A. exemption from regulatory oversight.

PETITION FOR RELEASE FROM REGULATION FOR MODIFIED T7 VARIETIES OF PROCESSING TOMATOES

The undersigned submits this petition to request that the Director, U.S.D.A., make a determination that modified T7 varieties of processing tomatoes having suppressed polygalacturonase enzyme activity no longer be considered a regulated article.

CERTIFICATION

The undersigned certifies, that to our best knowledge, this petition includes all information and views on which to base a determination, and that it includes relevant data and information known to the petitioner.

Submitted by:

Anne Mueller
Regulatory & Public Affairs Manager
Zeneca Plant Science (a business unit of Zeneca Inc.)
1800 Concord Pike
Wilmington, DE 19897
Tel: (302) 886-1224
Fax: (302) 886-1610

Charles E. Green
Vice President, New Technology
Petoseed Company Inc.
37437 State Highway 16
Woodland, CA 95695
Tel: (916) 666-0931
Fax: (916) 668-0219

Contains No Confidential Business Information

Petition for Release from Regulation for Modified Lines B, Da and F Derived from T7 Varieties Of Processing Tomatoes

Authors:

Wolfgang Schuch, Team Leader
Zeneca Plant Science

Charles E. Green
Petoseed Company, Inc.

Anna Trulson
Petoseed Company, Inc.

Date:

February 8, 1995

"Contains No Confidential Business Information"

Table of Contents

List of Tables

List of Figures

List of Appendices

Rationale for the Development of Low PG Processing Tomatoes

Tomato is currently one of the world's most versatile and important food crops. Grown on about seven million acres worldwide, the annual commercial world production of tomatoes is over seventy-six million tons, of which the United States produces 16%, followed by the former Soviet Union at 10%, Turkey and the Peoples Republic of China at 8% each, and Italy at 7%. There are two distinct tomato industries in the U.S., fresh market tomatoes and processing tomatoes. Each industry requires distinct tomato cultivars that were selected and bred over the years for the specific requirements of each market and production areas. The U.S. processing tomato industry grows 86% of the tomato volume, 90% of which is grown in California. The processing tomato crop in 1990 was valued at $700 million.

Eaten fresh, cooked in a variety of recipes, or consumed in one of its many processed forms, consumption of tomato in the U.S. is about 86 pounds per capita per annum. Over 80% of this is consumption of processed tomato food products such as catsup, juice, pizza sauce and salsa. Tomato's versatility and variety as a processed food contributed greatly to its prominent place in the American diet (Plummer, 1992). Even though tomato does not have high levels of vitamins, minerals and other dietary components, it makes a major contribution to the American diet for total vitamins and minerals due to its large per capita consumption.

Over the years tomato breeding provided the processing industry with improved varieties that are adapted to particular growing conditions, have stable yields in different environments and are resistant to pests and diseases. In addition, breeders have focused on development of tomato varieties that have appropriate quality determinants required by the processing industry. The major quality attributes for processing are solids, viscosity and color. Using conventional breeding it was possible to make progress toward developing tomato varieties in which these traits were improved.

The advent of biotechnology enabled the breeding community to make quantum improvements in specific quality attributes. One example is the introduction of tomato varieties with increased viscosity characteristics (Schuch *et al.*, 1991). This was achieved through introduction of genes into tomato that reduce the levels of the enzyme polygalacturonase (PG) (Smith *et al.*, 1988; Smith *et al.*, 1990). This enzyme is largely responsible for the breakdown of pectin in the tomato fruit. Pectin is a major contributor to viscosity characteristics of processed tomato products. Tomatoes in which PG was reduced allow improvements at all stages of production from harvesting to cooking and canning. Reduced PG enables the processors to work with thicker tomatoes which leads to increased yields and products with improved flavor and texture. The new varieties have improved consistency because of less breakdown of pectin.

Summary

The aim of this work was to introduce a known viscosity effect gene into commercial hybrid varieties. Over three hundred low PG tomato lines were generated. These were stringently evaluated for the following biochemical and molecular criteria: reduction of PG enzyme activity, presence of a single dominant effect gene, and stability of the gene in inbred lines and in a range of commercial hybrid varieties. In addition, experienced Petoseed tomato breeders evaluated the low PG lines for identity to the unmodified parental lines. Genetically modified lines B, Da and F fulfilled the criteria of identity to the unmodified inbred. Several commercial hybrids were made using these modified inbreds. They were extensively evaluated in field trials in California and Chile, as well as in Portugal and Australia.
In all trials, the genetically modified tomato inbreds and hybrids were evaluated by experienced tomato breeders, growers, agronomists and scientists. It was shown that the modified inbred lines used for the production of commercial hybrids do not have any unexpected characteristics that may contribute to increased outcrossing or weediness.

The low PG varieties that will be used for commercial production will be either hybrids tested so far or other hybrids derived from the inbred lines described here. This will involve traditional crossing, selection, hybrid evaluation, procedures that have been performed in tomato breeding for many decades.

References

Plummer, C. (1992) U.S. Tomato Statistics, 1960-90, U.S. Department of Agriculture Statistical Bulletin No. 841

Schuch, W., Kanczler, J., Robertson, D., Hobson, G., Tucker, G., Grierson, D., Bright, S., and Bird, C. (1991) Fruit quality characteristics of transgenic tomato fruit with altered polygalacturonase activity. HortScience 25, 1517-1520

Smith, C.J.S., Watson, C.F., Ray, J., Bird, C.R., Morris, P.C., Schuch, W., and Grierson, D. (1988) Antisense RNA inhibition of polygalacturonase gene expression in transgenic tomatoes. Nature 334, 724-726

Smith, C.J.S., Watson, C.F., Bird, C.R., Schuch, W., and Grierson, D. (1990a) Expression of a truncated tomato polygalacturonase gene inhibits expression of the endogenous gene in transgenic plants. Mol. Gen. Genet. 224, 477-481

Stevens, M.A. (1986) Inheritance of Tomato Fruit Quality Components, in Plant Breeding Reviews (J. Janick, ed.), Vol. 4, AVI Publishing, 273-311

The Tomato Family

Taxonomy

Commercial tomato is identified as *Lycopersicon esculentum* Mill. It is a member of the *Solanaceae* family which includes peppers, eggplants, potatoes, tobacco, and a variety of New World tropical fruits, as well as many medicinal and poisonous plants. The genus *Lycopersicon* consists of *L. esculentum* and seven closely related wild species all of which have the same chromosome number (2n=24) and chromosome morphology (Rick, 1976). The genus can be subdivided into two groups, those species that can be crossed readily with *L. esculentum* (the esculentum-complex) and those that can not (the peruvianum-complex). The species forming the esculentum-complex are *L. esculentum, L. pimpinellifolium, L. cheesmanii, L. parviflorum, L. chmielewskii,* and *L. hirsutum*. The species forming the peruvianum-complex are *L. chilense* and *L. peruvianum* (Taylor, 1986). All of these species have been and are being used for genetic improvement of commercial tomato cultivars and from the gene pool for today's commercial tomato varieties.

With the exception of *L. cheesmanii*, which is found only on the Galapagos Islands, all wild *Lycopersicon* species are native to the Andean region from Colombia to northern Chile. This region, centered in Peru, is the center of origin and diversity for the genus. The origin and early history of the cultivated tomato is less clear, but most of the evidence points to Mexico as the probable center of domestication and source of the cultivated tomatoes first brought to Europe. *L. esculentum* variety *cerasiforme* is the common weedy cherry tomato that occurs spontaneously throughout Mexico and Central America, and is widely accepted as the immediate ancestor of the cultivated tomato (Rick, 1976).

History of Use

The first record in the Old World describing tomato comes from the Italian herbalist Pier Andrea Mattioli in 1554 (Rick, 1976). Sturtevant cites numerous other references from European herbalists that indicate that tomato spread throughout Europe during the 16th century and was known in different languages as the gold apple (pomodoro), love apple (pommes d'amours), and tomatoes. Its arrival in England is put at 1596 when Gerarde says he received seed for his garden from Spain, Italy and other hot countries (Hedrick, 1919). Though this implies that tomato was accepted as a new food in Europe soon after the likely introduction from Mexico in early 16th century, this is not the case. The similarities of tomato to the known poisonous members of the *Solanaceae*, belladonna and mandrake, led to strong suspicions about its safety as food. As a result, tomato remained a curiosity for most of the people in Europe and elsewhere for many years. It is not until the late 1700's that tomato sauces began appearing in noted Italian cookbooks. Thus began the widespread use of tomato as a food in Italy.

Tomatoes were first mentioned in North America in the writings of William Salmon in 1710 (Rick, 1978). D.J. Brown in a U.S. Patent Office Report, 1854, said that until the year 1834 tomato was virtually unknown in the U.S. as a vegetable. The History of the Massachusetts Horticultural Society, 1880, states that not until 1844 did tomato start to become a popular vegetable. In 1847, at Lafayette College at Easton, Pennsylvania, the possibility of canning tomatoes was first demonstrated. Since then, tomato proved to be a very versatile processed food and became the leading processed vegetable crop and main staple in diet in the U.S.

Genetics of Processing Tomato Varieties

The development of improved tomato cultivars by selection from the original germplasm base imported from the New World to Europe began only after tomato was recognized as a safe and wholesome food. This happened first in the Mediterranean, and was followed by Northern Europe and North America. Many locally adapted cultivars were developed in Europe and North America by selection of chance variants resulting from mutation, or spontaneous outcrossing and recombination of existing varieties (Stevens and Rick, 1986).

Since the 1920's, tomato improvement proceeded at a much accelerated pace, hand in hand with the growing understanding of the biology of the species, techniques of plant breeding and the growth of the tomato industries and public demand. Dramatic improvements were made in yield, adaptation to specific growing conditions, plant habit, disease resistance and fruit quality, for both the fresh market and processing industries. Several single gene traits were of particular importance to tomato crop improvement. Many of these traits, of which resistance to diseases is one example, were often derived from related wild species. The development of the first machine harvested tomato cultivar by G.C. Hanna in the late 1950's and early 1960's utilizing the self pruning (*sp*) gene revolutionized the California processing tomato industry.

Today, modern day cultivars are bred to be specifically adapted to the production areas, industry requirements and consumer preferences. Breeders build on the genetic improvements of the past, as well as utilize new sources of genetic variation available from wild species and new molecular biology techniques, to develop superior quality tomato cultivars for the growers, tomato processing industries and consumers.

Many of today's varieties are inbred varieties. However, more recently production of single cross hybrids became the predominant method for production of cultivars that have desired environmental adaptation, disease tolerances and quality traits. The single cross hybrids allow the most efficient combination of desirable traits from the broadest range of germplasm.

Tomato is classified as a self-pollinated crop. The structure of tomato flower promotes autogamy: the anthers are arranged above the stigma in a tube into which pollen is released. This autogamy is a transition in cultivated tomato from exserted to inserted stigmas within the anther cone. Over the past 50 years, the change in style-length has been dramatic. This was brought about through selections made by breeders who desired improved self-pollination and fruit set. This development has virtually eliminated outcrossing (Rick, 1976). Taylor (1986) reports that all *L. esculentum* species are self-compatible and exclusively inbreeding. Hybrid varieties of commercial processing and fresh market tomatoes are produced by controlled hand pollinations.

Potential for Outcrossing

Outcrossing with Domesticated Tomato Species

The natural outcrossing of tomatoes was reviewed by Rick in 1992. The potential for outcrossing depends on several factors including flower morphology, genetic background, planting density, and environmental factors such as the availability of bee populations. Outcrossing among tomato cultivars was assessed using various methods (Rick, 1992). Low frequencies (between 0-5%) were reported for special experimental designs, in which outcrossing potential was optimized. In conventional production fields the frequencies of outcrossing are very low.
The potential for tomatoes to become weeds was reviewed previously (Redenbaugh, 1992). There is considerable historical evidence that despite the large acreage of plants grown annually the rate of

outcrossing between tomato varieties in conditions of intensive agricultural usage does not lead to weediness of tomatoes.

The potential for weediness of tomatoes in which PG enzyme activity was reduced using antisense constructs to PG (Hiatt *et al.*, 1988) was reviewed by the U.S.D.A. previously (Redenbaugh *et al.*, 1992; U.S.D.A., A.P.H.I.S., 1992). On July 14, 1992 A.P.H.I.S. received the petition from Calgene requesting that the low PG tomatoes be removed from regulatory oversight. This petition was granted on October 19, 1992.

Outcrossing with Wild Species

Pre- and post-fertilization barriers which prevent cross pollination between *Lycopersicon* species are well documented (Taylor, 1986). Tomato can only be crossed by hand-pollination to wild *Lycopersicon* species. Wide hybridization between members of the two subgenera, esculentum and peruvianum, typically leads to early embryo breakdown and non-viable seed. Sexual hybridization between the two subgenera can only be accomplished using embryo culture. The closest genetic relatives of *Lycopersicon* are in the genus *Solanum*. *L. esculentum* can also be crossed with *S. lycopersicoides* using controlled pollination techniques, although the hybrids are usually sterile (Stevens and Rick, 1986). Attempts to cross *L. esculentum* with *S. rickii* and *S. ochranthum* failed (Rick, 1979). Recently, a controlled cross between *L. esculentum* and *S. rickii* was successful using a sesquidiploid bridging hybrid (De Vena *et al.*, 1990), that may provide a means to move genes from *S. rickii* to commercial cultivars. No other member of the genus, including *S. nigrum*, a common weed in tomato fields, yielded any viable hybrids with tomato (Taylor, 1986).

Natural interspecific crossing occurs only in the tomato's natural habitat in South America and only within the esculentum subgenus. There is strong evidence, however, that even in the natural range, interspecific crossing does not occur. Esquinas-Alcazar (1981) stated that many species overlap but no evidence of natural introgression was found, with the exception of *L. pimpinellifolium* and *L. esculentum*.

Various bees (excluding honey bees) are the principal pollen vectors. Under field and greenhouse conditions airborne pollen is of little consequence for outcrossing. In fields in the major tomato growing regions, solitary and bumble bees seldom visit flowers of cultivated tomatoes as they are more attracted to the larger, better displayed flowers (usually with well-exserted stigmas) of other species. Thus, there is a very low likelihood of outcrossing to wild species.

The only wild *Lycopersicon* species that was found outside Latin America is *L. esculentum* variety cerasiforme. This species became established in south Florida and in southern Texas but is not considered a weed species. No natural outcrossing between *L. esculentum* and *L. esculentum* variety cerasiforme has been reported.

Outcrossing with Other Related Species

A number of weed species was identified by U.S.D.A. to occur in or in the vicinity of commercially grown tomato fields. *Solanum nigrum* is the only major weed pest related to tomato. Other members of the nightshade family that are weeds in tomato fields are: *S. sarrachoides, Physalis heterophylla, P. lanceifolia, P. xocarpa, P. acutifolia, Nicotiana bigelovii, Datura stramonium, D. meteloides,* and *D. ferox*. Other weedy *Solanaceae* are: *Hyoscyamus niger, Lycium ferocissimum, P. virginiana* variety *sonorae, P. viscosa, S. cardiophyllum S. carolinense, S. lanceolatum, S. marginatum* and *S. torvum*. Tomato (*L. esculentum*) is sexually incompatible with all of these weedy relatives.

References

De Verna, J., Rick, C., Chetelat, R., Lanini, B., Alpert, K. (1990) Sexual hybridization of *Lycopersicon esculentum* and *Solanum rickii* by means of a sesquidiploid bridging hybrid. Proc. Natl. Acad. Sci. USA 87:9486-9490

Esquinas-Alcazar, J. (1981) Genetic resources of tomatoes and wild relatives - a global report. Rome, Italy, International Board for Plant Genetic Resources. 65

Hedrick, U.P. (ed.) (1919) Sturtevant's Notes on Edible Plants, Report of the New York Agricultural Experiment Station, Vol. 2, Part II

Redenbaugh, K., Hiatt, W., Martineau, B., Kramer, M., Sheehy, R., Sanders, R., Houck, C., Emlay, D. (1992) Safety Assessment of Genetically Engineered Fruits and Vegetables: A Case Study of the FLAVR SAVR™ Tomatoes. CRC Press, Boca Raton

Rick, C. (1976) Tomato *Lycopersicon esculentum (Solanaceae)*. In: Evolution of Crop Plants (N. Simmonds, ed.). Longman Publications, New York. 268-273

Rick, C. (1979) Biosystematic studies in *Lycopersicon* and closely related species of Solanum. In: The Biology and Taxonomy of the *Solanaceae* (J. Hawkes, R. Lester and A. Skelding, ed.). Academic Press, New York. 667-679

Rick, C.M. (1992) Natural Cross-Pollination in Tomatoes, Workshop on Safeguards for Planned Introduction of Transgenic Tomatoes

Sheehy, R.E., Kramer, M., Hiatt, W.R. (1988) Reduction of polygalacturonase activity in tomato fruit by antisense RNA. Proc. Nat. Acad. Sci. 85, 8805-8809

Stevens, M.A. and Rick, C.M. (1986) Genetics and Breeding in the Tomato Crop (ed., J.G. Atherton and J. Rudich) Chapman and Hall. 35-109

Taylor, I.B. (1986) Biosystematics of the Tomato in the Tomato Crop (ed., J.G. Atherton and J. Rudich) Chapman and Hall. 1-34

U.S.D.A., A.P.H.I.S. (1992) Environmental Assessment and Finding of No Significant Impact on Tomato Containing an Antisense Polygalacturonase Gene. Permit Number 91-268-01

Description of the Transformation System and Vectors Used

Agrobacterium as a Delivery System

Ti Plasmids

A common soil-borne bacterium, *Agrobacterium tumefaciens* is known to cause a Crown Gall disease on many plant species. When this bacterium is present at a wound site on a susceptible plant, a complex series of events is initiated that results in transfer of genetic information from the bacterium to the plant and leads to gall formation. Several bacterial genes are required for successful infection. Some of these genes are encoded on the bacterial chromosomal DNA, whereas the majority are found on DNA plasmids called Ti plasmids. A series of genes in the so called "virulence" (*vir*) region of the Ti plasmid is activated by a phenolic compound, acetosyringone, which is present in the exudates from wounded plant tissue. The products of the *vir* genes cause another DNA segment on the same plasmid, called the "transfer" region (T-region or T-DNA), to be moved into plant cells. The molecular biology of Grown Gall disease was extensively reviewed by Koukolikova-Nicola *et al.* (1987).

The T-DNA genes function in plants but not in the bacteria from which they were transferred. The T-region is bordered by imperfect direct repeats called border sequences. Any DNA between these borders is transferred to plant cells where it is stably integrated into the chromosomal DNA. The newly integrated genes then direct the synthesis of substances that alter plant metabolism. Expression of auxin and cytokinin biosynthetic genes results in uncontrolled cell division leading to gall formation, whereas genes coding for opine biosynthesis provide nutrients for the bacteria.

Development of Disarmed Vectors

Disarmed Ti plasmids were constructed from natural Ti plasmids by deleting the tumor inducing and opine synthesis genes from the T-region. Other genes can then be inserted into this region using conventional cloning techniques. Any DNA inserted between the 25 base pair (bp) repeats (border sequences) is co-transferred and integrated into the plant nuclear genome. The T-region appears to integrate randomly into plant chromosomal DNA. Modified Ti plasmids are efficient vectors for transferring DNA inserts into dicotyledonous plant cells from which transformed fertile plants can be regenerated.

Binary Vector Systems

Binary vectors represent a further refinement of the DNA delivery system. The binary system requires two autonomously replicating plasmids in *A. tumefaciens*. One plasmid contains the virulence genes, whereas the second plasmid carries the genes of interest to be introduced into plant cells. The latter plasmid is referred to as a "binary vector" (Hoekema *et al.*, 1983). In this binary system the virulence properties can not be transferred to plant cells because the virulence genes and the effect genes are located on different plasmids. Thus, binary plasmids provide a greater degree of convenience and safety than systems in which the *vir* genes and the plant targeted genes are located on the same plasmid.

Tomato Transformation Involving Agrobacterium

Several methods for transformation of commercial tomato varieties were developed using disarmed binary *A. tumefaciens* vectors. These involve infection of sectioned stems, hypocotyls or cotyledons with Agrobacteria containing the transformation vectors. Tomato cotyledons were used as target tissue for the generation of the genetically modified tomato lines reported here.

Characteristics of the Unmodified Tomato Recipient

Petoseed proprietary inbred tomato line coded as T7 was the unmodified recipient of gene vectors. This inbred line has been used widely in production of elite processing hybrids for different growing regions both in the U.S. and abroad for many years. The success of this inbred line is due to the wide range of important characters that were combined into it by Petoseed breeders. This inbred is characterized by good combining ability and resistance to several important diseases, such as Alternaria Stem Canker and Fusarium Wilt. Seed sales of hybrids containing T7 as one of the parents amounted to approximately 15,000 pounds in 1990.

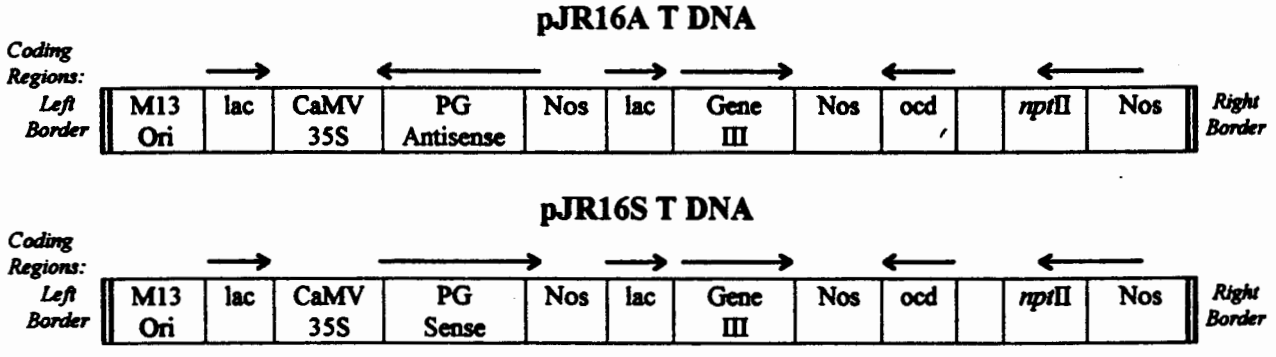
Construction of the Transformation Vectors

The vector constructs that were used to generate the genetically modified tomatoes subject to this submission are binary vectors based on the widely used plant transformation vector pBIN-19 (Bevan, 1984). This vector is characterized by the following features: it contains the left and the right border sequences that delineate the DNA sequences that are transferred to the recipient plant cells. The bacterial neomycin phosphotransferase (*npt*II) gene that is used as a selectable marker for selection of transformed plant cells is located between these border sequences. This gene is fused to the nopaline synthase (NOS) promoter of *Agrobacterium* to obtain expression in plant cells. The pBIN-19 vector also contains a multiple cloning site into which genes of interest, such as the polygalacturonase (PG) effect genes, can be inserted.

In plant transformation vector pJR16A part of the tomato PG gene is inserted in antisense orientation with respect to the CaMV 35S promoter, whereas in plant transformation vector pJR16S the same fragment of the tomato PG gene is inserted in sense orientation.

The construction of these vectors was described previously (pJR16A: Smith *et al.*, 1988, pJR16S: Smith *et al.*, 1990). Schematic maps of the vectors pJR16A and pJR16S are shown in Figure 1. DNA sequences of these vectors are shown in Appendices 1 and 2.

Figure 1. Structure of T-DNA in pJR16A and pJR16S



pJR16A: Contains the tomato PG sequences in antisense orientation.
pJR16S: Contains the tomato PG sequences in sense orientation.

Genetic elements found in these vectors between the border sequences are listed in Table 1. The details of these elements are shown in Table 2 and are further discussed in detail in the **Donor Genes to be Assessed for Non-Regulated Status** section.

Table 1. Genetic Elements of Plant Transformation Vectors pJR16A and pJR16S

Donor	Description of Donor DNA Sequences
Lycopersicon esculentum Mill. (variety Ailsa Craig)	Part of the polygalacturonase gene
Escherichia coli	Part of the beta-galactosidase gene
Agrobacterium tumefaciens	The left and right border sequence; part of the nopaline synthase gene; part of the ornithine cyclodeaminase gene
M13	Part of the origin or replication; part of Gene III
Tn5	Part of the neomycin phosphotransferase gene
CaMV	Part of the 35S promoter

Table 2. Summary of DNA Sequences Found in pJR16S

DNA Seq. Element	Size bp	Function	Origin	References
Left border	25	This border delineates the DNA transferred to plant cells.	*A. tumefaciens* Ti plasmid	Zambryski *et al.*, 1980
Origin of M13 fragment	406	This fragment is part of a larger region which is required for the replication of the M13 in bacteria.	Bacteriophage M13	Wezenbeek *et al.*, 1980 Yanisch-Perron *et.*, 1985
LacZ gene fragment	156	This fragment is derived from the lactose operon of *E. coli.*	*E. coli*	Beckwith and Singer, 1966; Bevan, 1984
CaMV 35S promoter	529	This fragment is responsible for the efficient expression of the PG effect genes (Smith *et al.*, 1988, 1990).	Cauliflower Mosaic Virus	Franck *et al.*, 1980; Odell *et al.*, 1985
PG sense	731	The expression of this fragment in sense orientation leads to down-regulation of PG.	Tomato, var. Ailsa Craig	Grierson *et al.*, 1986; Smith *et al.*, 1990
NOS 3' end	247	This fragment aids the termination of mRNA synthesis.	*A tumefaciens* Ti plasmid	Hernalsteens *et al.*, 1980; Bevan *et al.*, 1983
LacZ gene fragment	231	This fragment is derived from the lactose operon of *E. coli.*	*E. coli*	Beckwith and Singer, 1966; Bevan, 1984
M13 gene III fragment	440	This fragment is derived from gene III.	Bacteriophage M13	Wezenbeek *et al.*, 1980 Yanisch-Perron *et.*, 1985
NOS 3' end	258	This fragment aids the termination of mRNA synthesis.	*A. tumefaciens* Ti plasmid	Hernalsteens *et al.*, 1980; Bevan *et al.*, 1983
ocd gene fragment	209	This is a fragment derived from the OCD gene of *A. tumefaciens* Ti plasmids.	*A. tumefaciens* Ti plasmid	Sans *et al.*, 1987, 1988; Schindler *et al.*, 1989
*npt*II	800	This fragment is derived from the transposon Tn5 and allows the selection of genetically modified plants.	Bacterial transposon Tn5	Bert *et al.*, 1975; Bevan *et al.*, 1983, Bevan, 1984
NOS promoter	227	This fragment promotes the transcription of the *npt*II gene.	*A. tumefaciens* Ti plasmid	Hernalsteens *et al.*, 1980; Bevan *et al.*, 1983
Right border	26	This border delineates the DNA transferred to plant cells.	*A. Tumefaciens* Ti plasmid	Zambryski *et al.*, 1980
Various linkers	433			
Total nucleotides	**4718**			

Analysis of Open Reading Frames

The plant transformation vectors pJR16A and pJR16S were designed to express two functions: 1) the selectable marker gene under the control of the NOS promoter, and 2) the effect gene for the control of PG gene expression (PG antisense: PGA; partial PG sense: PGS) under the control of the CaMV 35S promoter.

A detailed computer analysis of the DNA sequences of both vectors was performed to identify whether additional open reading frames were present. Both strands of the vector DNA sequences were analyzed but open reading frames shorter than 50 amino acids were not covered.
Several potential open reading frames were detected in both transformation vectors. These potential open reading frames encoded proteins ranging in size from less than 10 kD to 29 kD. Potential open reading

frames larger than 10 kD are listed in the accompanying Tables 3 and 4. It should be noted that it is not unusual that DNA fragments contain small open reading frames and this is not a special characteristic of these plant transformation vectors. Detailed computer analyses of the open reading frames identified in pJR16A and pJR16S are shown in Appendices 3 and 4.

Table 3. Open Reading Frame Analysis of pJR16A

Normal Orientation

ORF	Size of Potential Peptide (kD)	Locations
1	19.5	*npt*II
2	15.6	35S Promoter
3	14.9	OCD Gene
4	12.8	OCD Gene
5	11.5	35S Promoter

Inverse Orientation

1	29.0	*npt*II (selectable marker gene)
2	25.7	Inverse PG
3	16.9	3' end *npt*II/OCD
4	16.4	*npt*II
5	15.9	*npt*II
6	14.1	*npt*II
7	12.9	*npt*II
8	12.3	Gene III
9	12.1	OCD
10	11.4	Gene III
11	10.5	OCD

Table 4. Open Reading Frame Analysis of pJR16S

Normal Orientation

ORF	Size of Potential Peptide (kD)	Locations
1	25.3	PG (effect gene)
2	19.5	Inverse *npt*II
3	15.6	35S Promoter
4	14.9	OCD
5	12.8	OCD
6	11.5	35S Promoter

Inverse Orientation

1	29.0	*npt*II (selectable marker gene)
2	16.9	3' end *npt*II/OCD
3	16.4	*npt*II
4	15.9	*npt*II
5	14.1	*npt*II
6	12.9	*npt*II
7	12.3	Gene III
8	12.1	OCD
9	11.4	Gene III
10	10.5	OCD

Several conclusions can be drawn from this computer analysis:

- The open reading frame of the selectable marker gene (*npt*II) was clearly identified. This selectable marker gene was used for the generation of the genetically modified tomato lines.
- The open reading frame covering part of the PG gene in pJR16S was also clearly identified. This open reading frame extends the partial sequence of the PG protein by 3 amino acids.
- There were no open reading frames identified in the normal orientation in the part of the PG gene sequences used in pJR16A.
- Several potential open reading frames were located within the coding sequence of the *npt*II gene or its inverse complement. There are no cited reports that any of these is expressed in genetically modified plants using the same promoter selectable marker gene cassette.
- Several potential open reading frames were located within the CaMV 35S promoter. There are no cited reports indicating that any of these potential open reading frames is translated into mRNA or peptides in genetically modified plants using the same promoter sequences.
- Several potential open reading frames were found in the OCD gene sequences from *Agrobacterium* Ti plasmid and the Gene III sequences from M13. These do not match the known open reading frames of the OCD and Gene III genes accept for cases discussed below.

In order to assess whether any of these potential open reading frames can be potentially transcribed into mRNA and proteins within the genetically modified tomato fruit, further computer analyses were carried out (the computer program EUKPROM was used for these analyses, this program is a part of the PC/GENE

suite of DNA sequence analysis programs by Intelligenetics, Inc.). These analyses focused on three essential features required for eukaryotic gene expression:

- The transcriptional activation region located around the TATA box of eukaryotic promoters (Bucher, 1990).
- The CAP signal sequence required for the modification of the 5' end of the mRNA (Bucher, 1990).
- The consensus sequences required for efficient translation (Lutcke *et al.*, 1987).

A summary of these analyses is shown in Appendix 5. These analyses demonstrated two potential regions of transcription adjacent to potential open reading frames. These are:

- Potential open reading frames 1, 3 and 4 in pJR16A and potential open reading frames 2, 4 and 5 in pJR16S. These potential open reading frames are equivalent between these vectors (e.g., 1 in pJR16A equals 2 in pJR16S, etc.) These potential open reading frames are located on the opposite strand of the transcription unit which expresses the *npt*II gene. There are no cited reports that any of the frames are expressed in genetically modified plants using the same promoter selectable marker gene cassette.
- Potential open reading frame 2 in pJR16A (inverse orientation). This potential open reading frame is located on the opposite strand of the transcription unit which expresses PG antisense.

In summary, we conclude that:

- Expression of the open reading frames found on the opposite strand of the PG and *npt*II effect genes is unlikely to occur. Any expression from the frames would almost certainly interfere with PG and *npt*II expression; this clearly does not occur.
- Other open reading frames are not associated with plant based transcriptional and translational control regions and are therefore highly unlikely to be expressed into proteins.

References

Bevan, M. (1984) *Agrobacterium* vectors for plant transformation. Nucl. Acids. Res. 12, 8711-8721

Bucher, P. (1990) Weight matrix descriptions of four eukaryotic RNA polymerase II promoter elements derived from 502 unrelated promoter sequences. J. Mol. Biol. 212, 563-578

Hoekema, A., Hirsch, P.R., Hooykaas, P.J.J., and Schilperoort, R.A. (1983) A binary plant vector strategy based in separation of vir- and T-region of the *Agrobacterium tumefaciens* Ti-plasmid. Nature 303, 179-182

Lutcke, H.A., Chow, K.C., Mickel, F.S., Moss, K.A., Kern, H.F., Scheele, G.A. (1987) Selection of AUG initiation codons differs in plants and animals. EMBO J. 6, 43-48

Smith, C.J.S., Watson, C.F., Ray, J., Bird, C.R., Morris, P.C., Schuch, W., and Grierson, D. (1988) Antisense RNA inhibition of polygalacturonase gene expression in transgenic tomatoes. Nature 334, 724-726

Smith, C.J.S., Watson, C.F., Bird, C.R., Schuch, W., and Grierson, D. (1990a) Expression of a truncated tomato polygalacturonase gene inhibits expression of the endogenous gene in transgenic plants. Mol. Gen. Genet. 224, 477-481

The Donor Genes to be Assessed for Non-Regulated Status

The PG Effect Genes

The effect gene sequence is derived from a tomato *(Lycopersicon esculentum* Mill. variety Ailsa Craig) and encodes the enzyme polygalacturonase (PG) gene. PG is a key enzyme in fruit ripening. It accumulates only during ripening due to *de novo* synthesis of the enzyme (Tucker and Grierson, 1982). It is responsible for the breakdown of pectin molecules in the cell walls of tomato fruit (Hobson, 1965). Pectin is a large polymer consisting of galacturonic acid residues to which rhamnose residues are attached at irregular intervals. Pectin is largely insoluble in green fruit. During ripening, the average size of pectin molecules significantly decreases with a coincident increase in soluble polygalacturonic acid molecules (Smith *et al.*, 1990b). The structure of pectin in tomatoes is a key determinant of tomato fruit texture and of the rheological characteristics of processed products. PG catalyses the cleavage of pectin chains by hydrolysis of bonds between adjacent galacturonic acid residues. Tomato fruit contains three related isoforms of endopolygalacturonase (PG1, PG2a and PG2b), all products of a single PG gene (Bird *et al.*, 1988). Purified PG isozymes were shown to degrade tomato cell walls *in vitro* and to reproduce cell wall softening changes that occur during natural ripening (Themmen *et al.*, 1982).

The PG gene was isolated and completely sequenced. In addition, the complementary DNA (cDNA) to PG was also cloned and completely sequenced (Grierson *et al.*, 1986). The cDNA clone encoding PG, pTOM6, was identified by comparison of the protein sequence predicted from DNA sequence analysis of this clone and direct sequencing of the purified PG protein (Grierson *et al.*, 1986). This clone is 1636 base pairs long and encodes the pre-PG protein with a molecular weight of 50,051 daltons and is comprised of 457 amino acid residues (Grierson *et al.*, 1986).

The first 731 bp of the cDNA clone pTOM6 were used in the construction of the two plant transformation vectors as indicated in Figure 1 and Appendix 2 (pJR16A: Smith *et al.*, 1988; pJR16S: Smith *et al.*, 1990). Plant transformation vector pJR16A contains the PG cDNA fragment inserted in antisense orientation with respect to the CaMV 35S promoter. Plant transformation vector pJR16S contains the same fragment in sense orientation. Both the antisense and partial sense genes down-regulate the endogenous PG gene in modified plants (Smith *et al.*, 1988; Smith *et al.*, 1990 a,b). The resulting tomatoes have improved processing characteristics (Schuch *et al.*, 1991).

The source of the genetic elements used in the design of the transformation vectors was *Lycopersicon esculentum* Mill variety Ailsa Craig (Darby *et al.*, 1977). This variety was first produced by a cross between tomato cultivars Fillbasket and Sunrise in 1910 (Lisman, 1961). This variety has vigorous indeterminate growth. It is a common gardener variety grown for over 30 years in the United Kingdom.

The *npt*II Selectable Marker Gene

The neomycin phosphotransferase gene (*npt*II) was derived from the bacterial transposon Tn5 isolated from *E. coli* (Berg *et al.*, 1975). This gene encodes the enzyme aminoglycoside (3') phosphotransferase II (APH [3'] II) that has a high specificity for kanamycin and neomycin. It catalyses the ATP dependent phosphorylation of the 3' hydroxyl group of the aminohexose ring. The enzyme consists of 264 amino acids with a molecular mass of 25,000. This gene has been used extensively as a selectable marker in transformation experiments in bacteria, mammalian and plant cells (Bevan *et al.*, 1983; Fraley *et al.*, 1983; Hoekema *et al.*, 1983; Bevan, 1984). The *npt*II gene used in plant transformation vectors pJR16A and pJR16S was fully sequenced (Auserwald *et al.*, 1981).

CaMV 35S Promoter

The Cauliflower Mosaic Virus (CaMV) belongs to a group of small isometric plant viruses with a double stranded DNA genomes called Caulimo viruses. CaMV has a narrow host range restricted to the *Cruciferae*, although experimentally, other species such as carrot or tobacco can be infected. In nature CaMV is transmitted via aphids of the species *Myzus persicae*. This virus is of agronomic importance, as crop losses were attributed to it in several environments. The molecular biology of CaMV was reviewed previously (Gronenborn, 1987).

A complete DNA sequence of CaMV has been determined (Franck *et al.*, 1980). This permitted identification of the functional structure of the virus. The viral genome is transcribed into two polycistronic mRNAs, one of which was termed the 35S RNA. The promoter that is responsible for the transcription of this RNA was termed the 35S promoter. This fragment is used widely by researchers as it provides high levels of expression of a wide variety of plant genes (Odell *et al.*, 1985). A 529 bp fragment of the 35S promoter was used in the construction of the plant transformation vectors pJR16A and pJR16S to drive both the partial PG antisense and sense constructs. (Smith *et al.*, 1988; Smith *et al.*, 1990).

NOS Promoter and Terminator Sequences

The Promoter of the Nopaline Synthase Gene

The promoter from the nopaline synthase gene was incorporated into the plant transformation vectors in order to promote transcription of the neomycin phosphotransferase gene (Bevan, 1984). It is derived from the *Agrobacterium* Ti plasmid pTiT37 (Hernalsteens *et al.*, 1980; Bevan *et al.*, 1983; Bevan, 1984). The DNA sequence was determined and its function as a promoter demonstrated by others (Bevan *et al.*, 1983; Fraley *et al.*, 1983).

The Terminator of the Nopaline Synthase Gene

The terminator sequence from the 3′ end of the nopaline synthase gene was included twice in the plant transformation vectors to ensure that transcription ceases after both the PG effect gene and the *npt*II selectable marker gene. It is derived from the *Agrobacterium* Ti plasmid pTiT37 and its sequence is fully characterized (Hernalsteens *et al.*, 1980; Bevan *et al.*, 1982; Bevan *et al.*, 1983; Bevan, 1984).

Other DNA Sequences

Sequences Derived from Agrobacterium Ti Plasmids

The Right and Left Border Sequences

The border sequences were derived from the Ti plasmid pTiT37 (Zambryski *et al.*, 1980; Hernalsteens *et al.*, 1980). This plasmid was derived from strain T37, a natural nopaline producing isolate. The Ti plasmid of this strain was mapped in detail by others (Zambryski *et al.*, 1980; Depicker *et al.*, 1982).

The function of these border sequences is to delineate the DNA that is transferred from *Agrobacterium* to plant cells. The left border sequence is 25 bp in length, whereas the right border has 26 bp.

Part of the Ornithine Cyclodeaminase Gene

The ornithine cyclodeaminase (OCD) gene is responsible for the catabolism of nopaline. It is a part of the noc region of the Ti plasmid which is localized just outside the right border region of the T-DNA (Sans *et al.*, 1987). The OCD gene is responsible for the conversion of nopaline to L-proline and is the last enzyme of this pathway. The gene was isolated from the nopaline Ti plasmids pTiC58 and the octopine Ti plasmids pTiAch5 and fully characterized by DNA sequence analysis (Sans *et al.*, 1988). A 209 bp internal fragment of the gene is present in the vector.

Part of the Beta-galactosidase Gene from Escherichia coli

During the construction of pBIN19, the starting point for the construction of plant transformation vectors pJR16A and pJR16S, a part of the beta-galactosidase gene was transferred from an intermediate bacteriophage cloning vector M13mp19 (Bevan 1984). This beta-galactosidase gene is derived from the bacterial strain ECO (Beckwith and Singer, 1966) which is a derivative of the wild type *E. coli* K12 strain. The K12 strain is used widely and many derivatives have been generated by the scientific community (Bachmann, 1972). The presence of this fragment permits application of a widely used color selection method. A blue colored compound is produced in the presence of an inducer of the lac operon, such as IPTG (isopropyl-thio-beta-d-galactoside), indicating the presence of active beta-galactosidase in the bacteriophage. When DNA fragments are inserted into this gene, bacterial colonies are colorless due to production of inactive beta-galactosidase protein. The lac operon is responsible for the metabolism of lactose in bacteria. The beta-galactosidase enzyme breaks down lactose into glucose and galactose.

Sequences Derived from the Bacteriophage M13

M13 belongs to a group of non-virulent single stranded DNA filamentous bacteriophages that are able to infect *E. coli*. This bacteriophage has found widespread use as a cloning vector to generate DNA sequence information from cloned DNA fragments. Development of a variety of such cloning vectors was described by others (Messing *et al.*, 1977; Yanisch-Perron *et al.*, 1985). The DNA sequences found in plant transformation vectors pJR16A and pJR16S are derived from two regions of the bacteriophage derivative named M13mp19 (Bevan, 1984).

Part of the Origin of Replication

This DNA sequence does not encode a protein. The function of the origin of replication is the initiation region for the replication of DNA synthesis. A highly specific mechanism involving M13 proteins and host proteins is required for replication of the M13 DNA. The complete sequence of the origin of replication was determined by others (Wezenbeek *et al.*, 1980; Yanisch-Perron *et al.*, 1985).

Part of Gene III

The Gene III is a component of the viral coat. The fragment of Gene III that was incorporated into the plant transformation vectors represents 455 bp of an internal fragment of the Gene III. The complete sequence of Gene III was determined by others (Wezenbeek *et al.*, 1980; Yanisch-Perron *et al.*, 1985).

Other Sources of DNA

Other small DNA sequences were included in the construction of the plant transformation vectors to act as linker fragments between above listed genetic elements. These DNA elements were added during the construction of the vectors as synthetic pieces of DNA.

The sequences represented in Appendices 1 and 2 were determined experimentally. The sequences of *Agrobacterium* that confer characteristics of hormone independent growth were eliminated from the vector. The remaining sequences do not confer weediness characteristics to the genetically modified plants. NPTII protein is the only protein that is expressed.

References

Auserwald, E.A., Ludwig, G., and Schaller, H. (1981) Structural Analysis of Tn5. CSHS QB 45, 107-113

Bachmann, B.J. (1972) Pedigrees of some mutant strains of *Escherichia coli* K-12. Bacteriol. Rev. 36, 525-557

Beckwith, J.R., Signer, E.R. (1966) Transposition of the Lac Region of *Escherichia coli*. J. Mol. Biol. 19, 254-265

Berg, D.E., Davies, J., Allet, B., and Rochaux, J.D. (1975) Transposition of R factor genes to bacteriophage. Proc. Nat. Acad. Sci. 72, 3628-3632

Bevan, M., Barnes, W.M., and Chilton, M.D. (1982) Structure and transcription of the nopaline synthase gene region of T-DNA. Nucl. Acids Res. 11, 369-385

Bevan, M.W., Flavell, R.B., and Chilton, M.D. (1983) A chimaeric antibiotic resistance gene as a selectable marker for plant cell transformation. Nature, 304, 184-186

Bevan, M. (1984) *Agrobacterium* vectors for plant transformation. Nucl. Acids Res. 12, 8711-8721

Bird, C.R., Smith, C.J.S., Ray, J.A., Moureau, P., Bevan, M.W., Bird, A.S., Hughes, S., Morris, P.C., Grierson, D., and Schuch, W. (1988) The tomato polygalacturonase gene and ripening specific expression in transgenic plants. Plant Mol. Biol. 11, 651-662

Darby, L.A., Richie, D.B., and Taylor, I.D. (1977) Isogenic lines of the tomato Ailsa Craig. The Glasshouse Crops Research Institute Annual Report 168-184

Depicker, A., Stachel, S., Dhaese, P., Zambryski, P., and Goodman, H.M. (1982) Nopaline synthase: transcript mapping and DNA sequence. J. Mol. Appl. Genetics 1, 561-573

Fraley, R.T., Rogers, S.G., Horsch, R.B., Sanders, P.R., Flick, J.S., Adams, S.P., Bittner, M.L., Brand, L.A., Fink, C.L., Fry, J.S., Galluppi, G.R., Goldberg, S.B., Hoffmann, N.L., and Woo, S.C. (1983) Expression of bacterial genes in plant cells. Proc. Natl. Acad. Sci. 80, 4803-4807

Franck, A., Guilley, H., Jonard, G., Richards, K., and Hirth, L. (1980) Nucleotide sequence of Cauliflower Mosaic Virus DNA. Cell 21, 285-294

Grierson, D., Tucker, G.A., Keen, G., Ray, J., Bird, C.R., and Schuch, W. (1986) Sequencing and Identification of a cDNA clone for tomato polygalacturonase. Nucleic Acids Research 14, 8595-8605

Gronenborn, B. (1987) The molecular biology of Cauliflower Mosaic Virus and its application as plant gene vector. In: Plant DNA Infectious Agents (eds., Hohn, T.H. and Schell, J.) Springer Verlag, 1-29

Hernalsteens, J.P., van Vliet, F., de Breuceleer, M., Depicker, A., Engler, G., Lemmers, M., Holsters, M., van Montagu, M., and Schell, J. (1980) The *Agrobacterium tumefaciens* Ti plasmid as a host vector system for introducing foreign DNA in plant cells. Nature 207, 654-665

Hobson, G.E. (1965) The firmness of tomato fruit in relation to polygalacturonase activity. J. Hort. Sci. 40, 66-72

Hoekema, A., Hirsch, P.R., Hooykaas, P.J.J., and Schilperoort, R.A. (1983) A binary plant vector strategy based in separation of *vir*- and T-region of the *Agrobacterium tumefaciens* Ti-plasmid. Nature 303, 179-182

Lisman, T.A. (1961) The romance of Ailsa Craig. The Grower 56, 582-583

Messing, J., Gronenborn, B., Muller-Hill, B., and Hofschneider, P.H. (1977) Filamentous coliphage M13 as a cloning vehicle: Insertion of a *Hind*II fragment of the lac regularity region in M13 replicative form *in vitro*. Proc. Natl. acad. Sci. 74, 3642-3646

Odell, J.T. (1985) Identification of DNA sequences required for activity of the Cauliflower Mosaic Virus 35S promoter. Nature 313, 810-812

Sans, N., Schroeder, G., and Schroeder, J. (1987) The Noc region of Ti plasmid C58 codes for arginase and ornithine cyclodeaminase. Eur. J. Biochem. 167, 81-87

Sans, N., Schindler, U., and Schroeder, J. (1988) Ornithine cyclodeaminase from Ti plasmid C58: DNA sequence, enzyme properties and regulation of activity by arginine. Eur. J. Biochem. 173, 123-130

Schuch, W., Kanczler, J., Robertson, D., Hobson, G., Tucker, G., Frierson, D., Bright, S., and Bird, C. (1991) Fruit quality characteristics of transgenic tomato fruit with altered polygalacturonase activity. HorScience 25, 1517-1520

Smith, C.J.S., Watson, C.F., Ray, J., Bird, C.R., Morris, P.C., Schuch, W., and Frierson, D (1988) Antisense RNA inhibition of polygalacturonase gene expression in transgenic tomatoes. Nature 334, 724-726

Smith, C.J.S., Watson, C.F., Bird, C.R., Schuch, W., and Frierson, D. (1990a) Expression of a truncated tomato polygalacturonase gene inhibits expression of the endogenous gene in transgenic plants. Mol. Gen. Genet. 224, 447-481

Smith, C.J.S., Watson, C.F., Morris, P.C., Bird, C.R., Seymour, G.B., Grey, J.E., Arnold, C., Tucker, G.A., Schuch, W., Harding, S., and Frierson, D. (1990b) Inheritance and effect on ripening of antisense polygalacturonase genes in transgenic tomatoes. Plant Mol. Biol. 14, 369-379

Themmen, A.P.N., Tucker, G.A., Grierson, D. (1982) Degradation of isolated tomato cells by purified polygalacturonase *in vitro*. Plant Physiology 69, 122-124

Tucker, G.A., and Grierson, D. (1982) Synthesis of polygalacturonase during tomato fruit ripening. Planta 155, 64-67

van Wezenbeek, P.M.G., Hulsebos, T.J.M., and Schoenmakers, J.G.G. (1980) Nucleotide sequence of the filamentous bacteriophage M13 DNA genome: comparison with phage fd. Gene 11, 129-148

Yanisch-Perron, C., Vieira, J., and Messing, J. (1985) Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene 33, 103-119

Zambryski, P., Holseters, M., Kruger, K., Depicker, A., Schell, J., van Montagu, M., and Goodman, H.M. (1980) Tumor DNA structure in plant cells transformed by *A. tumefaciens*. Science 209, 1385-1391

Genetic Analysis, Genetic Stability and Agronomic Performance

History of the Genetically Modified Inbred Lines B, Da and F

Generation of Primary Lines

Inbred lines B, Da and F were generated using *Agrobacterium*-mediated transformation of the Petoseed proprietary commercial inbred T7 using the plant transformation vectors pJR16A (PG-antisense; line B) and pJR16S (PG-sense; lines Da and F). These vectors contain the *npt*II gene for selection of transformed cells, and the PG effect genes as described in the **Donor Genes to be Assessed for Non-Regulated Status** section. The primary transformed lines (T_0 lines) were regenerated from tissue culture, then grown in the glasshouse and analyzed for PG enzyme levels. Lines that contained reduced levels of the PG enzyme were selected and characterized for the number of insertions of the effect gene cassette. Only lines with one insertion were advanced. A further selection was made during field trials conducted in 1991 and 1992 for lines that were agronomically identical to the parental line T7. The lines 66-51/08 (B), 87-22A/08 (Da) and 88-37/13 (F) were selected using this protocol.

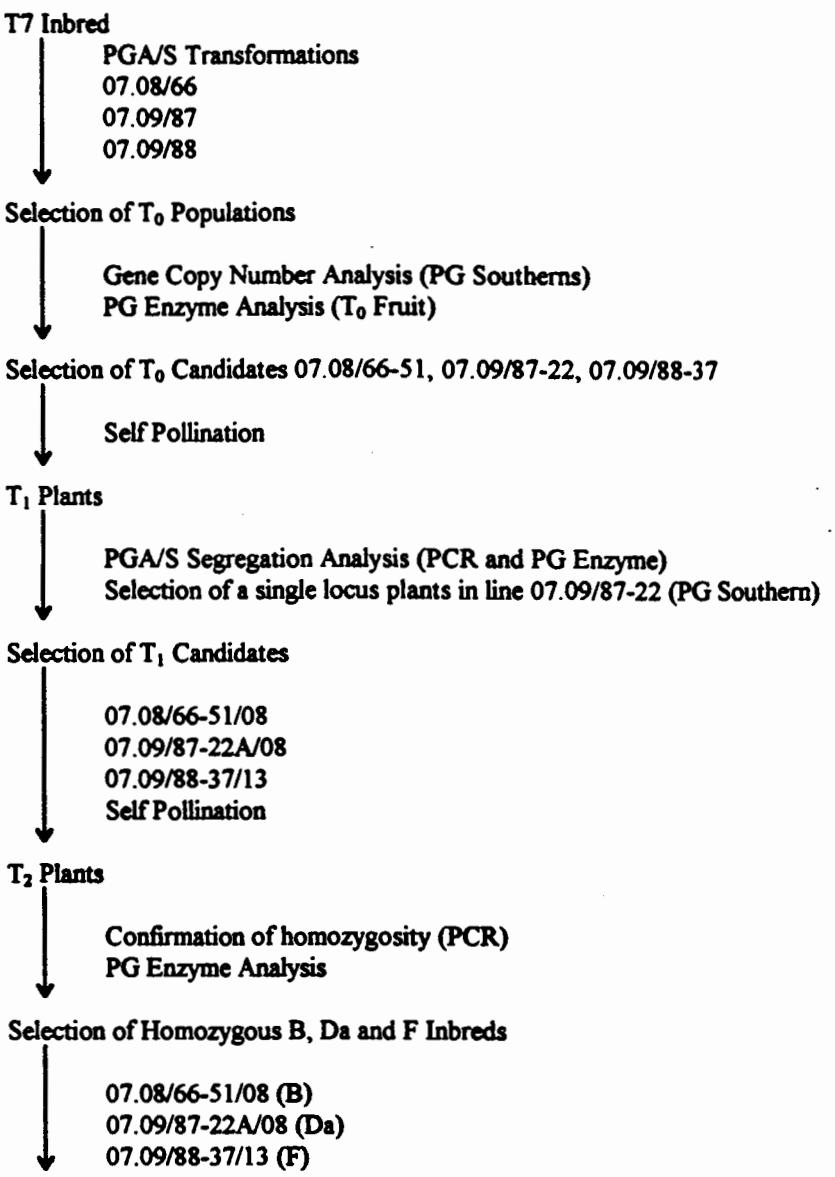
Molecular and Biochemical Analysis of Lines B, Da and F

Southern hybridizations of DNAs isolated from the primary transformants indicated single gene insertion sites in lines 66-51 and 88-37. Lines B and F were identified after selfing these primary transformants, selection of homozygous progeny lines, and selection of the best candidate out of the pool of homozygous lines. This identified plant 66-51/08 as line B and plant 88-37/13 as line F.

Southern hybridization of DNA isolated from line 87-22 identified two inserts at separate sites. These two inserts (designated as A and B) segregated after selfing of the T_0 plant. A single locus "A" was identified in T_1 plant 87-22/08. This plant was self pollinated and designated as a line Da. The letter A was added to this line experimental number (87-22A/08) and a subscript "a" was added to the inbred designation D, making it Da to indicate the single insertion at locus A. A scheme for the production and characterization of inbreds B, Da and F is shown in Figure 2. The gene copy number analysis for all three lines is presented in Appendix 6.

A protocol was developed for PCR analysis of the B, Da and F lines that allows identification of the PG antisense and PG sense plants. This protocol can be used to: 1) differentiate between PG antisense and PG sense plants; 2) differentiate genetically modified plants from control plants. This protocol is shown in Appendix 7.

Figure 2. Origin of Inbred Lines B, Da and F



Homozygous lines were identified as lines in which there was no segregation of the effect gene using PCR analysis. A further selection criterion was a consistently low level of PG enzyme activity. The details of the molecular and biochemical characterization of lines B, Da and F are presented in Table 5. The details of the PG activity in lines B, Da and F are shown in Appendix 8.

Table 5. Molecular and Biochemical Characterization of Lines B, Da and F. Determination of PG Enzyme Levels and Segregation Ratios

Inbred	T_0 (PG%)[1]	T_1 (PG%)[1]	T_2 (PCR + : PCR -)[2]	T_2 (PG%)[1]
T7 Control	100	--	--	--
B	3.4	2.6	24:0	1.4 ± 0.2
Da	3.2	0	24:0	0.7 ± 0.2
F	0.2	2.7	30:0	3.8 ± 0.4

1 PG activity expressed as percent of PG expression of non-transgenic control line. Individual plant analysis was performed on six pericarp discs (cut out using a No. 5 corkborer) collected from two red ripe fruits (three discs per fruit). T_0 and T_1 columns show PG expression in individual plants. Data in T_2 column shows an average of 17-20 plants ± standard error.

2 Segregation analysis was performed on DNA extracted from tomato leaves using a PCR amplification method.

Seeds of the homozygous inbred lines were transferred to the Petoseed production facility in Chile (line B in 1991, lines Da and F in 1992) where they were increased. At the same time seeds of genetically modified commercial hybrids Nema 1200, Nema 1400, Nema 1401 and H282 were produced. These genetically modified hybrids were produced using each of the modified inbreds, B, Da and F, as a hybrid parent. In this fashion genetically modified Nema 1200B, Nema 1200Da, Nema 1200F, Nema 1400B, Nema 1400Da, etc., were produced. The production of different genetically modified hybrids was possible because the inbred line T7 is used in production of these commercial hybrids as one of the hybrid parents. Thus, the genetically modified versions of inbred T7 could be tested as hybrid parents in a series of well known and extensively characterized commercial hybrids.

Analysis of Hybrids Derived from Genetically Modified Inbred Lines

The genetically modified hybrids were tested in a series of field trials in 1992 and 1993 in California, Chile, Portugal and Australia. The California trials were conducted in the major growing areas of processing tomatoes representing distinct climatic regions (Southern, Central and Northern California). The objectives of these trials were to evaluate the agronomic and processing characteristics of these hybrids. During trials conducted by Petoseed extensive field observations were carried out by experienced Petoseed breeders, agronomists and scientists. Trials conducted by Hunt Wesson were evaluated by Hunt Wesson staff and various commercial growers.

In addition to evaluation of the performance of these hybrids from the agronomic and processing point of view, extensive PG enzyme and PCR analyses were carried out. These analyses showed that PG enzyme levels were consistently low both in the inbreds and in the different hybrids, confirming the effectiveness and stability of the PG effect gene. These trials also demonstrated superior processing characteristics of the genetically modified versions of commercial hybrids. Detailed observations from these trials are presented in later sections of this document.

DNA Analysis of Genetically Modified Inbred Lines and Selected Hybrids

Genomic DNA was isolated from leaf tissue of the B, Da and F inbred lines and derived from the hybrids. PCR analysis was carried out to establish DNA sequences of the plant transformation vectors pJR16A (line B) and pJR16S (Da and F) that were present in the modified lines. PCR primers were designed in such a way that DNA sequences located between the borders could be visualized. The results are summarized in Table 6. Technical details of this analysis are shown in Appendix 9.

Table 6. PCR Analysis of Inbred Lines and Selected Hybrids Derived from Lines B, Da & F

Primer Pair	Inbreds			Hybrid Nema 1401			Hybrid H282		
	B	Da	F	B	Da	F	B	Da	F
Ori-2 + 35SC	+	+	+	+	+	+	+	+	+
35S + NOS	+	+	+	+	+	+	+	+	+
NOS-A + GIII-AR	+	+	+	+	+	+	+	+	+
GIII-A + OCD-AR	+	+	+	+	+	+	+	+	+
CD + *npt*II-RC	+	+	+	+	+	+	+	+	+
*npt*II + NOS-PR	+	+	+	+	+	+	+	+	+
*npt*II + RB	+	+	-	+	+	-	+	+	-

Key: + PCR fragment generated using primer pair
- No PCR fragment generated using primer pair

The following can be concluded from this analysis:

A complete insert is present in the inbred line B. No alteration in the structure of the DNA occurred during breeding and hybrid seed production.

A complete insert is present in the inbred line Da. No alteration in the structure of the DNA occurred during breeding and hybrid seed production..

A small deletion occurred in the right border sequence of inbred F. The precise location of the deletion was not determined. It is likely that the deletion is between the NOS promoter and the right border region of the insert because the *npt*II + NOS-PR primers indicate presence of a complete *npt*II selectable marker gene, and both the inbred F and derived from it hybrids have detectable levels of the NPTII protein (see Table 12 in the **Environmental Consequences of the Introduction of Genetically Modified Hybrids** section).

Agronomic Performance of the Modified Hybrids

Summary of Field Trials

Field trials that were carried out with genetically modified inbreds and derived from them hybrids are listed in Table 7.

Table 7. Summary of Field Trials

Trial Site	Year	U.S.D.A. Permit No.	Lines
Felda, Florida	1992[1]	91-205-02	B
Colina, Chile	1992[1]	91-351-01M*	B
Woodland, California	1992[1]	92-049-03	B
Colina, Chile	1993[1]	92-281-02M*	B, Da, F
Woodland, California	1993[1]	92-352-01	B, Da, F
Yolo County, California	1993[1,2]	92-352-01	B, Da, F
Fresno County, California	1993[1,2]	92-352-01	B, Da, F
Kern County, California	1993[1,2]	92-352-01	B, Da, F
Imperial County, California	1993[1,2]	92-352-01	B, Da, F
Woodland, California	1994[1]	92-352-01	B, Da, F
Fresno County, California	1994[2]	94-047-07N	B, Da, F
Merced County. California	1994[2]	94-083-06N	B, Da, F

1 Applied for by Petoseed 2 Applied for by Hunt Wesson * Courtesy/transportation permit only

Field Observations

Agronomic characteristics of the modified inbreds and hybrids were closely monitored during the field trials by experienced Petoseed breeders, Hunt Wesson staff, and commercial growers. Plants were observed for growth characteristics, disease resistance, flowering characteristics, and yield. Additionally, the fruit was evaluated for shape, firmness and processing characteristics. In some cases a modified Plant Variety Protection (PVP) form was used to obtain very detailed comparisons between genetically modified and control hybrids. The use of the PVP form was necessarily limited due to high labor required for such careful evaluations. An example of the PVP form with detailed evaluations performed on genetically modified and control hybrids Nema 1200 and Nema 1401 is given in Appendix 10.

The trials were conducted under standard growing conditions, using standard agronomic practices. The modified inbreds and hybrids did not show any alterations as compared to controls in any of these trials. Plant type, size, leaf cover, concentration of fruit set and overall fruit yield were judged to be identical to unmodified control plants.

The reduction of the PG enzyme levels in the genetically modified plants did not introduce any unexpected characteristics. Increased viscosity was the only difference between the genetically modified and control plants. The field observations are summarized in Tables 8 and 9.

Table 8. Field Observations from Tomato Breeders (Petoseed)

Observed Characteristics	Difference to Unmodified Control[1]
Seed Growth	None
Seedling Morphology	None
Plant Growth	None
Plant Morphology	None
Leaf Morphology	None
Inflorescence Morphology	None
Flower Morphology	None
Time to Flowering	None
Time to Fruit Set	None
Fruit Shape	None
Fruit Set	None
Fruit Quality	Yes[2]
Disease Susceptibility:	
Fusarium Wilt, Race 1	None
Fusarium Wilt, Race 2	None
Alternaria Stem Canker	None
Verticillium Wilt, Race 1	None
Pest Susceptibility	None
Seed Production	None
Volunteers	None
Yield	None

1 Data was gathered during the 1993 field trial held at Woodland, CA, using a PVP form. Observations were made on eight randomly selected plants in each control and genetically modified Nema 1200 and Nema 1401, as often as needed to determine the characteristic (a detailed summary of the observations and PVP forms are included in Appendix 10).

2 Viscosity only; other parameters not altered.

Table 9. Field Observations from Tomato Processor/Grower (Hunt Wesson)

Observed Characteristics	Difference to Unmodified Control
Vine Habit	None
Fruit Shape	None
Firmness	None
Peeling Potential	None
Maturity	None
Estimated Field Yield	None
Overall Field Ranking	None
Product Viscosity	Yes[1]
Product Sensory Properties	None
Product Nutritional Value	None
Product Glycoalkaloid Content	None
Grower Acceptance	None
Volunteer Plant (weed) Potential	None

1 Viscosity only; other parameters not altered.

The overall phenotypic assessment indicates that modified hybrids are not distinguishable from the unmodified, control hybrids. It was therefore concluded that the inbreds used for the hybrid production were not modified in any way other than that intended by the genetic modification.

The final field trial reports from trials carried out in 1992 and 1993 are shown in Appendix 11. Field observation reports from outside visitors are shown in Appendix 12.

Nutritional Analysis of Hybrids

The Role of Tomatoes in the U.S. Diet

Tomatoes are an important component of the American diet (see **The Tomato Family** section). Even though tomato has only intermediate levels of vitamins, minerals or other dietary components, it ranks number one as a contributor of vitamins and minerals due to its large consumption by the U.S. population. In particular, tomatoes provide significant amounts of both vitamin A and C to the American diet (Stevens, 1986).

Vitamin Content of Genetically Modified Hybrids

Modified tomato hybrids, grown in various locations in California, were used for the production of hot break tomato paste and pulp in the Hunt Wesson Process Engineering Laboratory at Fullerton, California. Tomato paste and pulp were produced using methods established for comparison of pre-commercial varieties and the levels of vitamins A and C were determined. This analysis showed that the levels of vitamins A and C found in the genetically modified varieties were not significantly different from those found in unmodified varieties in both paste and pulp (Table 10). Data are adjusted for moisture content, pulp to 7% dry matter and paste to 24% dry matter. Amounts are per 100 grams.

Table 10. Vitamin Content of Genetically Modified and Control Tomato Products

Product	Vitamin A	S.E.[1]	Vitamin C	S.E.[1]
Modified Paste[2]	428 I.U.	90.0	7.2 mg	4.71
Traditional Paste[4]	570 I.U.	91.7	12.5 mg	5.28
Modified Pulp[3]	262 I.U.	38.3	14.5 mg	1.67
Traditional Pulp[4]	281 I.U.	55.8	14.6 mg	0.51

1 S.E. (standard errors) are based on total variance among all varieties

2 Date represents an average of the following modified hybrids:
Nema 1200B n=1 Nema 1401B n=1
Nema 1400B n=1 H282B n =1

3 Date represent an average of the following modified hybrids:

Nema 1200B n=3	Nema 1200Da n=1	Nema 1200F n=1
Nema 1400B n=2	Nema 1400Da n=2	Nema 1400F n=1
Nema 1401B n=4	Nema 1401Da n=2	Nema 1401F n=2
H282B n=3	H282Da n=1	H282F n=1

4 Traditional Paste and Pulp = Non-transgenic paste and pulp

Compositional Analysis of Genetically Modified Processed Tomato Products

To determine the impact of genetic modification on other nutritional components of processed tomato products nutritional analysis of tomato paste and pulp was conducted. Samples were prepared as for determination of vitamins A and C (see the section above). All analyses were done according to methods established by the Association of Official Analytical Chemists as appropriate for each component of the respective tomato product. These analyses showed no alteration in any of the parameters measured when compared to the unmodified samples. Differences between means were not statistically significant at the 0.05 level by independent group test (Table 11). We therefore conclude that the genetic modification did not affect any of these characteristics. The list of Analytical Test Methods is shown in Appendix 13.

Table 11. Nutritional Analysis of Genetically Modified Tomato Products

	Tomato Type			
	Paste[1]		Pulp[1]	
	Modified[2]	Traditional	Modified[3]	Traditional
Calories	73.3	71.9	22.8	22.6
Fat gm	0.62	0.72	0.38	0.35
Na mg	59.3	40.6	20.1	25.3
Carbohydrate gm	15.8	12.1	4.35	4.39
Fructose gm	6.66	7.07	2.04	1.91
Glucose gm	7.35	6.62	1.77	1.54
Dietary Fiber gm	4.77	5.09	1.22	1.25
Protein gm	5.07	3.63	1.51	1.50
Ca mg	43.7	41.7	11.8	11.0
Fe mg	9.12	13.0	0.46	0.39

1 Amounts are per 100 mg

2 Date represents an average of the following modified hybrids:
Nema 1200B n=1 Nema 1401B n=1
Nema 1400B n=1 H282B n =1

3 Date represent an average of the following modified hybrids:

Nema 1200B n=3	Nema 1200Da n=1	Nema 1200F n=1
Nema 1400B n=2	Nema 1400Da n=2	Nema 1400F n=1
Nema 1401B n=4	Nema 1401Da n=2	Nema 1401F n=2
H282B n=3	H282Da n=1	H282F n=1

References

Stevens, MA. (1986) Inheritance of Tomato Fruit Quality Components, in Plant Breeding Reviews (ed., J. Janick), Vol. 4, AVI Publishing, 273-311

Environmental Consequences of the Introduction of Genetically Modified Hybrids

Expression of the *npt*II Gene

Fruit samples were collected from selected inbreds and hybrids grown in a field trial at Woodland, California, and levels of the NPTII protein were determined using an NPTII enzyme linked immunoabsorbant assay (ELISA). Levels of the NPTII protein varied among fruit samples of different lines, with line F showing the highest and line Da the lowest levels. The data from the NPTII ELISA is shown in Table 12.

Table 12. Levels of NPTII Protein in the Fruit of Genetically Modified Inbreds and Hybrids

Modified Inbreds & Hybrids[1]	Ng of NPTII Protein per mg Total Protein[2]
Inbred Da	0.7 ± 0.0
Hybrid Da	Not tested
Inbred F	9.7 ± 0.7
Nema 1200 F	2.6 ± 0.9
H282 F	3.5 ± 0.7

1 The NPTII proteins was quantified using an NPTII ELISA Kit (5 Prime → 3 Prime Co., Inc., Boulder, Colorado)

2 Each data point represents an average of 20 plants (one fruit per plant) ± standard error. The sample size was determined using a formula $n = 4\sigma^2/L2$ (Snedecor, G.W. & Cochran, W.G., "Statistical Methods", The Iowa State University Press, 1978) with the upper limit of error L = 100 picograms, at 95% probability.

The reported levels of NPTII protein are within the limits determined by U.S.D.A. and F.D.A. to be safe in genetically modified food. We also determined that upon processing the levels reported here are further reduced by more than 95%.

Expression of the PG Gene

Fruit samples were collected from selected inbreds and hybrids grown in Woodland, California, and analyzed for PG levels. The levels of the PG enzyme (expressed as percent of PG expression of glasshouse grown non-transgenic control T7 line) are shown in Table 13.

Table 13. PG Enzyme Levels in Field Grown Hybrids B, Da and F

	Tested Hybrids[1]			
Parent Line	Nema 1200	Nema 1400	Nema 1401	H282
T7 Control	62.2 ± 13.6	82.5 ± 31.0	46.1 ± 6.2	61.0 ± 8.8
B	3.5 ± 0.3	5.6 ± 4.5	1.3 ± 0.6	0.9 ± 0.4
Da	1.6 ± 1.0	2.6 ± 1.6	2.9 ± 1.7	1.9 ± 1.2
F	3.9 ± 1.4	2.6 ± 0.9	3.4 ± 0.5	3.8 ± 0.8

1 Each data point represents an average of four bulked samples ± standard error. Each bulked sample consisted of 15 paricarp discs collected from five plants (one fruit per plant, three discs per fruit). The discs were cut out using a No. 5 corkborer.

Disease and Pest Characteristics

Disease and pest incidence were monitored throughout the trials by experienced Petoseed breeders and field personnel as well as commercial growers. Their observations are summarized in Table 14.

Table 14. Comparison of Disease Resistance in Genetically Modified and Control Hybrids

Disease[1]	Difference
Fusarium Race 1	None
Fusarium Wilt, Race 2	None
Alternaria Stem Canker	None
Verticillium Wilt, Race 1	None

1 Disease ratings were performed by experienced tomato breeders on field-grown plants under natural disease pressure.

Agronomic Characteristics

Overall yield was determined using observational methods that are standard in the tomato industry. Yield was assessed to be comparable to unmodified lines. Previously described observations (Tables 8, 9 and 14) further support the conclusion that the modified lines are comparable to control lines in all monitored characteristics.

Processing Characteristics

Fruit from all trials run in California was evaluated for processing characteristics. This involved processing of tomato fruit samples on a laboratory scale, as well as on a pilot plant scale. In all instances, improved processing of the modified hybrids was observed in comparison to the unmodified hybrid samples. An increase in product yield ranging from 15% to 38% was demonstrated.

Weediness Potential

The following consensus of the traits common to many weeds was developed by Baker (1974): 1) good germination requirement in many environments; 2) discontinuous germination and great longevity of seed; 3) rapid growth through vegetative phase to flowering; 4) continuous seed production for as long as growing conditions permit; 5) self compatibility but not completely autogamous and apomictic; 6) when cross-pollinated, pollinated by unspecialized visitors, or wind pollinated; 7) high seed output in favorable environment and some seed production in a wide range of environments; 8) adaptation for short and long distance dispersal; 9) if perennial, vegetative production or regeneration from fragments, brittleness (not easily removed from the ground); 10) ability to compete interspecifically by special means (rosette formation and presence of allelochemicals). Not all weeds have all of these characteristics.

Tomatoes represent an annual crop that is highly domesticated and well characterized. Tomatoes possess only a few of the characteristics of plants that are successful as weeds. They are not persistent in the environment without human intervention. The unmodified inbred line T7 is not considered a weed; the introduction of the PG effect genes leading to a reduction of PG activity during fruit ripening does not affect "weediness" characteristics. In fact, many commercial fresh market tomato lines have reduced PG levels due to the introduction of the *rin* gene, this does not increase the weediness of these varieties. The reduction in PG activity in these fresh market lines is of similar magnitude as described here for the modified T7 varieties.

The introduction and expression of the selectable marker gene also does not affect the weediness characteristics. There were no increases in the number of produced seed, the rate of plant growth, fruit development, and disease susceptibility (see Final Reports 1992 and 1993, submitted to U.S.D.A., Appendix 11). The results of the agronomic and environmental testing described here provide no evidence that the genetic modification resulted in increased weediness of the genetically modified inbred lines. The vector constructs were analyzed in detail for the expression of novel polypeptides (see Appendices 3 and 4) to evaluate the possibility of expression of any new characteristics that could contribute to the weediness of the genetically modified lines, or impact on the food safety. This analysis showed that it is very unlikely that any novel polypeptides are expressed in these plants. The observations summarized in Tables 8, 9 and 14 clearly demonstrate no change in any of the phenotypic determinants of the modified lines.

The occurrence of volunteers in fields containing genetically modified plants was monitored as a part of standard practice of conventional breeding. These observations demonstrated that the rate of volunteers was not different from fields with unmodified plants (see field sheets/reports for comments in Appendices 11 and 12).

Low PG Tomato Products and Human/Animal Exposure

Tomato pomace remaining after processing is commonly fed to cattle. The pomace left after processing of modified lines was found to be nutritionally equivalent and unaltered when compared with traditional tomatoes. Please see the accompanying F.D.A. Advisory document for further details. The conclusion of the F.D.A. Advisory document was **no concern** as to safety for food or feed use.

Potential for Outcrossing

Outcrossing with Domesticated Tomato Varieties

The outcrossing potential of tomatoes has been reviewed in the **Tomato Family** section. The evidence provided here does not support the argument that the genetic modification altered the outcrossing potential of modified inbreds. Flower morphology, the major contributor to outcrossing, was not altered. Therefore, it can be concluded that the genetic modification does not effect the outcrossing characteristics.

The potential for tomatoes to become weeds was reviewed previously (Redenbaugh, 1992). There is considerable historical evidence that in conditions of intensive agricultural usage the rate of outcrossing between tomato varieties does not lead to weediness.

Tomatoes in which PG enzyme activity was reduced using an antisense constructs was previously reviewed by the U.S.D.A. (Redenbaugh *et al.*, 1992; U.S.D.A., A.P.H.I.S. 1992). On July 14, 1992, A.P.H.I.S. received a petition from Calgene requesting that the low PG tomatoes be removed from regulatory oversight. This petition was granted on October 19, 1992.

Outcrossing with Wild Species

Outcrossing with wild species was reviewed in the **Tomato Family** section. As described above the genetic modification of inbred T7 does not alter the behavior of this line.

Outcrossing with Other Related Species

Outcrossing with other related species was reviewed in the **Tomato Family** section. As described above the genetic modification does not alter the properties of the inbred line T7, which does not show outcrossing to related species.

A number of weed species was identified by the U.S.D.A. to occur in, or in the vicinity of, commercially grown tomato fields. *Solanum nigrum* is the only major weed pest related to tomato. Other members of the Nightshade family which are weeds in tomato fields are: *S. sarrachoides, Physalis heterophylla, P. lanceifolia, P. xocarpa, P. acutifolia, Nicotiana bigelovii, Datura stramonium, D. meteloides,* and *D. ferox.* Other weedy *Solanaceae* are: *Hyoscyamus niger, Lycium ferocissimum, P. virginiana* variety *sonorae, P. viscosa, S. cardiophyllum, S. carolinense, S. lanceolatum, S. marginatum* and *S. torvum.* Tomato (*L. esculentum*) is sexually incompatible with all these weedy relatives.

References

Barker, H.G. (1974) The Evolution of Weeds. Annual Review of Ecology and Systematics 5, 1-24

Redenbaugh, K., Hiatt, W., Martineau, B., Kramer, M., Sheehy, R., Sanders, R., Houck, C., Emlay, D. (1992) Safety Assessment of Genetically Engineered Fruits and Vegetables: A Case Study of the FLAVR SAVR™ Tomatoes. CRC Press, Boca Raton, 267

Snedecor, G.W. and Cochran, W.G. (1978) Statistical Methods, 6th Edition, Iowa State University Press

U.S.D.A., A.P.H.I.S. (1992) Environmental Assessment and Finding of No Significant Impact on Tomato Containing an Antisense Polygalacturonase Gene. Permit Number 91-268-01

Statement of Grounds Unfavorable

We have included all information and summaries available for the assessment of the parent lines B, Da and F. We have no knowledge of any reason why this petition should not be approved.

DNA Sequence of pJR16A (Antisense orientation)

(only sequences between the border regions are shown)

```
                10         20         30         40         50         60
                |          |          |          |          |          |
   1 TGGCAGGATA TATTGTGGTG TAAACAAATT GACGCTTAGA CAACTTAATA ACACATTGCG
     |Left border
  61 GACGTTTTTA ATGTACTGGG GTGGTTTTTC TTTTCACCAG TGAGACGGGC AACAGCTGAT
                                     |Ori from M13mp19      >
 121 TGCCCTTCAC CGCCTGGCCC TGAGAGAGTT GCAGCAAGCG GTCCACGCTG GTTTGCCCCA
 181 GCAGGCGAAA ATCCTGTTTG ATGGTGGTTC CGAAATCGGC AAAATCCCTT ATAAATCAAA
 241 AGAATAGCCC GAGATAGGGT TGAGTGTTGT TCCAGTTTGG AACAAGAGTC CACTATTAAA
 301 GAACGTGGAC TCCAACGTCA AAGGGCGAAA AACCGTCTAT CAGGGCGATG GCCCACTACG
 361 TGAACCATCA CCCAATCAA  GTTTTTTGGG GTCGAGGTGC CGTAAAGCAC TAAATCGGAA
 421 CCTAAAGGGA GCCCCCGATT TAGAGCTTGA CGGGGAAAGC CGGCGAACGT GGCGAGAAAG
 481 GAAGGGAAGA AAGCGAAAGG AGCGGGCGCC ATTCAGCGTG CGCAACTGTT GGGAAGGGCG
       < Ori from M13mp19    | Lac gene from E. coli    >
 541 ATCGGTGCGG GCCTCTTCGC TATTAGCCAG CTGGCGAAAG GGGGATGTGC TGCAAGGCGA
 601 TTAAGTTGGG TAACGCCAGG GTTTTCCCAG TCACGACGTT GTAAAACGAC GGCCAGTGAA
                                                          |ECO
 661 TTCCCATGGA GTCAAAGATT CAAATAGAGG ACCTAACAGA ACTCGCCGTA AAGACTGGCG
     RI  | CAMV 35s PROMOTER   >
 721 AACAGTTCAT ACAGACTCTC TTACGACTCA ATGACAAGAA GAAAATCTTC GTCAACATGG
 781 TGGAGCACGA CACGCTTGTC TACTCCAAAA ATATCAAAGA TACAGTCTCA GAAGACCAAA
 841 GGGCATTGA  GACTTTTCAA CAAAGGGTAA TATCCGGAAA CCTCCTCGGA TTCCATTGCC
 901 CAGCTATCTG TCACTTTATT GTGAAGATAG TGGAAAAGGA AGGTGGCTCC TACAAATGCC
 961 ATCATTGCGA TAAAGGAAAG GCCATCGTTG AAGATGCCTC TGCCGACAGT GGTCCCAAAG
1021 ATGGACCCCC ACCCACGAGG AGCATCGTGG AAAAAGAAGA CGTTCCAACC ACGTCTTCAA
1081 AGCAAGTGGA TTGATGTGAT ATCTCCACTG ACGTAAGGGA TGACGCACAA TCCCACTATC
1141 CTTCGCAAGA CCCTTCCTCT ATATAAGGAA GTTCATTTCA TTTGGAGAGG ACAGGTACCC
                                     > CaMV 35S  |
1201 ACTCAAATTT GATATGAATT TGTTGTGCAT TTTTACTCTT TAGATTATTC ACTTTCAAAT
     | PG antisense   >
1261 TTTTGCAATT CCAGAAGGTT AAGGCCGTTG GTGCATCCCT GCATGGCAGT GATTTATTTA
1321 TTTTGCAAGA ACTTGGCCAC CATACTTGTC CATTGCCATT GATAGTTCCT CCTCCTCCAA
1381 CAACTAAATT TTGAACACTA TCAAAAGCAA TCCAAAGCCT TCTATCTTTG TAGTCTGAAA
1441 TTTTACTAGA TGCTTCTAAG GATCCAAAAA TCTTTACTGA AATAGAAGAT CTGCATGGAC
1501 CTGAAAAGGT GATTTGCTTG AGAAGATAAT TCTTGTTTTT AGGAACCACA AATTGAACAG
1561 GTGTTCTAGA TGAACATGCT TCATTCCATG CTTGCTCAAA TGCAATATTA TCATATGTTT
1621 TTCCATCACC CTTAGCTCCA AAGCTAAGTA CATTAATCAC TTTAATCCCA TTTTTATCAA
1681 CCTTGTCAAT ATTATTGTTG CTTTCAATAT TTTTGCTCAA ATAAGAAAGA TAAGCTTGAA
1741 AATCATGAGC AAATTCTTGT TCAAGAATAT TATCATAAAC TTGTTTGAAT AAATTGTCAT
```

1801 CAATAACATT GCTTCTACAA GTTGAAATTG ATGAAGCAAA AATAATAATG AGAAGGAGAA

1861 TACTATTCCT TTGGATAACC ATGATATATT GTTATATGGT ATGGTTTTTA AACTTGTCTA

1921 TTGAAAAAGA TTGGATCCTC TAGAGTCGAC CTGCAGGTCG TTCAAACATT TGGCAATAAA

>PG Sense | | *nos* 3' >

1981 GTTTCTTAAG ATTGAATCCT GTTGCCGGTC TTGCGATGAT TATCATATAA TTTCTGTTGA

2041 ATTACGAATT GCATGTAATA ATTAACATGT AATGCATGAC GTTATTTATG AGATGGGTTT

2101 TTATGATTAG AGTCCCGCAA TTATACATTT AATACGCGAT AGAAAACAAA ATATAGCGCG

2161 CAAACTACCA TAAATTATCG CGCGCGGTGT CATCTATGTT ACTAGATCGG GAAGCTTGGC

> *nos* 3' | *Hin*dIII |

2221 GTAATCATGG TCATAGCTGT TTCCTGTGTG AAATTGTTAT CCGCTCACAA TTCCACACAA

2281 CATACGAGCC GGAAGCATAA AGTGTAAAGC CTGGGGTGCC TAATGAGTGA GCTAACTCAC

2341 ATTAATTGCG TTGCGCTCAC TGCCCGCTTT CCAGTCGGGA AACCTGTCGT GCCAGCTGCA

2401 TTAATGAATC GGCCAACGCG CGGGGAGAGG CGGTTTGCGT ATTGGGCCAA AGACAAAAGG

> *Lac* gene from *E. coli* || gene III from

2461 GCGACATTCA ACCGATTGAG GGAGGGAAGG TAAATATTGA CGGAAATTAT TCATTAAAGG

M13mp19 >

2521 TGAATTATCA CCGTCACCGA CTTGAGCCAT TTGGGAATTA GAGCCAGCAA AATCACCAGT

2581 AGCACCATTA CCATTAGCAA GGCCGGAAAC GTCACCAATG AAACCATCGA TAGCAGCACC

2641 GTAATCAGTA GCGACAGAAT CAAGTTTGCC TTTAGCGTCA GACTGTAGCG CGTTTTCATC

2701 GGCATTTTCG GTCATAGCCC CTTATTAGC GTTTGCCATC TTTTCATAAT CAAAATCACC

2761 GGAACCAGAG CCACCACCGG AACCGCCTCC CTCAGAGCCG CCACCCTCAG AACCGCCACC

2821 CTCAGAGCCA CCACCCTCAG AGCCGCCACC AGAACCACCA CCAGAGCCGC CGCCAGCATT

> gene III from

2881 GACAGGAGGC CCGATCTAGT AACATAGATG ACACCGCGCG CGATAATTTA TCCTAGTTTG

M13mp19|| *nos* 3' <

2941 CGCGCTATAT TTTGTTTTCT ATCGCGTATT AAATGTATAA TTGCGGGACT CTAATCATAA

3001 AAACCCATCT CATAAATAAC GTCATGCATT ACATGTTAAT TATTACATGC TTAACGTAAT

3061 TCAACAGAAA TTATATGATA ATCATCGCAA GACCGGCAAC AGGATTCAAT CTTAAGAAAC

3121 TTTATTGCCA AATGTTTGAA CGATCGGGGA TCATCCGGGT CTGTGGCGGG AACTCCACGA

< *Nos* 3' | | *ocd* gene from *A. tumefaciens* >

3181 AAATATCCGA ACGCAGCAAG ATATCGCGGT GCATCTCGGT CTTGCCTGGG CAGTCGCCGC

3241 CGACGCCGTT GATGTGGACG CCGGGCCCGA TCATATTGTC GCTCAGGATC GTGGCGTTGT

3301 GCTTGTCGGC CGTTGCTGTC GTAATGATAT CGGCACCTTC GACCGCCTGT TCCGCAGAGA

< *ocd* gene from *A. tumefaciens* |

3361 TCCCGTGGGC GAAGAACTCC AGCATGAGAT CCCCGCGCTG GAGGATCATC CAGCTCGGCG

3421 TCCCGGAAAA CGATTCCGAA GCCCAACCTT TCATAGAAGG CGGCGGTGGA ATCGAAATCT

3481 CGTGATGGCA GGTTGGGCGT CGCTTGGTCG GTCATTTCGA ACCCCAGAGT CCCGCTCAGA

| *npt*II <

3541 AGAACTCGTC AAGAAGGCGA TAGAAGGCGA TGCGCTGCGA ATCGGGAGCG GCGATACCGT

3601 AAAGCACGAG GAAGCGGTCA GCCCATTCGC CGCCAAGCTC TTCAGCAATA TCACGGGTAG

3661 CCAACGCTAT GTCCTGATAG CGGTCCGCCA CACCAGCCG GCCACAGTCG ATGAATCCAG

3721 AAAGCGGCC ATTTTCCACC ATGATATTCG GCAAGCAGGC ATCGCCATGG GTCACGACGA

```
3781  GATCTCGCC   GTCGGGCATG  CGCGCCTTGA  GCCTGGCGAA  CAGTTCGGCT  GGCGCGAGCC
3841  CCTGATGCTC  TTCGTCCAGA  TCATCCTGAT  CGACAAGACC  GGCTTCCATC  CGAGTACGTG
3901  CTCGCTCGAT  GCGATGTTTC  GCTTGGTGGT  CGAATGGGCA  GGTAGCCGGA  TCAAGCGTAT
3961  GCAGCCGCCG  CATTGCATCA  GCCATGATGG  ATACTTTCTC  GGCAGGAGCA  AGGTGAGATG
4021  ACAGGAGATC  CTGCCCCGGC  ACTTCGCCCA  ATAGCAGCCA  GTCCCTTCCC  GCTTGAGTGA
4081  CAACGTCGAG  CACAGCTGCG  CAAGGAACGC  CCGTCGTGGC  CAGCCACGAT  AGCCGCGCTG
4141  CCTCGTCCTH  CAGTTCATTC  AGGGCACCGG  ACAGGTCGGT  CTTGACAAAA  AGAACCGGGC
4201  GCCCCTGCGC  TGACAGCCGG  AACACGGCGG  CATCAGAGCA  GCCGATTGTC  TGTTGTGCCC
4261  AGTCATAGCC  GAATAGCCTC  TCCACCCAAG  CGGCCGGAGA  ACCTGCGTGC  AATCCATCTT
4321  GTTCAATCAT  GCGAAACGAT  CCAGATCCGG  TGCAGATTAT  TTGGATTGAG  AGTGAATATG
      < nptII  ||  nos promoter <
4381  AGACTCTAAT  TGGATACCGA  GGGGAATTTA  TTGGTAACGA  TTCAGTTGAG  CATTTTTTGA
4441  ACAAGAAATA  TTTCTAGCTG  ATAGTGACCT  TAGGCGCATT  TGAACGCGCA  ATAATGGTTT
4501  CTGACGTATG  TGCTTAGCTC  ATTAAACTCC  AGAAACCCGC  GGCTGAGTGG  CTCCTTCAAC
                                                    <  nos promoter |
4561  GTTGCGGTTC  TGTCAGTTCC  AAACGTAAAA  CGGCTTGTCC  CGCGTCATCG  GCGGGGGTCA
4621  TAACGTGACT  CCCTTAATTC  TCCGCTCATG  ATCAGATTGT  CGTTTCCCGC  CTTCAGTTTA
4681  AACTATCAGT  GTTTGACAGG  ATATATTGGC  GGGTAAAC
                  |Right Border
```

Total number of bases is: 4718

DNA Sequence of pJR16S (Sense orientation)

(only sequences between the border regions are shown)

```
                  10         20         30         40         50         60
                   |          |          |          |          |          |
   1 TGGCAGGATA TATTGTGGTG TAAACAAATT GACGCTTAGA CAACTTAATA ACACATTGCG
     |Left border
  61 GACGTTTTTA ATGTACTGGG GTGGTTTTTC TTTTCACCAG TGAGACGGGC AACAGCTGAT
                                      |Ori from M13mp19     >
 121 TGCCCTTCAC CGCCTGGCCC TGAGAGAGTT GCAGCAAGCG GTCCACGCTG GTTTGCCCCA
 181 GCAGGCGAAA ATCCTGTTTG ATGGTGGTTC CGAAATCGGC AAAATCCCTT ATAAATCAAA
 241 AGAATAGCCC GAGATAGGGT TGAGTGTTGT TCCAGTTTGG AACAAGAGTC CACTATTAAA
 301 GAACGTGGAC TCCAACGTCA AAGGGCGAAA AACCGTCTAT CAGGGCGATG GCCCACTACG
 361 TGAACCATCA CCCAATCAA  GTTTTTTGGG GTCGAGGTGC CGTAAAGCAC TAAATCGGAA
 421 CCTAAAGGGA GCCCCCGATT TAGAGCTTGA CGGGGAAAGC CGGCGAACGT GGCGAGAAAG
 481 GAAGGGAAGA AAGCGAAAGG AGCGGGCGCC ATTCAGCGTG CGCAACTGTT GGGAAGGGCG
     < Ori from M13mp19    | Lac gene from E. coli    >
 541 ATCGGTGCGG GCCTCTTCGC TATTAGCCAG CTGGCGAAAG GGGGATGTGC TGCAAGGCGA
 601 TTAAGTTGGG TAACGCCAGG GTTTTCCCAG TCACGACGTT GTAAAACGAC GGCCAGTGAA
                                                          |Bco
 661 TTCCCATGGA GTCAAAGATT CAAATAGAGG ACCTAACAGA ACTCGCCGTA AAGACTGGCG
     RI || CaMV 35S promoter   >
 721 AACAGTTCAT ACAGACTCTC TTACGACTCA ATGACAAGAA GAAAATCTTC GTCAACATGG
 781 TGGAGCACGA CACGCTTGTC TACTCCAAAA ATATCAAAGA TACAGTCTCA GAAGACCAAA
 841 GGGCATTGA  GACTTTTCAA CAAAGGGTAA TATCCGGAAA CCTCCTCGGA TTCCATTGCC
 901 CAGCTATCTG TCACTTTATT GTGAAGATAG TGGAAAAGGA AGGTGGCTCC TACAAATGCC
 961 ATCATTGCGA TAAAGGAAAG GCCATCGTTG AAGATGCCTC TGCCGACAGT GGTCCCAAAG
1021 ATGGACCCCC ACCCACGAGG AGCATCGTGG AAAAAGAAGA CGTTCCAACC ACGTCTTCAA
1081 AGCAAGTGGA TTGATGTGAT ATCTCCACTG ACGTAAGGGA TGACGCACAA TCCCACTATC
1141 CTTCGCAAGA CCCTTCCTCT ATATAAGGAA GTTCATTTCA TTTGGAGAGG ACAGGTACCC
                                          > CaMV 35S |
1201 AATCTTTTTC AATAGACAAG TTTAAAAACC ATACCATATA ACAATATATC ATGGTTATCC
     |PG Sense   >
1261 AAAGGAATAG TATTCTCCTT CTCATTATTA TTTTTGCTTC ATCAATTTCA ACTTGTAGAA
1321 GCAATGTTAT TGATGACAAT TTATTCAAAC AAGTTTATGA TAATATTCTT GAACAAGAAT
1381 TTGCTCATGA TTTTCAAGCT TATCTTTCTT ATTTGAGCAA AAATATTGAA AGCAACAATA
1441 ATATTGACAA GGTTGATAAA AATGGGATTA AAGTGATTAA TGTACTTAGC TTTGGAGCTA
1501 AGGGTGATGG AAAAACATAT GATAATATTG CATTTGAGCA AGCATGGAAT GAAGCATGTT
1561 CATCTAGAAC ACCTGTTCAA TTTGTGGTTC CTAAAAACAA GAATTATCTT CTCAAGCAAA
1621 TCACCTTTTC AGGTCCATGC AGATCTTCTA TTTCAGTAAA GATTTTTGGA TCCTTAGAAG
1681 CATCTAGTAA AATTTCAGAC TACAAAGATA GAAGGCTTTG GATTGCTTTT GATAGTGTTC
1741 AAAATTTAGT TGTTGGAGGA GGAGGAACTA TCAATGGCAA TGGACAAGTA TGGTGGCCAA
```


1801 GTTCTTGCAA AATAAATAAA TCACTGCCAT GCAGGGATGC ACCAACGGCC TTAACCTTCT
1861 GGAATTGCAA AAATTTGAAA GTGAATAATC TAAAGAGTAA AAATGCACAA CAAATTCATA
1921 TCAAATTTGA GTGGATCCTC TAGAGTCGAC CTGCAGGTCG TTCAAACATT TGGCAATAAA
>PG Sense
nos 3' >
1981 GTTTCTTAAG ATTGAATCCT GTTGCCGGTC TTGCGATGAT TATCATATAA TTTCTGTTGA
2041 ATTACGAATT GCATGTAATA ATTAACATGT AATGCATGAC GTTATTTATG AGATGGGTTT
2101 TTATGATTAG AGTCCCGCAA TTATACATTT AATACGCGAT AGAAAACAAA ATATAGCGCG
2161 CAAACTACCA TAAATTATCG CGCGCGGTGT CATCTATGTT ACTAGATCGG GAAGCTTGGC
> nos 3'
HindIII
2221 GTAATCATGG TCATAGCTGT TTCCTGTGTG AAATTGTTAT CCGCTCACAA TTCCACACAA
2281 CATACGAGCC GGAAGCATAA AGTGTAAAGC CTGGGGTGCC TAATGAGTGA GCTAACTCAC
2341 ATTAATTGCG TTGCGCTCAC TGCCCGCTTT CCAGTCGGGA AACCTGTCGT GCCAGCTGCA
2401 TTAATGAATC GGCCAACGCG CGGGGAGAGG CGGTTTGCGT ATTGGGCCAA AGACAAAAGG
> Lac gene from E. coli || gene III from
2461 GCGACATTCA ACCGATTGAG GGAGGGAAGG TAAATATTGA CGGAAATTAT TCATTAAAGG
M13mp19 >
2521 TGAATTATCA CCGTCACCGA CTTGAGCCAT TTGGGAATTA GAGCCAGCAA AATCACCAGT
2581 AGCACCATTA CCATTAGCAA GGCCGGAAAC GTCACCAATG AAACCATCGA TAGCAGCACC
2641 GTAATCAGTA GCGACAGAAT CAAGTTTGCC TTTAGCGTCA GACTGTAGCG CGTTTTCATC
2701 GGCATTTTCG GTCATAGCCC CTTATTAGC GTTTGCCATC TTTTCATAAT CAAAATCACC
2761 GGAACCAGAG CCACCACCGG AACCGCCTCC CTCAGAGCCG CCACCCTCAG AACCGCCACC
2821 CTCAGAGCCA CCACCCTCAG AGCCGCCACC AGAACCACCA CCAGAGCCGC CGCCAGCATT
> gene III from
2881 GACAGGAGGC CCGATCTAGT AACATAGATG ACACCGCGCG CGATAATTTA TCCTAGTTTG
M13mp19|| nos 3' <
2941 CGCGCTATAT TTTGTTTTCT ATCGCGTATT AAATGTATAA TTGCGGGACT CTAATCATAA
3001 AAACCCATCT CATAAATAAC GTCATGCATT ACATGTTAAT TATTACATGC TTAACGTAAT
3061 TCAACAGAAA TTATATGATA ATCATCGCAA GACCGGCAAC AGGATTCAAT CTTAAGAAAC
3121 TTTATTGCCA AATGTTTGAA CGATCGGGGA TCATCCGGGT CTGTGGCGGG AACTCCACGA
< Nos 3' | ocd gene from A. tumefaciens >
3181 AAATATCCGA ACGCAGCAAG ATATCGCGGT GCATCTCGGT CTTGCCTGGG CAGTCGCCGC
3241 CGACGCCGTT GATGTGGACG CCGGGCCCGA TCATATTGTC GCTCAGGATC GTGGCGTTGT
3301 GCTTGTCGGC CGTTGCTGTC GTAATGATAT CGGCACCTTC GACCGCCTGT TCCGCAGAGA
< ocd gene from A. tumefaciens |
3361 TCCCGTGGGC GAAGAACTCC AGCATGAGAT CCCCGCGCTG GAGGATCATC CAGCTCGGCG
3421 TCCCGGAAAA CGATTCCGAA GCCCAACCTT TCATAGAAGG CGGCGGTGGA ATCGAAATCT
3481 CGTGATGGCA GGTTGGGCGT CGCTTGGTCG GTCATTTCGA ACCCCAGAGT CCCGCTCAGA
| nptII <
3541 AGAACTCGTC AAGAAGGCGA TAGAAGGCGA TGCGCTGCGA ATCGGGAGCG GCGATACCGT
3601 AAAGCACGAG GAAGCGGTCA GCCCATTCGC CGCCAAGCTC TTCAGCAATA TCACGGGTAG
3661 CCAACGCTAT GTCCTGATAG CGGTCCGCCA CACCAGCCG GCCACAGTCG ATGAATCCAG
3721 AAAGCGGCC ATTTTCCACC ATGATATTCG GCAAGCAGGC ATCGCCATGG GTCACGACGA

```
3781  GATCTCGCC   GTCGGGCATG  CGCGCCTTGA  GCCTGGCGAA  CAGTTCGGCT  GGCGCGAGCC
3841  CCTGATGCTC  TTCGTCCAGA  TCATCCTGAT  CGACAAGACC  GGCTTCCATC  CGAGTACGTG
3901  CTCGCTCGAT  GCGATGTTTC  GCTTGGTGGT  CGAATGGGCA  GGTAGCCGGA  TCAAGCGTAT
3961  GCAGCCGCCG  CATTGCATCA  GCCATGATGG  ATACTTTCTC  GGCAGGAGCA  AGGTGAGATG
4021  ACAGGAGATC  CTGCCCCGGC  ACTTCGCCCA  ATAGCAGCCA  GTCCCTTCCC  GCTTGAGTGA
4081  CAACGTCGAG  CACAGCTGCG  CAAGGAACGC  CCGTCGTGGC  CAGCCACGAT  AGCCGCGCTG
4141  CCTCGTCCTH  CAGTTCATTC  AGGGCACCGG  ACAGGTCGGT  CTTGACAAAA  AGAACCGGGC
4201  GCCCCTGCGC  TGACAGCCGG  AACACGGCGG  CATCAGAGCA  GCCGATTGTC  TGTTGTGCCC
4261  AGTCATAGCC  GAATAGCCTC  TCCACCCAAG  CGGCCGGAGA  ACCTGCGTGC  AATCCATCTT
4321  GTTCAATCAT  GCGAAACGAT  CCAGATCCGG  TGCAGATTAT  TTGGATTGAG  AGTGAATATG
      < nptI  ||  nos promoter  <
4381  AGACTCTAAT  TGGATACCGA  GGGGAATTTA  TTGGTAACGA  TTCAGTTGAG  CATTTTTTGA
4441  ACAAGAAATA  TTTCTAGCTG  ATAGTGACCT  TAGGCGCATT  TGAACGCGCA  ATAATGGTTT
4501  CTGACGTATG  TGCTTAGCTC  ATTAAACTCC  AGAAACCCGC  GGCTGAGTGG  CTCCTTCAAC
                                                    < nos promoter  |
4561  GTTGCGGTTC  TGTCAGTTCC  AAACGTAAAA  CGGCTTGTCC  CGCGTCATCG  GCGGGGGTCA
4621  TAACGTGACT  CCCTTAATTC  TCCGCTCATG  ATCAGATTGT  CGTTTCCCGC  CTTCAGTTTA
4681  AACTATCAGT  GTTTGACAGG  ATATATTGGC  GGGTAAAC
                  | Right Border
```

Total number of bases is: 4718

Analysis of Open Reading Frames in the Plant Transformation Vector pJR16A

Using the universal genetic code.
The initiation codon(s) are: AUG/ATG only.
The minimal size for an ORF is set to 50 amino acids.

Normal Orientation. Total number of bases: 4718.

List of Open Reading Frames in decreasing size order.

	ORFs	Size (bp)	From Base	To Base
1	N2-11	534	3485	4018
2	N3-4	426	666	1091
3	N3-18	408	3075	3482
4	N3-19	351	3132	3482
5	N3-5	315	777	1091
6	N2-12	252	3767	4018
7	N3-20	231	3252	3482
8	N2-13	174	3845	4018
9	N1-16	168	2323	2490
10	N3-21	159	3324	3482

Inverse Orientation. Total number of bases: 4718.

List of Open Reading Frames in decreasing size order.

	ORFs	Size (bp)	From Base	To Base
1	N2-3	792	389	1180
2	N2-28	702	2837	3538
3	N1-4	462	1204	1665
4	N2-4	447	734	1180
5	N2-5	435	746	1180
6	N3-6	384	561	944
7	N2-6	351	830	1180
8	N3-14	336	1980	2315
9	N2-9	330	1265	1594
10	N3-15	312	2004	2315
11	N2-10	285	1310	1594
12	N2-11	261	1334	1594
13	N2-7	261	920	1180
14	N3-7	204	741	944
15	N2-8	204	977	1180
16	N1-28	192	4207	4398
17	N1-9	189	2014	2202
18	N1-10	183	2020	2202
19	N3-26	177	3066	3242
20	N1-7	165	1840	2004
21	N3-3	156	402	557
22	N3-27	150	3093	3242
23	N2-12	150	1445	1594

Analysis of Open Reading Frames in the Plant Transformation Vector pJR16S

Using the universal genetic code.
The initiation codon(s) are: AUG/ATG only.
The minimal size for an ORF is set to 50 amino acids.

Normal Orientation. Total number of bases: 4718.

List of Open Reading Frames in decreasing size order.

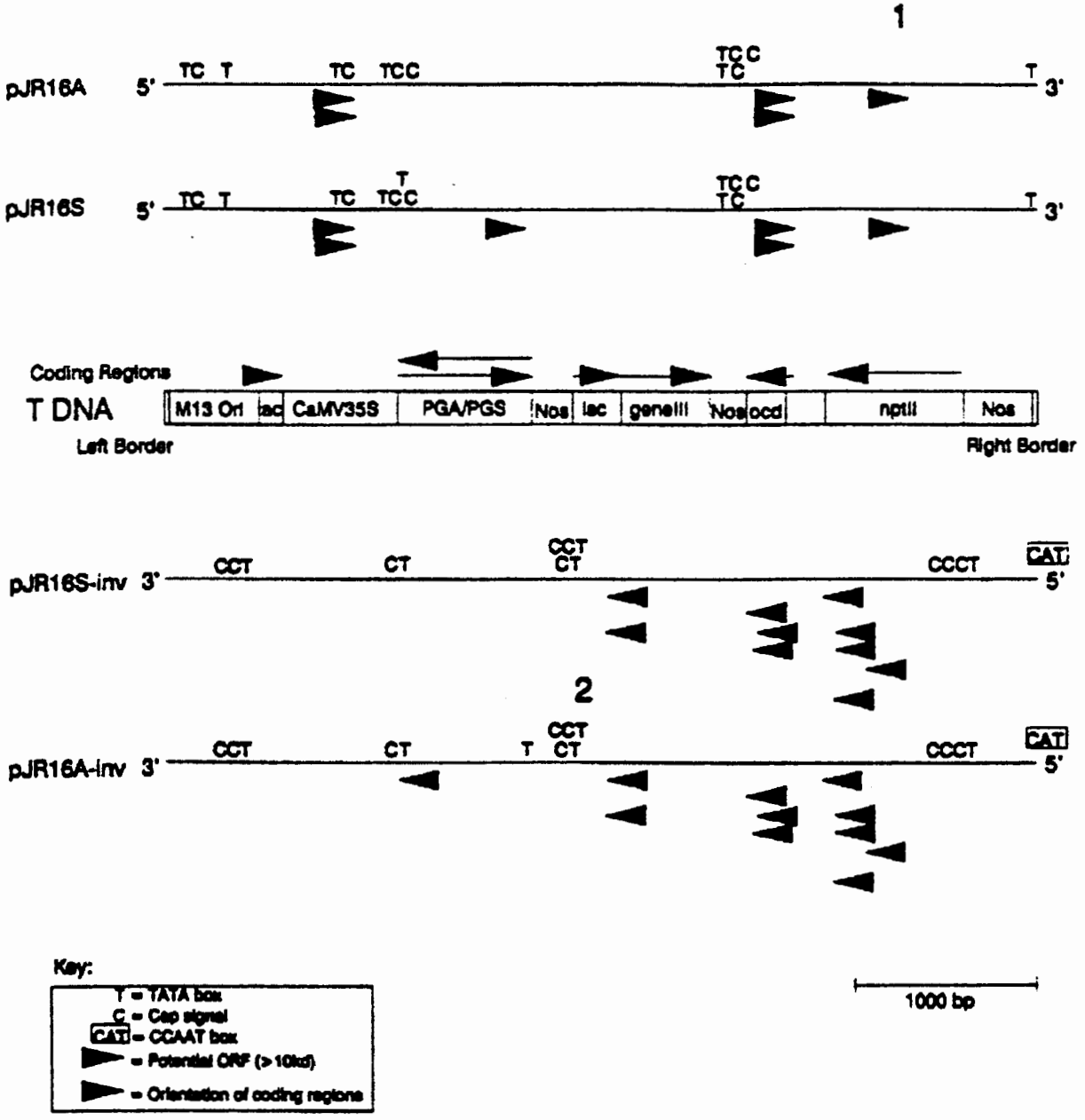
	ORFs	Size (bp)	From Base	To Base
1	N3-6	690	1251	1940
2	N2-13	534	3485	4018
3	N3-4	426	666	1091
4	N3-16	408	3075	3482
5	N3-17	351	3132	3482
6	N3-5	315	777	1091
7	N2-14	252	3767	4018
8	N3-18	231	3252	3482
9	N1-10	177	1480	1656
10	N2-15	174	3845	4018
11	N1-20	168	2323	2490
12	N3-19	159	3324	3482
13	N1-11	150	1507	1656

Inverse Orientation. Total number of bases: 4718.

List of Open Reading Frames in decreasing size order.

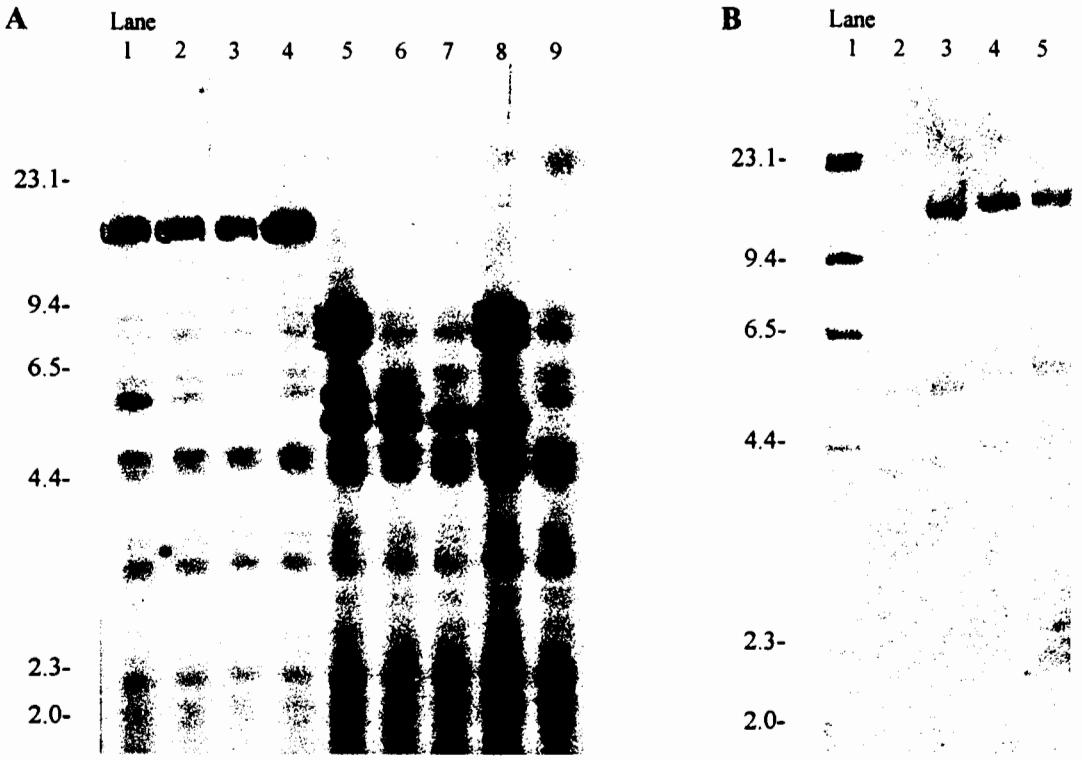
	ORFs	Size (bp)	From Base	To Base
1	N2-3	792	389	1180
2	N1-4	462	1204	1665
3	N2-4	447	734	1180
4	N2-5	435	746	1180
5	N3-6	384	561	944
6	N2-6	351	830	1180
7	N3-14	336	1980	2315
8	N2-9	330	1265	1594
9	N3-15	312	2004	2315
10	N2-10	285	1310	1594
11	N2-11	261	1334	1594
12	N2-7	261	920	1180
13	N3-7	204	741	944
14	N2-8	204	977	1180
15	N1-26	192	4207	4398
16	N1-9	189	2014	2202
17	N1-10	183	2020	2202
18	N1-7	165	1840	2004
19	N3-3	156	402	557
20	N2-12	150	1445	1594

Promoter Analysis of pJR16A and pJR16S



Computer analysis was performed to identify; open reading frames, TATA boxes, cap sites and translational initiation sites. The direction of the arrows indicate the direction of the open reading into the frame. Lines associated with the arrows indicate the length of the open reading frames shown in Tables 3-4. Symbols identify the following sequence elements, T: TATA box, C: cap sites, LAT: translational sequences. The conclusions of this analysis are summarized in the **Analysis of Open Reading Frames** section.

Copy Number Analysis of Line 66-51 (B)



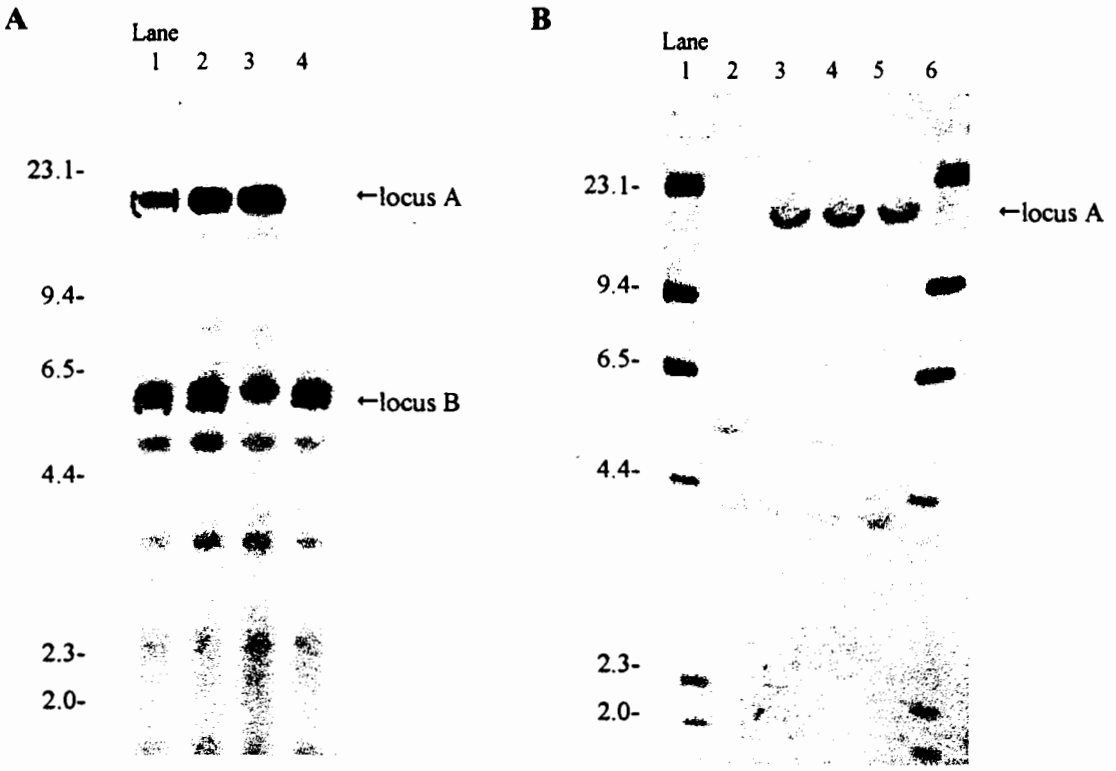
Panel A: Autoradiogram of genomic DNAs isolated from T_1 plants derived from selfing plant 66-51. Genomic DNAs were digested with *Eco*RI, resolved on a 1% agarose gel, transferred to a membrane and incubated with a [α-^{32}P] dCTP-labeled probe generated from a 647 base pair fragment of the PG coding sequence. Molecular size markers are located on the left side of the autoradiogram and are expressed in Kilobase pairs. The probe was amplified by PCR and labeled using a random priming kit (Boehringer Mannheim). The probe hybridizes to several areas of the tomato genome from the non-transgenic parental control (negative control, lane 9). A transgenic insert is indicated by an additional band that is not found in the parental control. *Eco*RI cuts once in the pJR16A plasmid; the recognition site is found immediately upstream of the CaMV 35S promoter. As a result, every insertion will result in a novel band of a size greater than 4060 base pairs (the distance between the *Eco*RI site and the right T-DNA border). The exact size of the novel band is determined by the distance from the right border insertion site in the tomato genome to the next *Eco*RI site in the tomato DNA. Lanes 1-4 are DNAs from pJR16A transformed T_1 plants that were produced by selfing the original 66-51 primary transformant. Note that in each lane (1-4), there is a single, novel band of approximately 16 Kbp that is highlighted with parenthesis. This novel 16 Kbp band contains approximately 12 Kbp of DNA from the tomato genome and 4060 base pairs of the inserted DNA, including both the 35S-PGS-Nos and NOS-*npt*II-Nos chimeric genes. Lanes 5-8 areEcoRI cut DNAs isolated from unrelated T_0 plants. Lane 9 is EcoRI cut DNA from non-transformed control T7 plant.

Panel B: Fluorogram of genomic DNAs isolated from T_5 plants derived from the generational selfing of T_0 plant 66-51. DNA from bacteriophage lambda that has been digested with *Hin*dIII and labeled with digoxigenin serves as molecular weight markers and are shown in lane 1. The respective sizes of these marker bands are shown to the left of lane 1 and are expressed in kilobase pairs. Genomic DNAs from three T_5 plants (lanes 3,4 and 5) and a non-transformed parental control plant (lane 2) were electrophoresed and blotted as described in Panel A. The blot was incubated with a dUTP digoxigenin-labeled probe

prepared from a 478 base pair fragment of the PG coding region. The probe was labeled and amplified by PCR. Reagents for probe preparation, markers and for detection were purchased from Boehringer Mannheim. Although both probes used in Panels A and B are from the PG coding region, variation in the probes is reflected by the amount of hybridization to the non-transformed parental control line (lane 2 in Panel B and lane 9 in Panel A). Using the 478 bp probe, the amount of hybridization to the non-transformed parental control DNA is reduced; however, bands of approximately 5 Kb and 4 Kb can be observed in both the non-transformed control (lane 2) and the transgenic plants (lanes 3-5).

T_0 plant 66-51 was selfed in subsequent generations; during this process, the single insertion of PG was taken to homozygosity. Lanes 3-5 show the PG banding patterns from three plants of the T_5 generation. In each plant, the transgenic PG insert is represented by a novel, approximately 16 Kbp band. This is the same size band that was observed in the T_0 generation (data not shown), T_1 generation (Panel A) and all subsequent generations (data not shown), and is an indication of the generational stability of the introduced transgenic PG locus.

Copy Number Analysis of Line 87-22A (Da)



Panel A: Autoradiogram of genomic DNAs isolated from 87-22 derived T_1 plants transformed with pJR16S (PGS). Genomic DNAs were digested with *Eco*RI, resolved on a 1% agarose gel, transferred to a membrane and incubated with a [α-^{32}P] dCTP labeled probe generated from a 647 base pair fragment of the PG coding sequence. Molecular size markers are located on the left side of the autoradiogram, and are expressed in Kilobase pairs. The probe was amplified by PCR and labeled using a random priming kit (Boehringer Mannheim). The probe hybridizes to several areas of the tomato genome from the non-transgenic parental control .

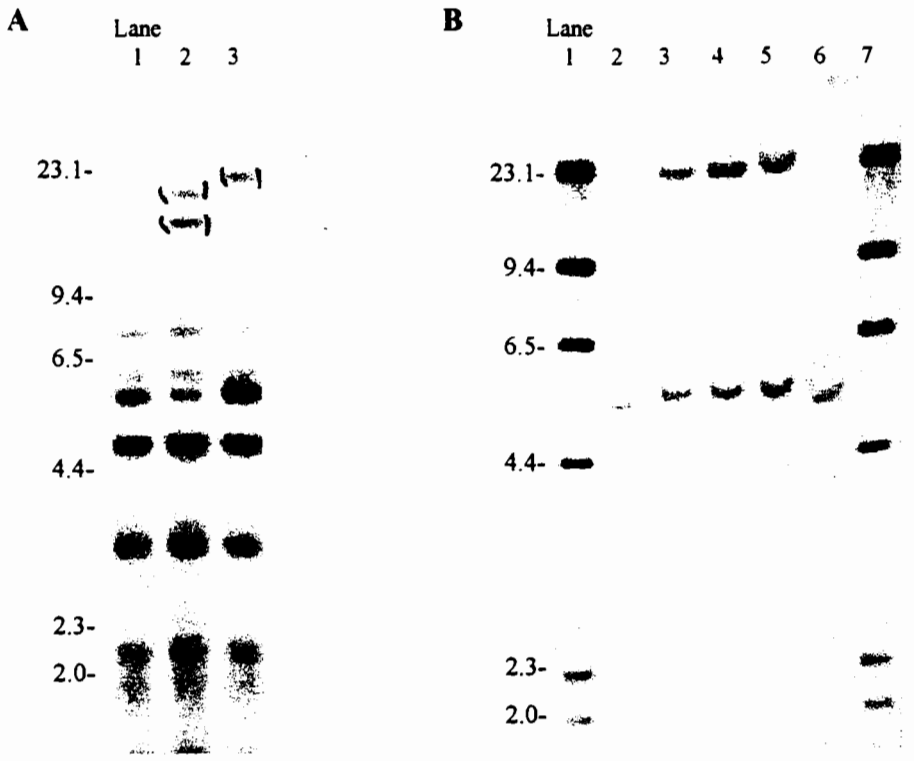
The 87-22 T_0 plant was found to have two copies of the transgenes (35S-PGS-Nos, NOS-*npt*II-Nos) incorporated into the genome (data not shown). Plant 87-22 was selfed and the progeny were analyzed to determine whether the two copies could be separated. The autoradiogram shows the DNA hybridization patterns for four T_1 plants derived from plant 87-22 (lanes 1-4). One of the insertions yields a band of approximately 20 Kbp; the second insert yields a band of approximately 5 Kbp. Both novel bands, representing separate insertions of the transgenes, are highlighted by parentheses. In some of the T_1 plants, the two insertions have segregated. For example, while lanes 1 and 2 have both of the transgenic bands, lane 3 retained only the larger of the two bands (locus A), and lane 4 has retained only the smaller of the two bands (locus B). The plant in lane 3 (87-22A/08) that carried locus A but not locus B was selected for further study.

Panel B: Fluorogram of genomic DNAs isolated from T_5 plants derived from the generational selfing of T_1 plant 87-22A/08. DNA from bacteriophage lambda that has been digested with *Hin*dIII and labeled with digoxigenin serves as molecular weight markers and is shown in lanes 1 and 6. The respective sizes of these marker bands are shown to the left of lane 1 and are expressed in Kilobase pairs. Genomic DNAs from three T_5 plants and a non-transformed parental control plant were electrophoresed and blotted as described

in Panel A. The blot was incubated with a dUTP digoxigenin-labeled probe prepared from a 478 base pair fragment of the PG coding region. The probe was labeled and amplified by PCR. Reagents for probe preparation, markers and for detection were purchased from Boehringer Mannheim. Although both probes used in Panels A and B are from the PG coding region, variation in the probes is reflected by the amount of hybridization to the non-transformed parental control line (lane 2). Using the 478 bp probe, the amount of hybridization to the non-transformed parental control DNA is reduced; however, bands of approximately 5 Kb and 4 Kb can be observed in both the non-transformed control (lane 2) and the transgenic plants (lanes 3-5).

T_1 plant 87-22A/08 was selfed in subsequent generations; during this process, the single insertion of PG was taken to homozygosity. Lanes 3-5 show the PG banding patterns from three plants of the T_5 generation. In each plant, the transgenic PG insert is represented by a novel, approximately 20 Kbp band. This is the same size band that was observed in the T_1 plant (Panel A, lane 3) and all subsequent generations (data not shown), and is an indication of the generational stability of the introduced transgenic PG locus.

Copy Number Analysis of Line 88-37 (F)



Panel A: Autoradiogram of genomic DNAs isolated from T_0 plants transformed with pJR16S (PGS). Genomic DNAs were digested with *Eco*RI, resolved on a 1% agarose gel, transferred to a membrane and incubated with a [α-^{32}P] dCTP-labeled probe generated from a 647 base pair fragment of the PG coding sequence. Molecular size markers are located on the left side of the autoradiogram, and are expressed in Kilobase pairs. The probe was amplified by PCR and labeled using a random priming kit (Boehringer Mannheim). Hybridization patterns are shown for non-transgenic parental control (lane 1) and two T_0 plants (lanes 2 and 3). Unique bands that are not found in the non-transgenic parental control indicate insertional events and are shown parenthetically in lanes 2 and 3. The plant in lane 2 has had two copies inserted, while the plant in lane 3 (88-37) has had a single copy inserted into the plant chromosome, and is represented by the unique 23Kbp band.

Panel B: Fluorogram of genomic DNAs isolated from T_5 plants derived from the generational selfing of T_0 plant 88-37. DNA from bacteriophage lambda that has been digested with *Hin*dIII and labeled with digoxigenin serves as molecular weight markers and is shown in lanes 1 and 7. The respective sizes of these marker bands are shown to the left of lane 1 and are expressed in Kilobase pairs. Genomic DNAs from three T_5 plants (lanes 3,4 and 5) and a non-transformed parental control plant (lanes 2 and 6) were electrophoresed and blotted as described in Panel A. The blot was incubated with a dUTP digoxigenin-labeled probe prepared from a 478 base pair fragment of the PG coding region. The probe was labeled and amplified by PCR. Reagents for probe preparation, markers and for detection were purchased from Boehringer Mannheim. Although both probes used in Panels A and B are from the PG coding region, variation in the probes is reflected by the amount of hybridization to the non-transformed parental control line (lanes 2 and 6 in Panel B and lane 1 in Panel A). Using the 478 bp probe, the amount of hybridization to the non-transformed parental control DNA is reduced; however, bands of approximately 5 Kb and 4 Kb can be observed in both the non-transformed control (lanes 2 and 6) and the transgenic plants (lanes 3-5).

T_0 plant 88-37 was selfed in subsequent generations; during this process, the single insertion of PG was taken to homozygousity. Lanes 3-5 show the PG banding patterns from three plants of the T_5 generation. In each plant, the transgenic PG insert is represented by a novel, approximately 23 Kbp band. This is the same size band that was observed in the T_0 generation (Panel A, lane 3) and all subsequent generations (data not shown), and is an indication of the generational stability of the introduced transgenic PG locus.

PCR Confirmation of PG Sense, PG Antisense and Gene Orientation

Tomato plants transformed with the plasmid constructs pJR16A (PG antisense) and pJR16S (PG sense) can be differentiated using two PCR primer sets. One primer of each set will anneal to a specific nucleotide sequence *within* the PGA or PGS gene respectively. The other primer will anneal to a specific nucleotide sequence *outside* of the PGA or PGS gene within the CaMV 35S promoter. The presence or absence of the correct size PCR product for a particular primer set is a simple and rapid method to confirm sense or antisense PG gene orientation. In addition, a third set of PCR primers can be used to detect the presence of either orientation of the PG gene. The expected PCR products for plants transformed with the PGA and PGS genes and for non-transformed control plants are listed below.

Table 1. PCR Products for Plants Transformed with the PGA and PGS genes and for non-transformed control plants

	PCR Products (bp)		
	Primers 1010 & 1011	**Primers 1010 & 1012**	**Primers PGR & PGL**
PGA Plants	472	None	180 and 380[1]
PGS Plants	1100[1]	478	180 and 380[1]
Non-transgenic Control Plants	1100[1]	None	380[1]

1 Product of amplification of the endogenous PG gene.

Detailed description:

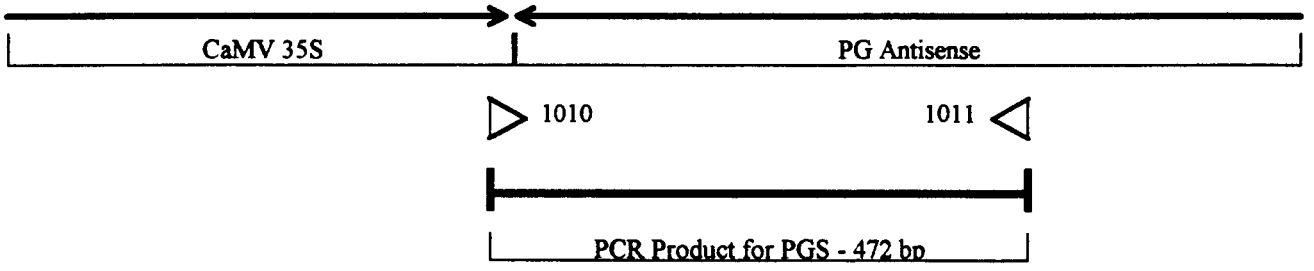
To confirm the antisense (pJR16A insert) orientation, the following primer set is used:

Primer 1010: (5'-AGTTCATTTCATTTGGAGAGGACA-3') is a 24 mer that anneals within the CaMV 35S promoter at nucleotides 1170 to 1193.

Primer 1011: (5'-TTTGGAGCTAAGGGTGATGGA-3') is a 21 mer that anneals within the PG gene at nucleotides 1622 to 1642.

The positioning of primers 1010 and 1011 is shown schematically below in Figure 1.

Figure 1.



When DNA from tomato lines transformed with PG antisense (pJR16A) are used in a PCR reaction with the above primer set, a 472 bp fragment is amplified (see schematic figure above, and lane 6 in Figure 4). In contrast, when DNA from tomato lines transformed with PG sense (pJR16S) are used in a PCR reaction with the above primer set, a larger, approximate 1100 bp fragment is amplified (see lanes 9 and 12 of Figure 4). This is an endogenous PCR product of the line since an identically sized PCR product is produced when non-transformed tomato DNA is used with these primers (see lane 2 of Figure 4).

Using primers PGR and PGL, a 380 bp fragment is obtained in all cases due to detection of the endogenous PG gene.

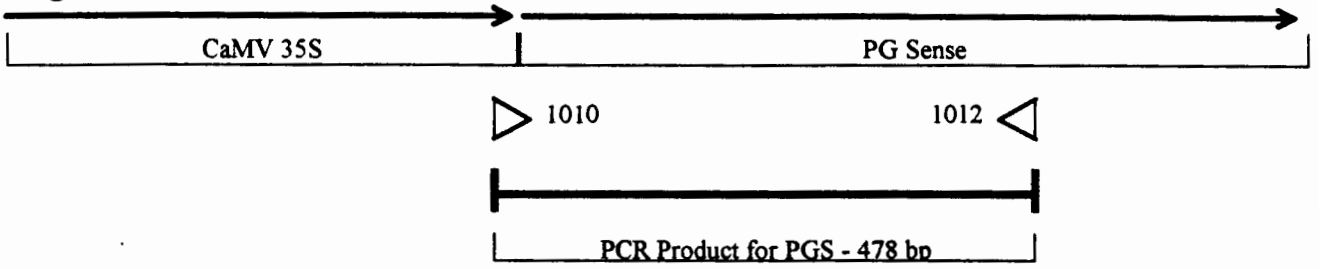
To confirm the sense (pJR16S insert) orientation, the following primer set is used:

Primer 1010: (5'-AGTTCATTTCATTTGGAGAGGACA-3') is a 24 mer that anneals within the CaMV 35S promoter at nucleotides 1170 to 1193

Primer 1012: (5'-GAAGATCTGCATGGACCTGAAAA-3') is a 23 mer that anneals within the PG gene at nucleotides 1626 to 1648.

The positioning of primers 1010 and 1012 is shown below schematically below in Figure 2.

Figure 2.



When DNA from tomato lines transformed with PG sense (pJR16S insert) are used in a PCR reaction with the above primer set, a 478 bp fragment is amplified (see schematic figure above, and lanes 10 and 13 in Figure 4). In contrast, when DNA from tomato lines transformed with PG antisense (pJR16A) are used in a PCR reaction with the above primer set, no amplification product is produced (see lane 7 of Figure 4).

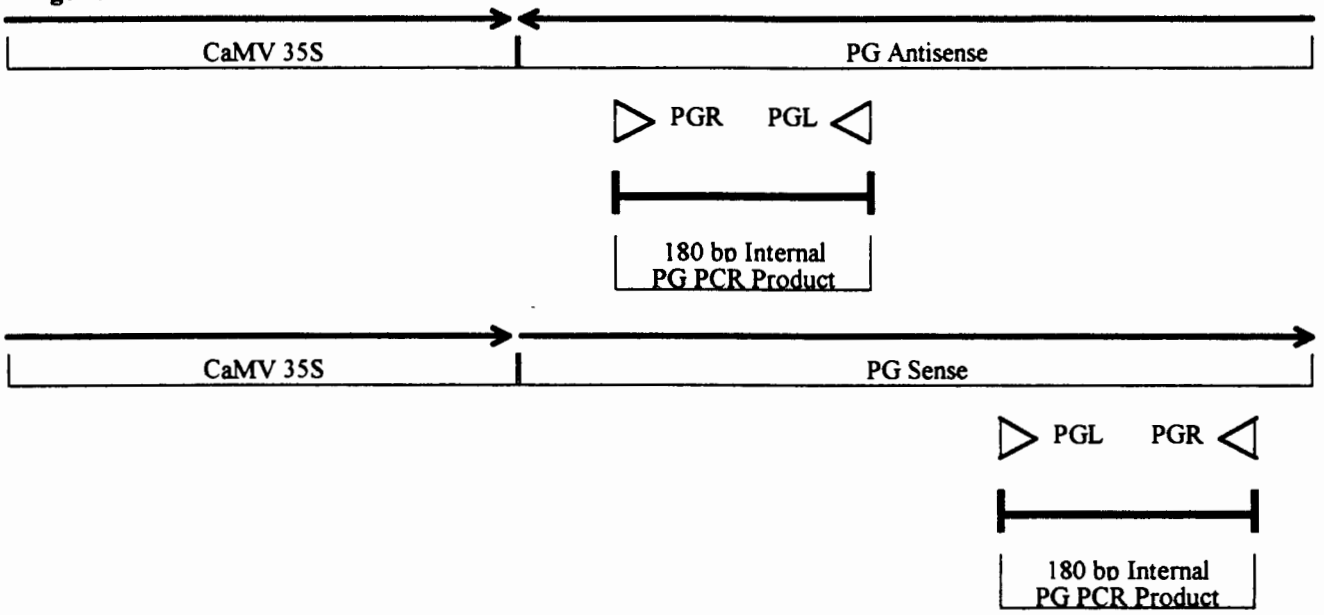
To confirm the presence of the PG gene, irrespective of orientation (either pJR16A or pJR16S), the following primer set can be used:

Primer PGR: (5'-CGTTGGTGCATCCCTGCATGG-3') is a 21 mer that anneals to nucleotides 1285-1306 of the PGA gene (pJR16A), and to nucleotides 1827-1848 of the PGS gene (pJR16S).

Primer PGL: (5'-GGATCCTTAGAAGCATCTAGT-3') is a 21 mer that anneals to nucleotides 1445-1465 of the PGA gene (pJR16A), and to nucleotides 1668-1688 of the PGS gene (pJR16S).

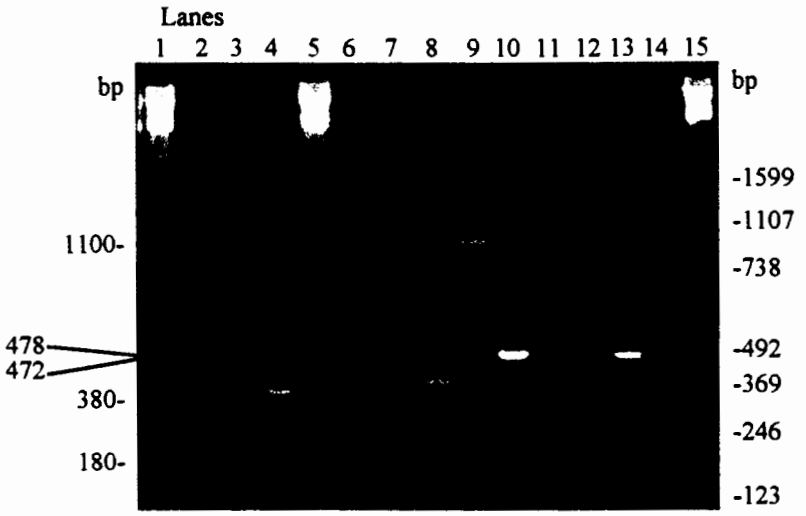
The positioning of primers PGR and PGL is shown schematically below in Figure 3.

Figure 3.



When DNA from tomato lines transformed with either PG antisense (pJR16A) or PG sense (pJR16S) are used in a PCR reaction with the above primer set, a 180 bp fragment from the PG gene insert is amplified (see schematic figure shown above, and the faint bands in lanes 8, 11 and 14 in Figure 4.). In addition, an approximate 380 bp fragment is also amplified from an endogenous PG gene sequence (see lanes 8, 11, and 14 in Figure 4.). In contrast, when DNA from a non-transformed tomato line are used in a PCR reaction with the above primer set, only the endogenous 380 bp PG gene fragment is amplified (see lane 4 of Figure 4).

Figure 4. PCR Based Differentiation of PGA and PGS Transformed Plants



A PCR based assay is used to distinguish transformed and non-transformed lines, and to identify lines transformed with the PG antisense (pJR16A) or PG sense (pJR16S) gene. Aliquots from several PCR reactions were resolved on a 2% agarose gel and compared to DNA size standards from Gibco-BRL (123 bp ladder; lanes 1,5 and 15).

Three primer combinations are used to synthesize a specific sized fragment using substrate DNA from either a non-transformed parental line (lanes 2-4), PG antisense (pJR16A) transformed line B (lines 6-8), and PG sense (pJR16S) transformed lines Da (lanes 9-11) and F (lanes 12-14).

To distinguish the non-transformed parental control line from transgenic PG lines, primers PGR and PGL are used. These primers will synthesize a 380 bp fragment in both non-transgenic (lane 4) and transgenic lines (lanes 8,11 and 14). These primers will also uniquely synthesize a 180 bp fragment in the transgenic lines, irrespective of PG orientation (lanes 8,11 and 14). This 180 bp fragment is not present in the non-transformed parental control (lane 4). Thus this assay can be used to distinguish the transgenic lines from the non-transformed parental control.

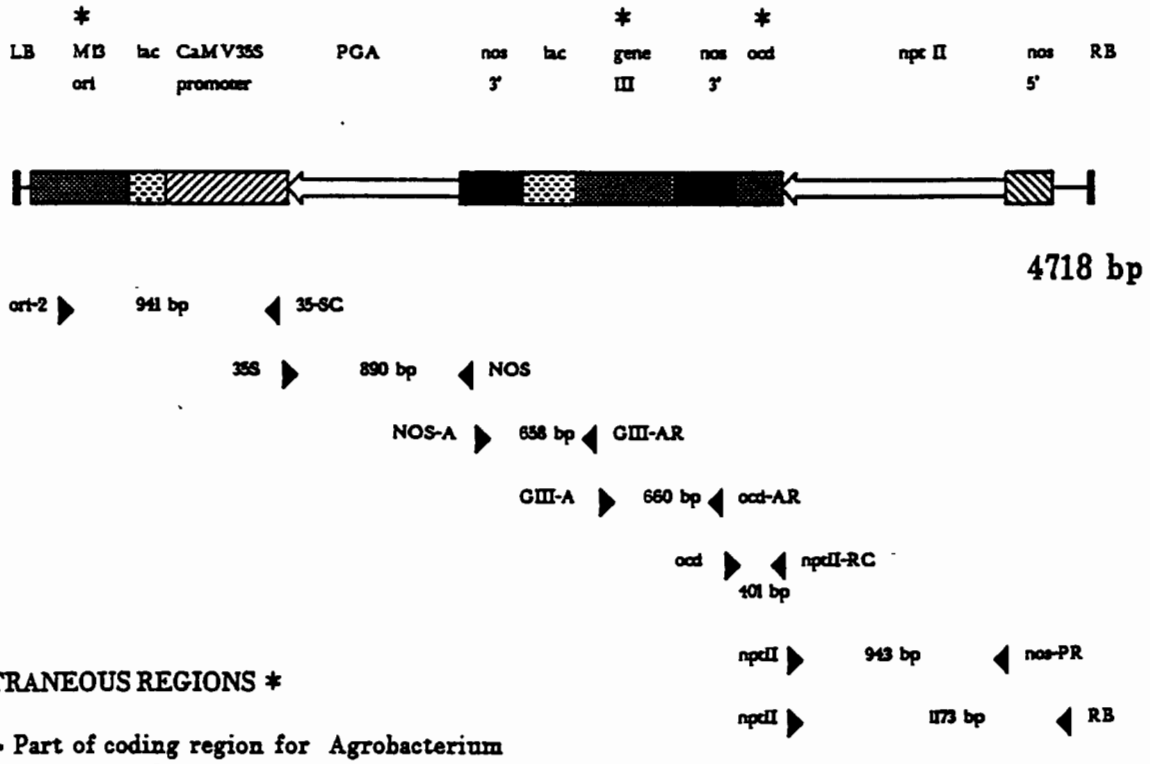
To distinguish PG antisense and PG sense transgenic lines, primer combinations 1010/1011 and 1010/1012 are used. Primers 1010 and 1011 are used to synthesize a 472 bp fragment in lines transformed with PG antisense (pJR16A, lane 6). In either the non-transgenic parental control line (lane 2) or lines transformed with PG sense (pJR16S, lanes 9 and 12), these primers synthesize an approximate 1100 bp fragment. Primers 1010 and 1012 are used to synthesize a 478 bp fragment in lines transformed with PG sense (pJR16S, lanes 10 and 13). No amplification product is observed with this primer set in either the non-transformed parental control line (lane 3) or PG antisense (pJR16A, lane 7).

PG Activity in T2 Homozygous Inbred Lines (% of glasshouse standard)

66-51/08 (B)	88-22A/08 (Da)	87-37/13 (F)
0.0	0.2	5.4
1.0	0.7	2.4
4.0	0.3	2.3
1.4	0.6	3.8
1.3	0.3	2.7
0.1	0.0	1.9
3.4	0.2	2.7
0.7	0.9	3.1
2.2	0.4	7.0
1.2	0.3	2.1
2.2	0.2	5.9
1.9	2.5	3.3
1.0	0.5	5.9
2.8	0.8	4.7
1.4	0.4	5.1
0.8	2.6	4.2
0.0		3.1
1.5		2.0
0.0		
1.4 ± 0.2	**0.7 ± 0.2**	**3.8 ± 0.4**

Individual plant analyses were performed on six pericarp discs (cut out using a No. 5 corkborer) collected from two red-ripe fruits (three discs per fruit).

PCR ANALYSIS OF PGA INSERT(pJR16A)



EXTRANEOUS REGIONS *

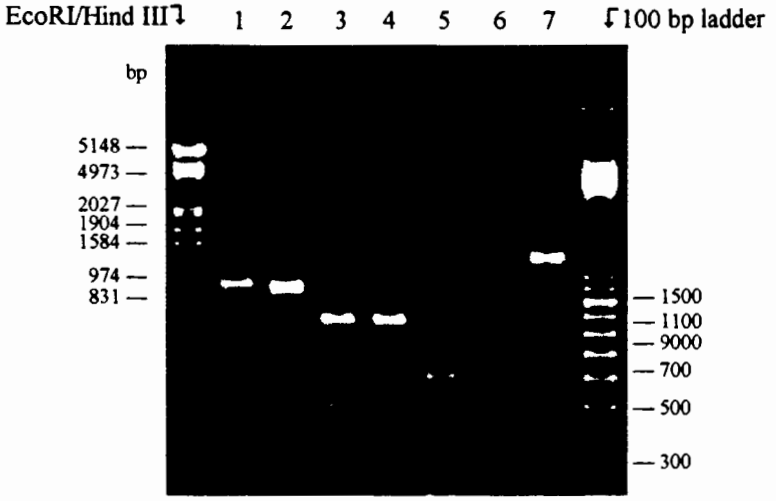
ocd - Part of coding region for Agrobacterium Ti plasmid C58 ornithine cyclodeaminase gene

gene III - Part of coding region of M13mp19 coat protein Gene III

M13 ori - M13mp19 origin of replication

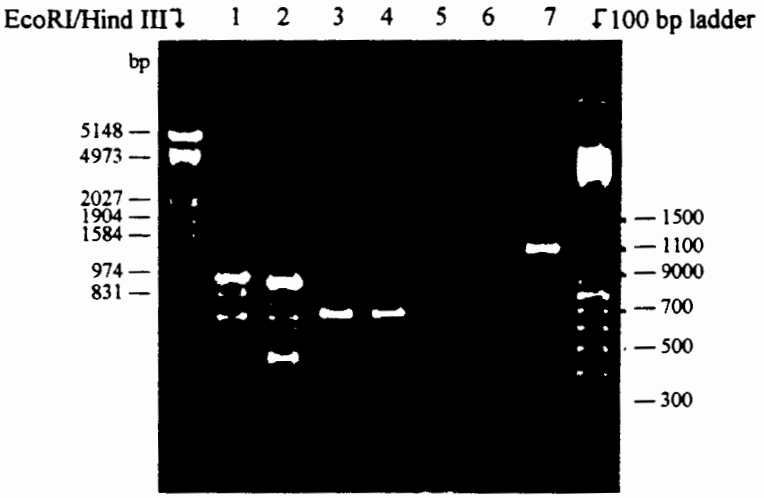
Zeneca Seeds
May 1994

PCR Analysis of PGA Insert (pJR16A) in Inbred B



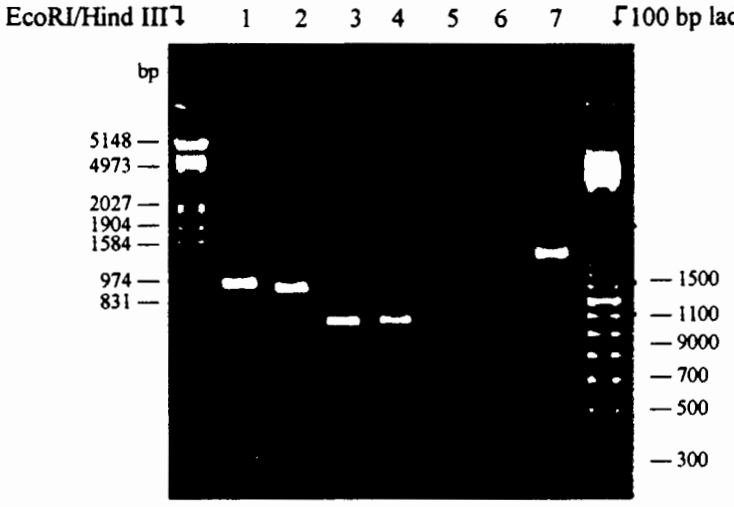
Lane	Primer Pair	PCR Product (bp)	PCR (+/-)
1	ori-2 + 35-SC	941	+
2	35S + NOS	890	+
3	nos-A + GIII-AR	658	+
4	GIII-A + ocd-AR	660	+
5	ocd + *npt*II-RC	401	+
6	*npt*II + nos-PR	943	+
7	*npt*II + RB	1173	+

PCR Analysis of PGA Insert (pJR16A) in Hybrid Nema 1401B



Lane	Primer Pair	PCR Product (bp)	PCR (+/-)
1	ori-2 + 35-SC	941	+
2	35S + NOS	890	+
3	nos-A + GIII-AR	658	+
4	GIII-A + ocd-AR	660	+
5	ocd + *npt*II-RC	401	+
6	*npt*II + nos-PR	943	+
7	*npt*II + RB	1173	+

PCR Analysis of PGA Insert (pJR16A) in Hybrid H282B



Lane	Primer Pair	PCR Product (bp)	PCR (+/-)
1	ori-2 + 35-SC	941	+
2	35S + NOS	890	+
3	nos-A + GIII-AR	658	+
4	GIII-A + ocd-AR	660	+
5	ocd + *npt*II-RC	401	+
6	*npt*II + nos-PR	943	+
7	*npt*II + RB	1173	+

PCR Analysis of PGA Insert (pJR16A)

PCR primers used, as follows:

Name	Sequence
ori-A	5' - AAT CGG AAC CTA AAG GGA GCC - 3'
35-SC	5' - GAT TGT GCG TCA TCC CTT ACG - 3'
ori-2	5' - ATC CTG TTT GAT GGT GGT TCC - 3'
35S	5' - CAA TCC CAC TAT CCT TCG C - 3'
NOS	5' - CAT CGC AAG ACC GGC AAC AG - 3'
NOS-A	5' - TGA ATC CTG TTG CCG GTC TTG - 3'
GIII-AR	5' - CTA CTG ATT ACG GTG CTG CTA - 3'
GIII-A	5' - TAG CAG CAC CGT AAT CAG TAG - 3'
ocd-AR	5' - CGA TCC TGA GCG ACA ATA TGA - 3'
ocd	5' - CTG TGG CGG GAA CTC CAC GA - 3'
nptII-RC	5' - TAT CGC CTT CTT GAC GAG TTC - 3'
*npt*II	5' - GAA CTC GTC AAG AAG GCG ATA - 3'
nos-PR	5' - GTT CAA ATG CGC CTA AGG TCA - 3'
RB	5' - TAC CCG CCA ATA TAT CCT GTC - 3'

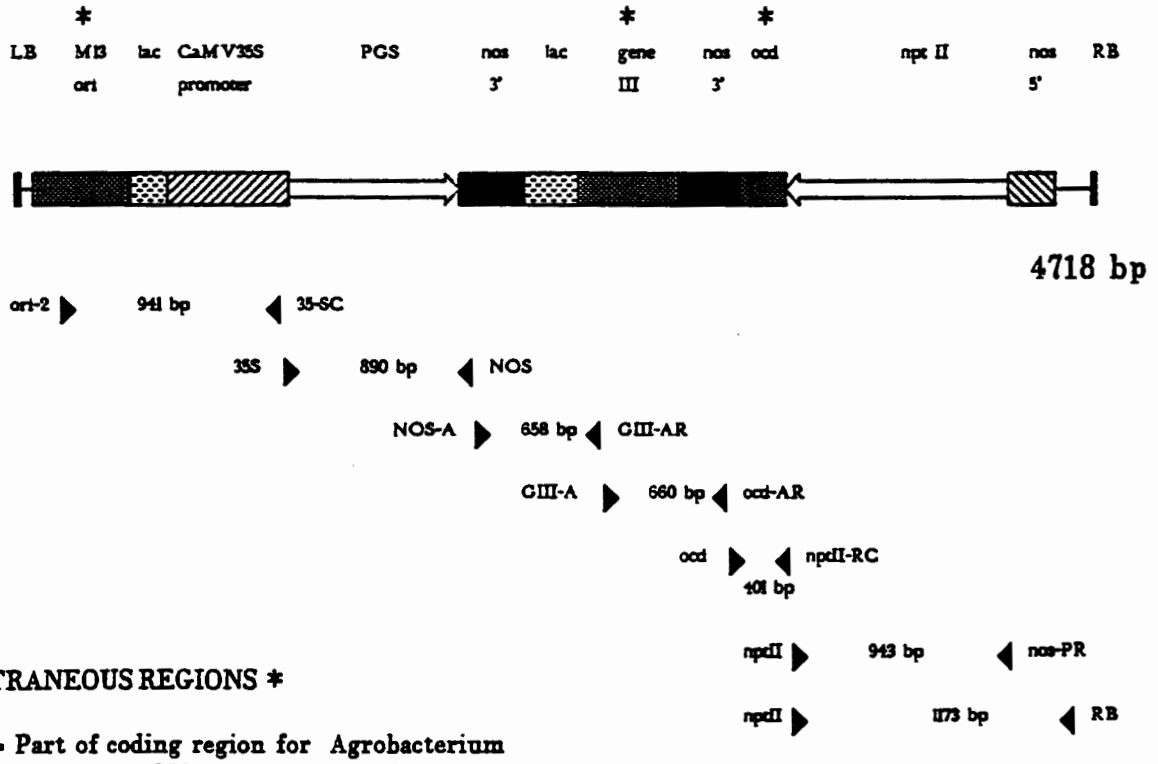
Amplification Conditions

Primer Pair	Annealing T°C	Cycling Parameters
ori-2 + 35-SC	60	Dissociation 94°C for 1.0 min.
35S + NOS	60	Annealing X°C for 0.2 min.
NOS-A + GIII-AR	64	Extension 73°C for 1.5 min.
GIII-A + ocd-AR	63	
ocd + *npt*II-RC	59	FOR 35 CYCLES
*npt*II + nos-PR	64	
*npt*II + RB	60	

Summary of Analysis of PGS Insert (pJR16A)

The presence of all regions comprising the PGA insert, except the left border region (see PGS analysis in following pages in this Appendix), has been confirmed by PCR analysis for both the inbred PGA lines B and the two hybrids H282B and Nema 1401B.

PCR ANALYSIS OF PGS INSERT (pJR16S)



EXTRANEOUS REGIONS *

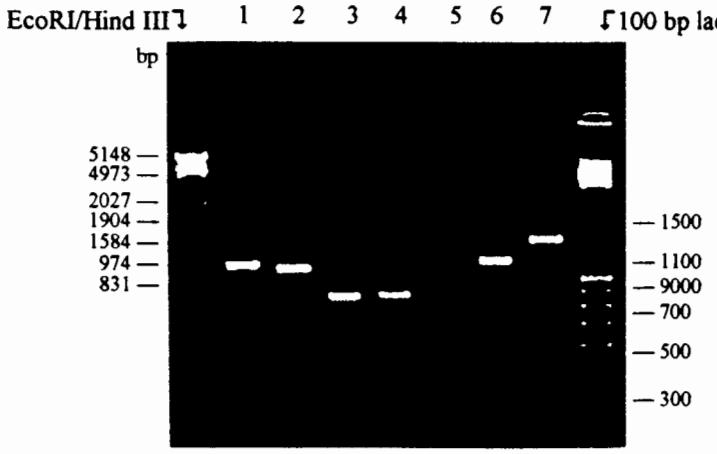
ocd = Part of coding region for Agrobacterium Ti plasmid C58 ornithine cyclodeaminase gene

gene III = Part of coding region of M13mp19 coat protein Gene III

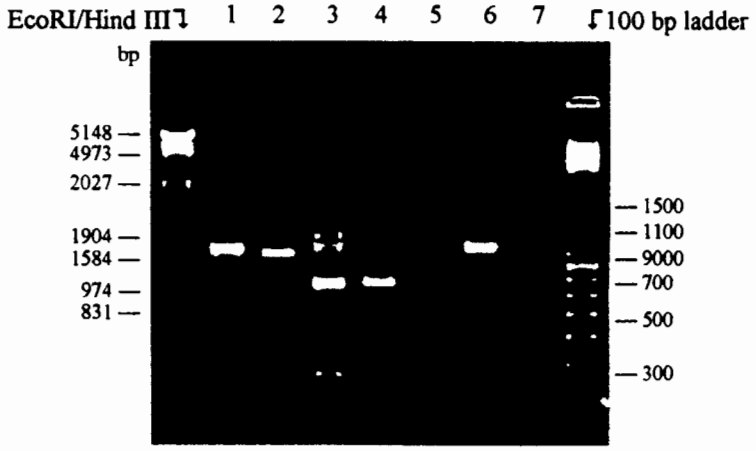
M13 ori = M13mp19 origin of replication

Zeneca Seeds
May 1994

PCR Analysis of PGS Insert (pJR16S) in Inbreds Da and F



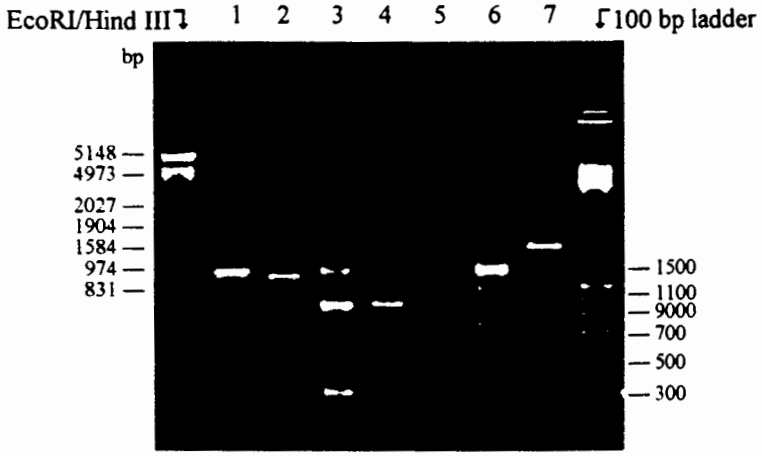
Line Da



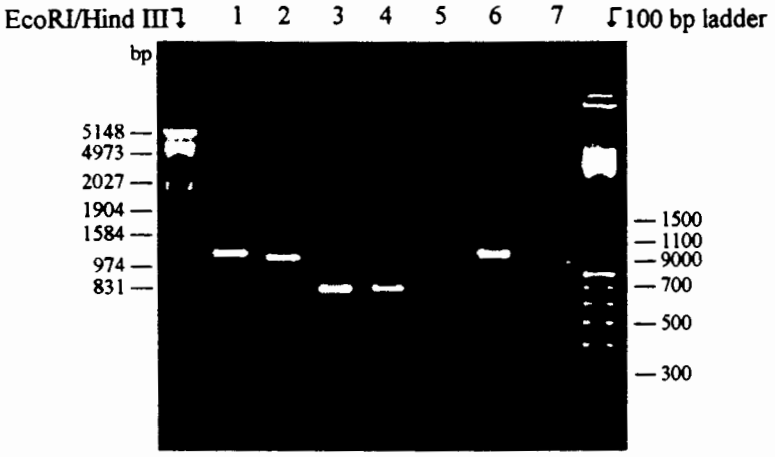
Line F

Lane	Primer Pair	PCR Product (bp)	PCR (+/-) Da	PCR (+/-) F
1	ori-2 + 35-SC	941	+	+
2	35S + NOS	890	+	+
3	nos-A + GIII-AR	658	+	+
4	GIII-A + ocd-AR	660	+	+
5	ocd + *npt*II-RC	401	+	+
6	*npt*II + nos-PR	943	+	+
7	*npt*II + RB	1173	+	-

PCR Analysis of PGS Insert (pJR16S) in Hybrids Nema 1401Da and Nema 1401F



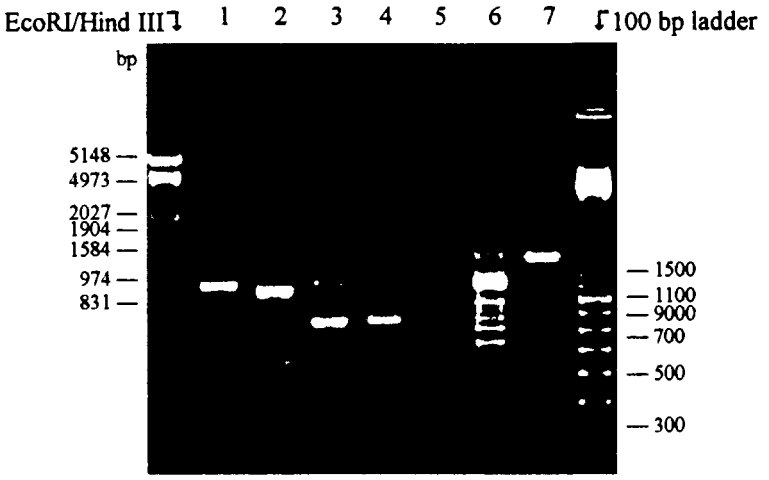
Nema 1401Da



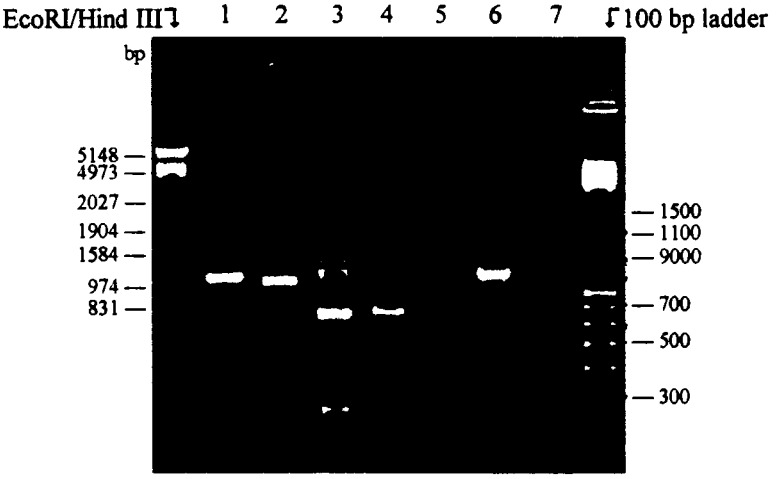
Nema 1401F

Lane	Primer Pair	PCR Product (bp)	PCR (+/-) Da	PCR (+/-) F
1	ori-2 + 35-SC	941	+	+
2	35S + NOS	890	+	+
3	nos-A + GIII-AR	658	+	+
4	GIII-A + ocd-AR	660	+	+
5	ocd + *npt*II-RC	401	+	+
6	*npt*II + nos-PR	943	+	+
7	*npt*II + RB	1173	+	-

PCR Analysis of PGS Insert (pJR16S) in Hybrids H282Da and H282F



H282Da



H282F

Lane	Primer Pair	PCR Product (bp)	PCR (+/-) Da	PCR (+/-) F
1	ori-2 + 35-SC	941	+	+
2	35S + NOS	890	+	+
3	nos-A + GIII-AR	658	+	+
4	GIII-A + ocd-AR	660	+	+
5	ocd + *npt*II-RC	401	+	+
6	*npt*II + nos-PR	943	+	+
7	*npt*II + RB	1173	+	-

PCR Analysis of PGS and PGA Insert (pJR16S, pJR16A)

PCR primers used, as follows:

Name	Sequence
35-SC	5' - GAT TGT GCG TCA TCC CTT ACG - 3'
ori-2	5' - ATC CTG TTT GAT GGT GGT TCC - 3'
35S	5' - CAA TCC CAC TAT CCT TCG C - 3'
NOS	5' - CAT CGC AAG ACC GGC AAC AG - 3'
NOS-A	5' - TGA ATC CTG TTG CCG GTC TTG - 3'
GIII-AR	5' - CTA CTG ATT ACG GTG CTG CTA - 3'
GIII-A	5' - TAG CAG CAC CGT AAT CAG TAG - 3'
ocd-AR	5' - CGA TCC TGA GCG ACA ATA TGA - 3'
ocd	5' - CTG TGG CGG GAA CTC CAC GA - 3'
nptII-RC	5' - TAT CGC CTT CTT GAC GAG TTC - 3'
*npt*II	5' - GAA CTC GTC AAG AAG GCG ATA - 3'
nos-PR	5' - GTT CAA ATG CGC CTA AGG TCA - 3'
RB	5' - TAC CCG CCA ATA TAT CCT GTC - 3'

Amplification Conditions

Primer Pair	Annealing T°C	Cycling Parameters
ori-2 + 35-SC	60	Dissociation 94°C for 1.0 min.
35S + NOS	59	Annealing X°C for 0.2 min.
NOS-A + GIII-AR	63	Extension 73°C for 1.5 min.
GIII-A + ocd-AR	62	
ocd + *npt*II-RC	59	FOR 35 CYCLES
*npt*II + nos-PR	62	
*npt*II + RB	60	

Summary of Analysis of PGS Insert (pJR16S)

a) Presence of the left border region could not be confirmed by PCR for any of the lines under analysis due to priming difficulties. Consequentially, optimization of cycling parameters was ineffective.

b) All regions of the PGS insert were PCR positive for the Da lines and the two hybrids, H282Da and Nema 1401Da. This confirms the presence of all regions comprising pJR16S, not allowing for the left border.

c) All regions of the PGS insert were not PCR positive for the F lines. Inbred line F and hybrids H282F and Nema 1401F were PCR negative for the presence of the right border region. This indicates a possible deletion 3' of the NOS promoter of the *npt*II gene. All other regions were PCR positive thus confirming their presence.

d) Despite the presence of non-specific PCR products in some of the tracks, a feature commonly associated with PCR, the specific product is always clearly visible as the more intense band.

Summary of Observations Made on Genetically Modified (line F) and Control Hybrids Nema 1200 and Nema 1401.

Characteristic	Nema 1200F	Nema 1200	Nema 1401F	Nema 1401
Seedling				
Anthocyanin in hypocotyl of 15 cm seedling	present	present	present	present
Habit of 3-4 week old seedling	normal	normal	normal	normal
Mature plant				
Height	55 cm	53.1 cm	59 cm	61 cm
Growth	indeterminate	indeterminate	indeterminate	indeterminate
Form	normal	normal	normal	normal
Size of canopy	medium	medium	medium	medium
Habit	semi-erect	semi-erect	semi-erect	semi-erect
Stem				
Branching	profuse	profuse	profuse	profuse
Branching at cotyledonary or first leafy node	present	present	present	present
No. of nodes below the first inflorescence	4-7	4-7	4-7	4-7
No. of nodes between early inflorescences	1-4	1-4	1-4	1-4
No. of nodes between later-developing inflorescences	1-4	1-4	1-4	1-4
Pubescence on younger stems	moderately hairy	moderately hairy	moderately hairy	moderately hairy
Leaf				
Type	tomato	tomato	tomato	tomato
Margins of major leaflets	nearly entire	nearly entire	nearly entire	nearly entire
Marginal rolling or wiltiness	absent	absent	absent	absent
Morphology	type 3	type 3	type 3	type 3
Surface of major leaflets	rugose	rugose	rugose	rugose
Pubescence	normal	normal	normal	normal
Inflorescence				
Type	simple	simple	simple	simple
Number of flowers	6	6	6	6
Leafy inflorescence	absent	absent	absent	absent
Flower				
Calyx	normal	normal	normal	normal
Calyx lobes	shorter than corolla	shorter than corolla	shorter than corolla	shorter than corolla
Corolla color	yellow	yellow	yellow	yellow
Style pubescence	sparse	sparse	sparse	sparse
Anthers	all fused into tube	all fused into tube	all fused into tube	all fused into tube
Fasciation	absent	absent	absent	absent

Characteristic	Nema 1200F	Nema 1200	Nema 1401F	Nema 1401
Fruit (3rd fruit of 2nd or 3rd cluster)				
Typical fruit shape	type 4	type 4	type 4	type 4
Shape of transverse section	angular	angular	angular	angular
Shape of blossom end	flat	flat	flat	flat
Shape of stem end	flat	flat	flat	flat
Shape of pistil scar	dot	dot	dot	dot
Abscission layer	absent	absent	absent	absent
Point of detachment of fruit	at pedicel	at pedicel	at pedicel	at pedicel
Length of pedicel	11.9 mm	12.1 mm	11.7 mm	11.4 mm
Length of mature fruit	56.5 mm	57.3 mm	57.5 mm	62.1 mm
Diameter of fruit	58.4 mm	56.5 mm	55.0 mm	56.5 mm
Weight of mature fruit	88 gm	84 gm	90 gm	92 gm
No. of locules	three and four	three and four	three and four	three and four
Fruit surface	smooth	smooth	smooth	smooth
Fruit base color	light green	light green	light green	light green
Fruit pattern	green shouldered	green shouldered	uniform green	uniform green
Shoulder color	dark green	dark green	dark green	dark green
Fruit color, full ripe	red	red	red	red
Flesh color, full ripe	red/crimson	red/crimson	red/crimson	red/crimson
Flesh color	uniform	uniform	uniform	uniform
Locular gel color	red	red	red	red
Ripening	uniform	uniform	uniform	uniform
Ripening	uniformly	uniformly	uniformly	uniformly
Epidermis color	yellow	yellow	yellow	yellow
Epidermis	normal	normal	normal	normal
Epidermis texture	tough	tough	tough	tough
Thickness of pericarp	6-9 mm	6-9 mm	6-9 mm	6-9 mm
Stem scar size	small	small	small	small
Core	coreless	coreless	coreless	coreless
Resistance to fruit disorder	not assessed	not assessed	not assessed	not assessed
Disease and pest reaction				
Fusarium wilt, Race 1	resistant	resistant	resistant	resistant
Fusarium wilt, Race 2	resistant	resistant	resistant	resistant
Verticillium wilt, Race 1	resistant	resistant	resistant	resistant
Alternaria Stem Canker	resistant	resistant	resistant	resistant
Southern root knot nematode	resistant	resistant	resistant	resistant
Chemistry and composition of full-ripe fruit				
Juice Bostwick cold break	21.5	27.9	15.1	17.2
Brix (raw puree)	5.4	5.7	5.4	5.6
pH (raw puree)	4.3	4.3	4.2	4.3
Agtron color (raw puree)	25.7	26.3	25.8	26.9

Characteristic	Nema 1200F	Nema 1200	Nema 1401F	Nema 1401
Phenology				
Fruiting season	very concentrated	very concentrated	very concentrated	very concentrated
Relative maturity in areas tested	early	early	medium	medium
Adaptation				
Culture	field	field	field	field
Principal use	concentrated products	concentrated products	whole pack canning; concentrated products	whole pack canning; concentrated products
Machine harvest	adapted	adapted	adapted	adapted
Regions to which adaptation has been demonstrated	All regions in California	All regions in California	All regions in California	All regions in California

Hunt - Peto - Zeneca
TG Tomato Field Trial Data Form

Year 1993 APHIS Permit # _____

Location WOODLAND, CA Evaluator _____

NEMA 1401 Check

Choose responses for the following characters which best fit your variety. Complete this form as fully as possible for best characterization of the variety. When a single quantitative value is requested (e.g., fruit weight), your answer should be the mean of an adequate-sized, unbiased sample of plants. Use leading zeroes when necessary (e.g., [0 1 9] or [0 1 3 1 1], etc.). The applicant variety should be compared with at least one well-known standard check variety of the same type (see list of recommended check varieties below), and grown in the same trials. The characters on this form should be described from plants grown under normal conditions of culture for the variety. Indicate by a check whether trial data are from greenhouse _____ or field _____ planting. Trials direct-seeded _____ or transplanted X ; raised _____ or unraised _____. Give locations and dates of seeding and transplanting here: _____

Seeded 4-2-93 Transplanted 4-29-93

COMPARISONS SHOULD BE MADE TO ONE OR MORE CHECK VARIETIES IN THE FOLLOWING LIST. IF AT ALL POSSIBLE, ENTER THE NUMBER OF THE CHECK IN BOXES WHERE IDENTITY OF CHECK IS REQUESTED.

1 = Ace 55 VF	7 = Homestead 24	13 = Red Rock	19 = VF 134
2 = Campbell 27	8 = Marglobe	14 = Roma VF	20 = US 82
3 = Chico III	9 = Muneta	15 = Rutgers	21 = VF 145 B 7879
4 = Flora Dade	10 = New Yorker	16 = Sunray	22 = Other (Specify) Nema 1401
5 = Florida MH-1	11 = Ohio MR-13	17 = Tropic	
6 = Heinz 1350	12 = Red Cherry Large	18 = UC 82	

1. SEEDLING:

[2] Anthocyanin in hypocotyl of 2-15 cm. seedling: 1 = Absent 2 = Present [1] Habit of 3-4 week old seedling: 1 = Normal 2 = Compact

2. MATURE PLANT (at maximum vegetative development):

[0 1 6 1] Cm. Height

[1] Growth: 1 = Indeterminate 2 = Determinate

[2] Form: 1 = Lax, open 2 = Normal 3 = Compact 4 = Dwarf 5 = Bracteate

[2] Size of canopy (compared to others of similar type): 1 = Small 2 = Medium 3 = Large

[2] Habit: 1 = Sprawling (decumbent) 2 = Semi-erect 3 = Erect ("Dwarf Champion")

STEM:

[3] Branching: 1 = Scarce ("Grenm's Solid Red", "Precail") 2 = Intermediate ("Nestover") 3 = Profuse ("UC 82")

[1] Branching at cotyledonary or first leafy node: 1 = Present 2 = Absent

[2] No. of nodes below the first inflorescence: 1 = 1-4 2 = 4-7 3 = 7-10 4 = 10 or more

[1] No. of nodes between early (1st - 2nd, 2nd - 3rd) inflorescences. [1] No. of nodes between later-developing inflorescences.

[3] Pubescence on younger stems: 1 = Smooth (no long hairs) 2 = Sparsely hairy (scattered long hairs) 3 = Moderately hairy 4 = Densely hairy or woolly

LEAF (mature leaf beneath the 3rd inflorescence):

[1] Type: 1 = Tomato 2 = Potato ("Trip-L-Crop") [3] Morphology (choose illustration on pg. 5 of this form that is most similar)

[1] Margins of major leaflets: 1 = Nearly entire 2 = Shallowly toothed or scalloped 3 = Deeply toothed or cut, esp. towards base

[1] Marginal rolling or wilting: 1 = Absent 2 = Slight 3 = Moderate 4 = Strong

[ ] Onset of leaflet rolling: 1 = Early-season 2 = Mid-season 3 = Late season

4. LEAF (mature leaf beneath the 3rd inflorescence – continued):

[2] Surface of major leaflets: 1 = Smooth 2 = Rugose (bumpy or veiny)

[2] Pubescence: 1 = Smooth (no long hairs) 2 = Normal 3 = Hirsute 4 = Woolly

5. INFLORESCENCE (make observations on 3rd inflorescence):

[1] Type: 1 = Simple 2 = Forked (2 major axes) 3 = Compound (much branched)

[0][6] Number of flowers in inflorescence, average

[1] Leafy or "running" inflorescences: 1 = Absent 2 = Occasional 3 = Frequent

6. FLOWER:

[1] Calyx: 1 = Normal, lobes awl-shaped 2 = Macrocalyx, lobes large, leaflike 3 = Fleshy

[1] Calyx-lobes: 1 = Shorter than corolla 2 = Approx. equalling corolla 3 = Distinctly longer than corolla

[1] Corolla color: 1 = Yellow 2 = Cla gold 3 = White or tan

[2] Style pubescence: 1 = Absent 2 = Sparse 3 = Dense

[1] Anthers: 1 = All fused into tube 2 = Separating into 2 or more groups at anthesis

[1] Fasciation (1st flower of 2nd or 3rd inflorescence): 1 = Absent 2 = Occasionally present 3 = Frequently present

7. FRUIT (3rd fruit of 2nd or 3rd cluster): *For the first 5 characters below, match your variety with the most similar illustration on pg. 5 of this form.*

[4] Typical fruit shape:

[3] Shape of transverse section:

[2] Shape of blossom end:

[1] Shape of stem end:

[1] Shape of distal scar:

---

[2] Abscission layer: 1 = Present (pedicellate) 2 = Absent (jointless)

[1] Point of detachment of fruit at harvest: 1 = At pedicel joint 2 = At calyx attachment

[1][1] mm length of pedicel (from joint to calyx attachment) (11.4)

[0][6][2] mm length of mature fruit (stem axis) (62.1).

[2][5][7] mm diameter of fruit at widest point (56.5).

[0][9][2] g weight of mature fruit

[ ][ ][ ] mm length, check var. no. [2][2]

[ ][ ][ ] mm diameter, check var. no. [2][2]

[ ][ ][ ] g weight, check var. no. [2][2]

[2] No. of locules: 1 = Two 2 = Three and four 3 = Five or more

[1] Fruit surface: 1 = Smooth 2 = Slightly rough 3 = Moderately rough or ribbed

[1] Fruit base color (mature-green stage): 1 = Light green ("Lanai", "VF145-F5") 2 = Light gray-green ("Westover") 3 = Apple or medium green ("Heinz 1439 VF") 4 = Yellow green 5 = Dark green

[1] Fruit pattern (mature-green stage): 1 = Uniform green 2 = Green-shouldered 3 = Radial stripes on sides of fruit

[1] Shoulder color if different from base: 1 = Dark green 2 = Gray green 3 = Yellow green

[5] Fruit color, full-ripe: 1 = White 2 = Yellow 3 = Orange 4 = Pink 5 = Red 6 = Brownish 7 = Greenish 8 = Other (Specify) _____

[3] Flesh color, full-ripe: 1 = Yellow 2 = Pink 3 = Red/Crimson 4 = Orange 5 = Other (Specify) _____

[1] Flesh color: 1 = Uniform 2 = With lighter and darker areas in walls

[3] Locular gel color of table-ripe fruit: 1 = Green 2 = Yellow 3 = Red

[2] Ripening: 1 = Blossom-to-stem end 2 = Uniform

7. FRUIT (3rd fruit of 2nd or 3rd cluster): Continued

[2] Ripening: 1 = Inside out 2 = Uniformity 3 = Outside in

[2] Epidermis color: 1 = Colorless 2 = Yellow

[1] Epidermis: 1 = Normal 2 = Easy-peel

[3] Epidermis texture: 1 = Tender 2 = Average 3 = Tough

[3] Thickness of pericarp . . . . . [3] Thickness of pericarp, check var. no. [2 2]

1 = Under 3 mm 2 = 3-6 mm 3 = 6-9 mm 4 = Over 9 mm

[1] Stem scar size: 1 = Small ("Roma") 2 = Medium ("Rutgers") 3 = Large

[1] Core: 1 = Coreless (absent or smaller than 6x6 mm) 2 = Present

8. RESISTANCE TO FRUIT DISORDERS (Use code: 0 = Unknown, 1 = Susceptible, 2 = Resistant)

[ ] Blossom end rot
[ ] Blotchy ripening
[ ] Bursting
[ ] Catface
[ ] Cracking, concentric
[ ] Cracking, radial
[ ] Fruit pox
[ ] Gold fleck
[ ] Graywall
[ ] Zippering
[ ] Other (Specify) _____

9. DISEASE AND PEST REACTION (Use code: 0 = Not tested, 1 = Susceptible, 2 = Resistant). NOTE: If claim of novelty is based wholly or in substantial part upon disease resistance, trial data should be appended. These should specify the method of testing, the reaction of the application variety, and reaction of well-known check varieties grown in the trial (identified by name).

VIRAL DISEASES:

[ ] Cucumber mosaic
[ ] Curly top
[ ] Potato-Y virus
[ ] Other virus (Specify) _____
[ ] Tobacco mosaic, Race 0
[ ] Tobacco mosaic, Race 1
[ ] Tobacco mosaic, Race 2
[ ] Tomato spotted wilt
[ ] Tomato yellows

BACTERIAL DISEASES:

[ ] Bacterial canker (*Corynebacterium michiganense*)
[ ] Bacterial soft rot (*Erwinia carotovora*)
[ ] Bacterial speck (*Pseudomonas tomato*)
[ ] Bacterial spot (*Xanthomonas vesicatorium*)
[ ] Bacterial wilt, (*Pseudomonas solanacearum*)
[ ] Other bacterial disease (Specify) _____

FUNGAL DISEASES:

[ ] Anthracnose (*Colletotrichum* sp.)
[ ] Brown root rot or corky root, (*Pyrenochaeta lycopersici*)
[ ] Collar rot or stem canker, (*Alternaria solani*)
[ ] Early blight defoliation, (*Alternaria solani*)
[2] Fusarium wilt, Race 1, (*F. oxysporum f. lycopersici*)
[2] Fusarium wilt, Race 2
[ ] Fusarium wilt, Race 3
[ ] Gray leaf spot (*Stemphylium* sp.)
[ ] Late blight, Race 0, (*Phytophthora infestans*)
[ ] Late blight, Race 1
[ ] Leaf mold, Race 1 (*Cladosporium fulvum*)
[ ] Leaf mold, Race 2
[ ] Leaf mold, Race 3
[ ] Leaf mold, other races (Specify) _____
[ ] Nailhead spot (*Alternaria tomato*)
[ ] Septoria leafspot (*S. lycopersici*)
[ ] Target leafspot (*Corynespora cassiicola*)
[2] Verticillium wilt, Race 1 (*V. albo-atrum*)
[ ] Verticillium wilt, Race 2
[2] Other fungal disease Alternaria Stem Canker
[ ] Other fungal disease _____

INSECTS AND PESTS:

[ ]	Colorado potato beetle (*Leptinotarsa decemlineata*)	[ ]	Tomato hornworm (*Manduca quinquemaculata*)
[2]	Southern root knot nematode (*Meloidogyne incognita*)	[ ]	Tomato fruitworm (*Heliothis zea*)
[ ]	Spider mites (*Tetranychus* spp.)	[ ]	Whitefly (*Trialeurodes vaporariorum*)
[ ]	Sugar beet army worm (*Spodoptera exigua*)	[ ]	Other (Specify) _____
[ ]	Tobacco flea beetle (*Epitrix hirtipennis*)		_____

POLLUTANTS:

[ ]	Ozone	[ ]	Sulfur dioxide
[ ]	Other (Specify) _____		

10. CHEMISTRY AND COMPOSITION OF FULL-RIPE FRUITS: Suggested test methods may be found in "Tomato Products," 3rd ed., National Canners Assn. Bull. 27-L. Please specify test methods or give a reference to methods used. Fill in table below with values for the new variety and for at least one well-known check variety or similar type grown in the same trial. Specify names or numbers of check varieties.

	SUBMITTED VARIETY	Check Variety _____	Check Variety _____	Check Variety _____
	Nema 1401			
JUICE BOSTWICK COLD BREAK	17.2			
BRIX (raw puree)	5.6°			
PH (raw puree)	4.3			
AGTRON COLOR (raw puree)	26.9			

11. PHENOLOGY: Express length of developmental stages either as calendar days or as heat units (growing degree days), in degrees Celsius. If heat units are used, indicate the base temperature used in their calculation here _____°C. See paper by Warnock under "References" for method. Give comparative data for at least one check variety; identify checks by name or by number from table on page 1.

	APPLICATION VARIETY	Check variety _____	Check variety _____	Check variety _____
...aging to 50% flower (1 open flower on 50% of plants)				
...to one-over harvest (if applicable)				

[4] Ripening season: 1 = Long ("Marglobe") 2 = Medium ("Nemador") 3 = Short, concentrated ("VF 145") 4 = Very concentrated ("UC 82")

[3] Relative maturity in areas tested: 1 = Early 2 = Medium early 3 = Medium 4 = Medium late 5 = Late 6 = Variable (If relative maturity is known to differ by location or environment, please explain on separate sheet.)

12. ADAPTATION: If more than one category applies, list all in rank order.

[1] Culture: 1 = Field 2 = Greenhouse

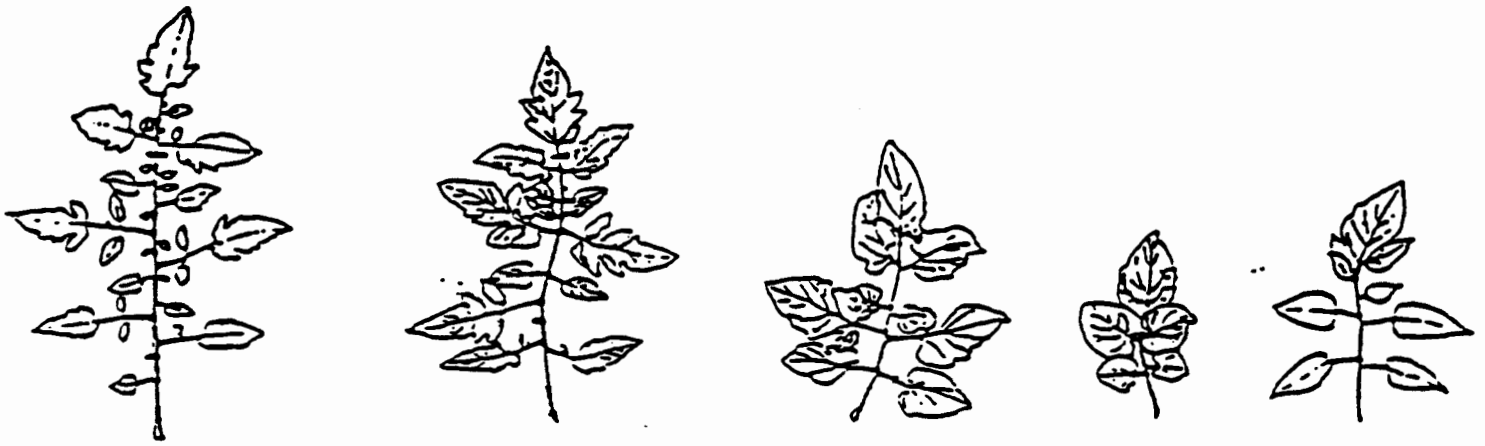
[3 4] Principal use(s): 1 = Home garden 2 = Fresh market 3 = Whole-pack canning 4 = Concentrated products 5 = Other (Specify) _____

[2] Machine harvest: 1 = Not adapted 2 = Adapted

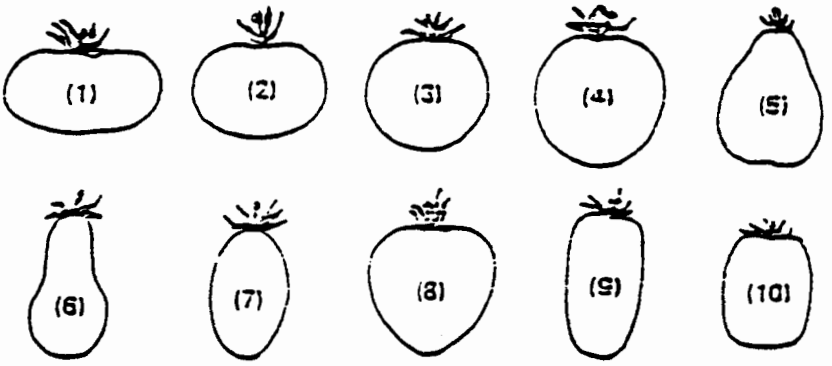
[9 10 11] Regions to which adaptation has been demonstrated:
1 = Northeast 2 = Mid Atlantic 3 = Southeast 4 = Florida
5 = Great Plains 6 = South-central 7 = Intermountain West 8 = Northwest
9 = California: Sacramento and Upper San Joaquin Valley
10 = California: Coastal areas 11 = California: Southern San Joaquin Valley & desert

ILLUSTRATIONS OF TOMATO LEAF AND FRUIT CHARACTERISTICS

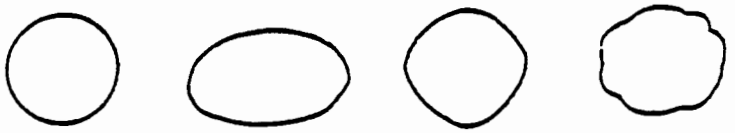
4. LEAF: Morphology:



7. FRUIT: Typical fruit shape:

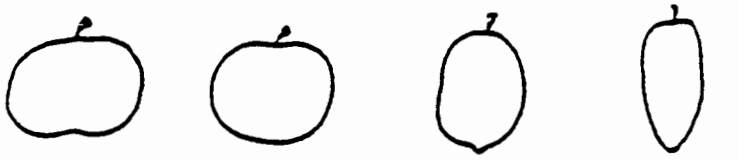


Shape of transverse section:

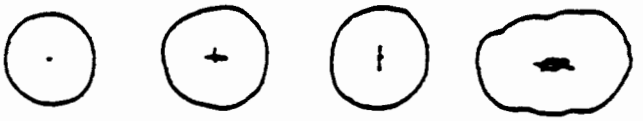


Shape of stem end:

Shape of blossom end:



Shape of pistil scar:



REFERENCES

Anonymous, 1976. All About Tomatoes. Ortho Books, Chevron Chemical Co., San Francisco. In three volumes: Midwest/Northeast Edition, West Edition, and South Edition

Ware, G.W. & J. P. McCollum, 1968. Producing Vegetable Crops. The Interstate Printer & Publishers, Inc., Danville, Illinois. Chapter 30, pp. 451-472. "Tomatoes".

Warnock, S.J. 1978. Using Tomato Heat Units. Leaflet No. 5, Campbell Institute for Agricultural Research, Camden, N.J. 10 p.

Webb, R.E., T. H. Bartsdale, & A. K. Stoner, 1973. "Tomatoes", pp. 344-361. In: Nelson, R.R. (Ed.), Breeding Plants for Disease Resistance. Pennsylvania State University Press, University Park.

Young, P.A. & J.W. MacArthur, 1947. Horticultural characters of tomatoes. Bull. Texas Agric. Exper. Station No. 698.

Hunt - Peto - Zeneca
TG Tomato Field Trial Data Form

Year 1993 APHIS Permit # _____

Location WOODLAND, CA Evaluator _____

NEMA 1401 PGS

Choose responses for the following characters which best fit your variety. Complete this form as fully as possible for best characterization of the variety. When a single quantitative value is requested (e.g., fruit weight), your answer should be the mean of an adequate-sized, unbiased sample of plants. Use leading zeros when necessary (e.g., 0 1 9 or 0 1 3 1 1, etc.). The applicant variety should be compared with at least one well-known standard check variety of the same type (see list of recommended check varieties below), and grown in the same trial. The characters on this form should be described from plants grown under normal conditions of culture for the variety. Indicate by a check whether trial data are from greenhouse _____ or field _____ plantings; trial direct-seeded _____ or transplanted _____; staked _____ or unstaked _____. Give locations and dates of seeding and transplanting here: _____

Seeded 4-2-93 Transplanted 4-29-93

COMPARISONS SHOULD BE MADE TO ONE OR MORE CHECK VARIETIES IN THE FOLLOWING LIST, IF AT ALL POSSIBLE. ENTER THE NUMBER OF THE CHECK IN BOXES WHERE IDENTITY OF CHECK IS REQUESTED.

1 = Ace 55 VF	7 = Homestead 24	13 = Red Rock	19 = VF 134
2 = Cumberland 37	8 = Marglobe	14 = Roma VF	20 = US 82
3 = Ohio 7111	9 = Muneta	15 = Rutgers	21 = VF 145 B 7879
4 = Flora Dade	10 = New Yorker	16 = Sunray	22 = Other (Specify) Nema 1401
5 = Florida MH-1	11 = Ohio MR-13	17 = Tropic	
6 = Heinz 1350	12 = Red Cherry Large	18 = UC 82	

SEEDLING:

2 Anthocyanin in hypocotyl at 2-15 cm. seedling: 1 = Absent 2 = Present

1 Habit of 3-4 week old seedling: 1 = Normal 2 = Compact

MATURE PLANT (at maximum vegetative development):

0 5 9 Cm. Height

1 Growth: 1 = Indeterminate 2 = Determinate

2 Form: 1 = Lax, open 2 = Normal 3 = Compact 4 = Dwarf 5 = Erect-type

2 Size of canopy (compared to others of similar type): 1 = Small 2 = Medium 3 = Large

2 Habit: 1 = Sprawling (decumbent) 2 = Semi-erect 3 = Erect ("Dwarf Champion")

STEM:

3 Branching: 1 = Sparse ("Bremm's Solid Red", "Precoce") 2 = Intermediate ("Rearover") 3 = Profuse ("UC 82")

1 Branching at cotyledonary or first leafy node: 1 = Present 2 = Absent

2 No. of nodes below the first inflorescence: 1 = 1-4 2 = 4-7 3 = 7-10 4 = 10 or more

1 No. of nodes between early (1st - 2nd, 2nd - 3rd) inflorescences.

1 No. of nodes between later-developing inflorescences.

3 Pubescence on younger stems: 1 = Smooth (no long hairs) 2 = Sparsely hairy (scattered long hairs) 3 = Moderately hairy 4 = Densely hairy or woolly

LEAF (mature leaf beneath the 2nd inflorescence):

1 Type: 1 = Tomato 2 = Potato ("Trip-L-Crop")

3 Morphology (choose illustration on pg. 5 of this form that is most similar)

1 Margins of major leaflets: 1 = Nearly entire 2 = Shallowly toothed or scalloped 3 = Deeply toothed or cut, esp. towards base

1 Marginal rolling or wilting: 1 = Absent 2 = Slight 3 = Moderate 4 = Strong

_ Onset of leaflet rolling: 1 = Early-season 2 = Mid-season 3 = Late season

LEAF (mature leaf beneath the 3rd inflorescence – continued):

[2] Surface of major leaflets: 1 = Smooth 2 = Rugose (bumpy or veiny)

[2] Pubescence: 1 = Smooth (no long hairs) 2 = Normal 3 = Hirsute 4 = Woolly

5. INFLORESCENCE (make observations on 3rd inflorescence):

[1] Type: 1 = Simple 2 = Forked (2 major axes) 3 = Compound (much branched)

[0|6] Number of flowers in inflorescence, average

[1] Leafy or "running" inflorescences: 1 = Absent 2 = Occasional 3 = Frequent

6. FLOWER:

[1] Calyx: 1 = Normal, lobes awl-shaped 2 = Macrocalyx, lobes large, leaflike 3 = Fleshy

[1] Calyx-lobes: 1 = Shorter than corolla 2 = Approx. equalling corolla 3 = Distinctly longer than corolla

[1] Corolla color: 1 = Yellow 2 = Old gold 3 = White or tan

[2] Style pubescence: 1 = Absent 2 = Sparse 3 = Dense

[1] Anthers: 1 = All fused into tube 2 = Separating into 2 or more groups at anthesis

[1] Fasciation (1st flower of 2nd or 3rd inflorescence): 1 = Absent 2 = Occasionally present 3 = Frequently present

7. FRUIT (3rd fruit of 2nd or 3rd cluster): *For the first 5 characters below, match your variety with the most similar illustration on pg. 5 of this form.*

[4] Typical fruit shape:

[3] Shape of transverse section:

[2] Shape of blossom end:

[1] Shape of stem end:

[1] Shape of distal scar:

---

[2] Abscission layer: 1 = Present (pedicellate) 2 = Absent (jointless)

[1] Point of detachment of fruit at harvest: 1 = At pedicel joint 2 = At calyx attachment

[1|2] mm length of pedicel (from joint to calyx attachment) (11.7)

[0|5|8] (57.5)	mm length of mature fruit (stem axis)	[0|6|2] (62.1)	mm length, check var. no. [2|2]
[0|5|5]	mm diameter of fruit at widest point	[0|5|7] (56.5)	mm diameter, check var. no. [2|2]
[0|9|0]	g weight of mature fruit	[0|9|2]	g weight, check var. no. [2|2]

[2] No. of locules: 1 = Two 2 = Three and four 3 = Five or more

[1] Fruit surface: 1 = Smooth 2 = Slightly rough 3 = Moderately rough or ribbed

[1] Fruit base color (mature-green stage): 1 = Light green ("Lanai", "VF145-F5") 2 = Light gray-green ("Westover") 3 = Apple or medium green ("Heinz 1439 VF") 4 = Yellow green 5 = Dark green

[1] Fruit pattern (mature-green stage): 1 = Uniform green 2 = Green-shouldered 3 = Radial stripes on sides of fruit

[1] Shoulder color if different from base: 1 = Dark green 2 = Gray green 3 = Yellow green

[5] Fruit color, full-ripe: 1 = White 2 = Yellow 3 = Orange 4 = Pink 5 = Red 6 = Brownish 7 = Greenish 8 = Other (Specify) _____

[3] Flesh color, full-ripe: 1 = Yellow 2 = Pink 3 = Red/Crimson 4 = Orange 5 = Other (Specify) _____

[1] Flesh color: 1 = Uniform 2 = With lighter and darker areas in walls

[3] Locular gel color of table-ripe fruit: 1 = Green 2 = Yellow 3 = Red

[2] Ripening: 1 = Blossom-to-stem end 2 = Uniform

7. FRUIT (3rd fruit of 2nd or 3rd cluster): Continued

[2] Ripening: 1 = Inside out 2 = Uniformly 3 = Outside in

[2] Epidermis color: 1 = Colorless 2 = Yellow

[1] Epidermis: 1 = Normal 2 = Easy-peel

[3] Epidermis texture: 1 = Tender 2 = Average 3 = Tough

[3] Thickness of pericarp . . . . . [3]

1 = Under 3 mm 2 = 3-6 mm 3 = 6-9 mm 4 = Over 9 mm

[1] Stem scar size: 1 = Small ('Roma') 2 = Medium ('Rutgers') 3 = Large

[1] Core: 1 = Coreless (absent or smaller than 6x6 mm) 2 = Present

Thickness of pericarp, check var. no. [2 2]

8. RESISTANCE TO FRUIT DISORDERS (Use code: 0 = Unknown, 1 = Susceptible, 2 = Resistant)

- [ ] Blossom end rot
- [ ] Blotchy ripening
- [ ] Bursting
- [ ] Catface
- [ ] Cracking, concentric
- [ ] Cracking, radial
- [ ] Fruit pox
- [ ] Gold fleck
- [ ] Graywall
- [ ] Zippering
- [ ] Other (Specify) _____

9. DISEASE AND PEST REACTION (Use code: 0 = Not tested, 1 = Susceptible, 2 = Resistant). NOTE: If claim of novelty is based wholly or in substantial part upon disease resistance, trial data should be appended. These should specify the method of testing, the reaction of the application variety, and reaction of well-known check varieties grown in the trial (identified by name).

VIRAL DISEASES:

- [ ] Cucumber mosaic
- [ ] Curly top
- [ ] Potato-Y virus
- [ ] Other virus (Specify) _____
- [ ] Tobacco mosaic, Race 0
- [ ] Tobacco mosaic, Race 1
- [ ] Tobacco mosaic, Race 2
- [ ] Tobacco mosaic, Race 2[2]
- [ ] Tomato spotted wilt
- [ ] Tomato yellows

BACTERIAL DISEASES:

- [ ] Bacterial canker (*Corynebacterium michiganense*)
- [ ] Bacterial soft rot (*Erwinia carotovora*)
- [ ] Bacterial speck (*Pseudomonas tomato*)
- [ ] Bacterial spot (*Xanthomonas vesicatorium*)
- [ ] Bacterial wilt, (*Pseudomonas solanacearum*)
- [ ] Other bacterial disease (Specify) _____

FUNGAL DISEASES:

- [ ] Anthracnose (*Colletotrichum sp.*)
- [ ] Brown root rot or corky root, (*Pyrenochaeta lycopersici*)
- [ ] Collar rot or stem canker, (*Alternaria solani*)
- [ ] Early blight defoliation, (*Alternaria solani*)
- [2] Fusarium wilt, Race 1, (*F. oxysporum f. lycopersici*)
- [2] Fusarium wilt, Race 2
- [ ] Fusarium wilt, Race 3
- [ ] Gray leaf spot (*Stemphylium sp.*)
- [ ] Late blight, Race 0, (*Phytophthora infestans*)
- [ ] Late blight, Race 1
- [ ] Leaf mold, Race 1 (*Cacosporium fulvum*)
- [ ] Leaf mold, Race 2
- [ ] Leaf mold, Race 3
- [ ] Leaf mold, other races (Specify) _____
- [ ] Nailhead spot (*Alternaria tomato*)
- [ ] Septoria leafspot (*S. lycopersici*)
- [ ] Target leafspot (*Corynespora cassiicola*)
- [2] Verticillium wilt, Race 1 (*V. albo-atrum*)
- [ ] Verticillium wilt, Race 2
- [2] Other fungal disease Alternaria Stem Canker
- [ ] Other fungal disease _____

INSECTS AND PESTS:

[ ] Colorado potato beetle (*Leptinotarsa decemlineata*)
[2] Southern root knot nematode (*Meloidogyne incognita*)
[ ] Spider mites (*Tetranychus* spp.)
[ ] Sugar beet army worm (*Spodoptera exigua*)
[ ] Tobacco flea beetle (*Epitrix hirtipennis*)
[ ] Tomato hornworm (*Manduca quinquemaculata*)
[ ] Tomato fruitworm (*Heliothis zea*)
[ ] Whitefly (*Trialeurodes vaporariorum*)
[ ] Other (Specify) _____

POLLUTANTS:

[ ] Ozone
[ ] Sulfur dioxide
[ ] Other (Specify) _____

10. CHEMISTRY AND COMPOSITION OF FULL-RIPE FRUITS: Suggested test methods may be found in "Tomato Products" 3rd ed., National Canners Assn. Bull. 31-L. Please specify test methods or give a reference to methods used. Fill in table below with values for the new variety and for at least one well-known check variety or similar type grown in the same trial. Specify names or numbers of check varieties.

	SUBMITTED VARIETY	Check Variety Nema 1401 nontransformed	Check Variety	Check Variety
JUICE BOSTWICK COLD BREAK	15.1	17.2		
BRIX (raw puree)	5.4°	5.6°		
PH (raw puree)	4.2	4.3		
AGTRON COLOR (raw puree)	25.8	26.9		

11. PHENOLOGY: Express origin of developmental stages either as calendar days or as heat units (growing degree days), in degrees Celsius. If heat units are used, indicate the base temperature used in their calculation here _____°C. See Baker or Warnock under "References" for method. Give comparative data for at least one check variety; identify checks by name or by number from table on page 1.

	APPLICATION VARIETY	Check Variety	Check Variety	Check Variety
Going to 50% flower (1 open flower on 50% of plants)				
Ready to cut-over harvest (if applicable)				

[4] Ripening season: 1 = Long ("Marglobe") 2 = Medium ("Westover") 3 = Short, concentrated ("VF 145") 4 = Very concentrated ("UC 82")

[3] Relative maturity in areas tested: 1 = Early 2 = Medium early 3 = Medium 4 = Medium late 5 = Late 6 = Variable (If relative maturity is known to differ by location or environment, please explain on separate sheet.)

12. ADAPTATION: If more than one category applies, list all in rank order.

[1] Culture: 1 = Field 2 = Greenhouse

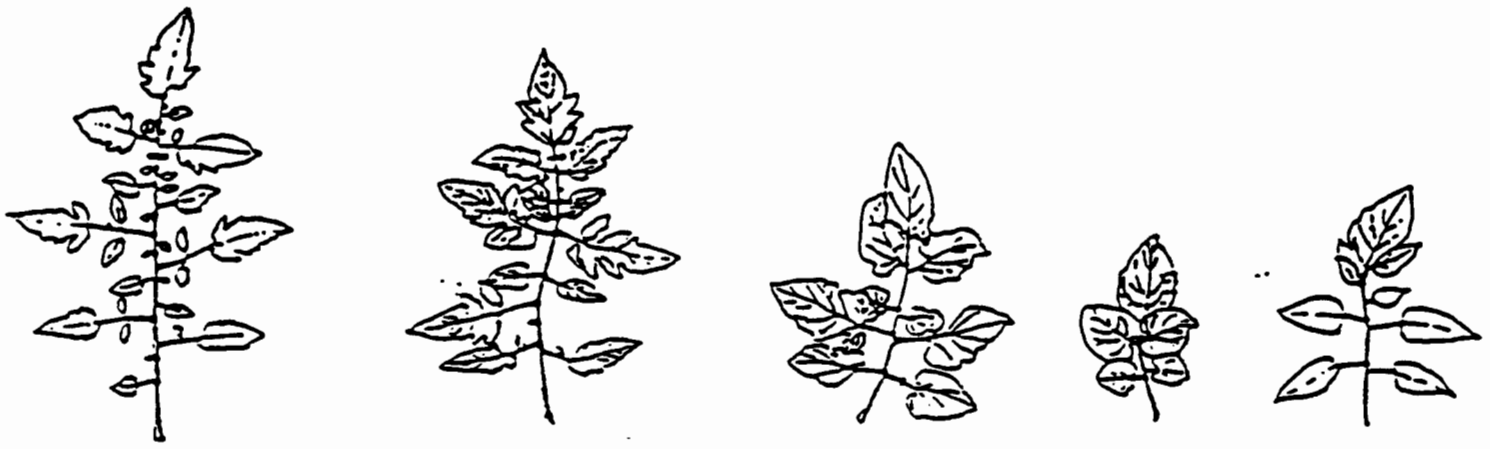
[3 4] Principal use(s): 1 = Home garden 2 = Fresh market 3 = Whole-pack canning 4 = Concentrated products 5 = Other (Specify) _____

[2] Machine harvest: 1 = Not adapted 2 = Adapted

[9 10 11] Regions to which adaptation has been demonstrated:
1 = Northeast 2 = Mid Atlantic 3 = Southeast 4 = Florida
5 = Great Plains 6 = South-central 7 = Intermountain West 8 = Northwest
9 = California: Sacramento and Upper San Joaquin Valley
10 = California: Coastal areas 11 = California: Southern San Joaquin Valley & deserts

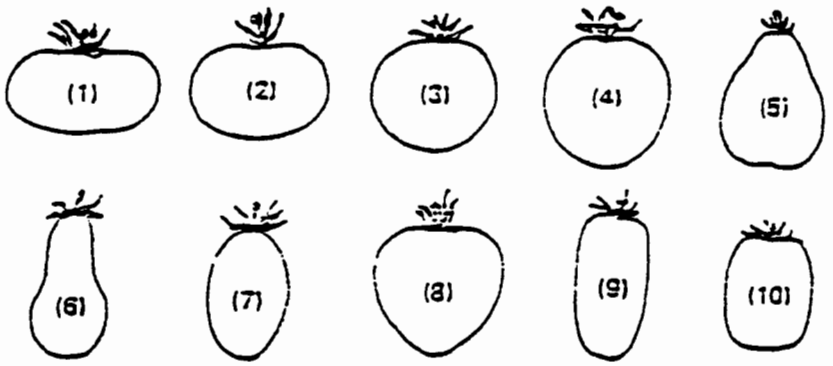
ILLUSTRATIONS OF TOMATO LEAF AND FRUIT CHARACTERISTICS

4. LEAF: Morphology:

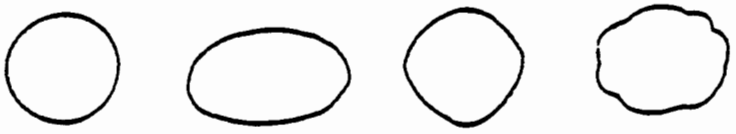


(1) (2) (3) (4) (5)

7. FRUIT: Typical fruit shape:



Shape of transverse section:

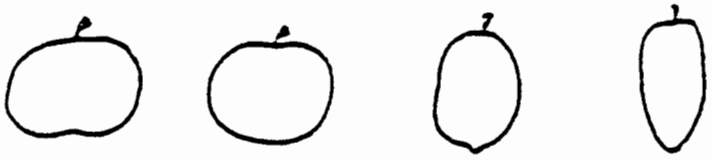


1=round 2=flattened 3=angular 4=irregular

Shape of stem end:

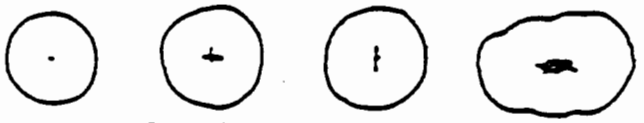
1=flat 2=indented

Shape of blossom end:



1=indented 2=flat 3=nipple 4=tapered

Shape of pistil scar:



1=dot 2=stellate 3=linear 4=irregular

REFERENCES

Anonymous, 1976. All About Tomatoes. Orbis Books, Chevron Chemical Co., San Francisco. In three volumes: Midwest/Northeast Edition, West Edition, and South Edition

Ware, G.W. & J. P. McCollum, 1968. Producing Vegetable Crops. The Interstate Printer & Publishers, Inc., Danville, Illinois. Chapter 20, pp. 451-473. "Tomatoes".

Warnock, S.J. 1978. Using Tomato Heat Units. Leaflet No. 5, Campbell Institute for Agricultural Research, Camden, N.J. 10 p.

Webb, R.E., T. H. Barksdale, & A. K. Stoner, 1973. "Tomatoes", pp. 344-381. In: Nelson, R.R. (Ed.), Breeding Plants for Disease Resistance. Pennsylvania State University Press, University Park.

Young, P.A. & J.W. MacArthur, 1947. Horticultural characters of tomatoes. Bull. Texas Agric. Exper. Station No. 698.

Hunt - Peto - Zeneca
TG Tomato Field Trial Data Form

Year 1993 APHIS Permit # _____

Location WOODLAND, CA Evaluator _____

NEMA 1200 Check

oose responses for the following characters which best fit your variety. Complete this form as fully as possible for best characterization of the variety.
en a single quantitative value is requested (e.g., fruit weight), your answer should be the mean of an adequate-sized, unbiased sample of plants. Use leading
es when necessary (e.g., 0 1 9 or 0 1 3 . 1 , etc.). The applicant variety should be compared with at least one well-known standard check
iety of the same type (see list of recommended check varieties below), and grown in the same trials. The characters on this form should be examined from
nts grown under normal conditions of culture for the variety. Indicate by a check whether trial data are from greenhouse _____ or field _____ plantings
ds direct-seeded _____ or transplanted _____; staked _____ or unstaked _____. Give locations and dates of seeding and transplanting here: _____

SEEDED 4-2-93 TRANSPLANTED 4-29-93

MPARISONS SHOULD BE MADE TO ONE OR MORE CHECK VARIETIES IN THE FOLLOWING LIST. IF AT ALL POSSIBLE, ENTER THE NUMBER
THE CHECK IN BOXES WHERE IDENTITY OF CHECK IS REQUESTED.

Ace 55 VF	7 = Homestead 24	13 = Red Rock	19 = VF 134
Comcast 27	8 = Marglobe	14 = Roma VF	20 = US 82
Ohio 7111	9 = Murietta	15 = Rutgers	21 = VF 145 B 7879
Flora Dade	10 = New Yorker	16 = Sunray	22 = Other (Specify) NEMA 1200
Florida MH-1	11 = Ohio MR-13	17 = Tropic	
Heinz 1350	12 = Red Cherry Large	18 = UC 82	

SEEDLING:

[2] Anthocyanin in hypocotyl of 2-15 cm. seedling: 1 = Absent 2 = Present [1] Habit of 3-4 week old seedling: 1 = Normal 2 = Compact

MATURE PLANT (at maximum vegetative development):

[0153] cm. Height (53.1)

[1] Growth: 1 = Indeterminate 2 = Determinate

[2] Form: 1 = Lax, open 2 = Normal 3 = Compact 4 = Dwarf 5 = Bracteate

[2] Size of canopy (compared to others of similar type): 1 = Small 2 = Medium 3 = Large

[2] Habit: 1 = Sprawling (decumbent) 2 = Semi-erect 3 = Erect ("Dwarf Champion")

STEM:

[3] Branching: 1 = Sparse ("Brenn's Solid Red", "Firecall") 2 = Intermediate ("Nestover") 3 = Profuse ("UC 82")

[1] Branching at cotyledonary or first leafy node: 1 = Present 2 = Absent

[2] No. of nodes below the first inflorescence: 1 = 1-4 2 = 4-7 3 = 7-10 4 = 10 or more

[1] No. of nodes between leafy (1st - 2nd, 2nd - 3rd) inflorescences. [1] No. of nodes between later-developing inflorescences.

[3] Pubescence on younger stems: 1 = Smooth (no long hairs) 2 = Scarcely hairy (scattered long hairs)
3 = Moderately hairy 4 = Densely hairy or woolly

LEAF (mature leaf between the 2nd inflorescence):

[1] Type: 1 = Tomato 2 = Potato ("Trip-L-Crop") [3] Morphology (choose illustration on pg. 5 of this form that is most similar)

[1] Margins of major leaflets: 1 = Nearly entire 2 = Shallowly toothed or scalloped
3 = Deeply toothed or cut, esp. towards base

[1] Marginal rolling or wilting: 1 = Absent 2 = Slight 3 = Moderate 4 = Strong

[ ] Onset of leaflet rolling: 1 = Early-season 2 = Mid-season 3 = Late season

. LEAF (mature leaf beneath the 3rd inflorescence – continued):

[2] Surface of major leaflets: 1 = Smooth 2 = Rugose (bumpy or veiny)

[2] Pubescence: 1 = Smooth (no long hairs) 2 = Normal 3 = Hirsute 4 = Woolly

5. INFLORESCENCE (make observations on 3rd inflorescence):

[1] Type: 1 = Simple 2 = Forked (2 major axes) 3 = Compound (much branched)

[0|6] Number of flowers in inflorescence, average

[1] Leafy or "running" inflorescences: 1 = Absent 2 = Occasional 3 = Frequent

6. FLOWER:

[1] Calyx: 1 = Normal, lobes awl-shaped 2 = Macrocalyx, lobes large, leaflike 3 = Fleshy

[1] Calyx-lobes: 1 = Shorter than corolla 2 = Approx. equalling corolla 3 = Distinctly longer than corolla

[1] Corolla color: 1 = Yellow 2 = Old gold 3 = White or tan

[2] Style pubescence: 1 = Absent 2 = Sparse 3 = Dense

[1] Anthers: 1 = All fused into tube 2 = Separating into 2 or more groups at anthesis

[1] Fasciation (1st flower of 2nd or 3rd inflorescence): 1 = Absent 2 = Occasionally present 3 = Frequently present

7. FRUIT (3rd fruit of 2nd or 3rd cluster): *For the first 5 characters below, match your variety with the most similar illustration on pg. 5 of this form.*

[4] Typical fruit shape:

[3] Shape of transverse section:

[2] Shape of blossom end:

[1] Shape of stem end:

[1] Shape of distal scar:

---

[2] Abscission layer: 1 = Present (pedicellate) 2 = Absent (jointless)

[1] Point of detachment of fruit at harvest: 1 = At pedicel joint 2 = At calyx attachment

[1|2] mm length of pedicel (from joint to calyx attachment) (12.1)

[0|5|7]	mm length of mature fruit (stem axis) (57.3).	[| |]	mm length, check var. no. [2|2]
[0|5|7]	mm diameter of fruit at widest point (56.5).	[| |]	mm diameter, check var. no. [2|2]
[0|8|4]	g weight of mature fruit	[| |]	g weight, check var. no. [2|2]

[2] No. of locules: 1 = Two 2 = Three and four 3 = Five or more

[1] Fruit surface: 1 = Smooth 2 = Slightly rough 3 = Moderately rough or ribbed

[1] Fruit base color (mature-green stage): 1 = Light green ("Lanai", "VF145-F5") 2 = Light gray-green ("Westover") 3 = Apple or medium green ("Heinz 1439 VF") 4 = Yellow green 5 = Dark green

[2] Fruit pericarp (mature-green stage): 1 = Uniform green 2 = Green-shouldered 3 = Radial stripes on sides of fruit

[1] Shoulder color if different from base: 1 = Dark green 2 = Gray green 3 = Yellow green

[5] Fruit color, full-ripe: 1 = White 2 = Yellow 3 = Orange 4 = Pink 5 = Red 6 = Brownish 7 = Greenish 8 = Other (Specify) _____

[3] Flesh color, full-ripe: 1 = Yellow 2 = Pink 3 = Red/Crimson 4 = Orange 5 = Other (Specify) _____

[1] Flesh color: 1 = Uniform 2 = With lighter and darker areas in walls

[3] Locular gel color of table-ripe fruit: 1 = Green 2 = Yellow 3 = Red

[2] Ripening: 1 = Blossom-to-stem end 2 = Uniform

7. FRUIT (3rd fruit of 2nd or 3rd cluster): Continued

[2] Ripening: 1 = Inside out 2 = Uniformly 3 = Outside in

[2] Epidermis color: 1 = Colorless 2 = Yellow

[1] Epidermis: 1 = Normal 2 = Easy-peel

[3] Epidermis texture: 1 = Tender 2 = Average 3 = Tough

[3] Thickness of pericarp . . . . . [3] Thickness of pericarp, check var. no. [22]

1 = Under 3 mm 2 = 3-6 mm 3 = 6-9 mm 4 = Over 9 mm

[1] Stem scar size: 1 = Small ("Roma") 2 = Medium ("Rutgers") 3 = Large

[1] Core: 1 = Coreless (absent or smaller than 6x6 mm) 2 = Present

8. RESISTANCE TO FRUIT DISORDERS (Use code: 0 = Unknown, 1 = Susceptible, 2 = Resistant)

- [ ] Blossom end rot
- [ ] Blotchy ripening
- [ ] Bursting
- [ ] Catface
- [ ] Cracking, concentric
- [ ] Cracking, radial
- [ ] Fruit pox
- [ ] Gold fleck
- [ ] Graywall
- [ ] Zippering
- [ ] Other (Specify) _____

9. DISEASE AND PEST REACTION (Use code: 0 = Not tested, 1 = Susceptible, 2 = Resistant). NOTE: If claim of novelty is based wholly or in substantial part upon disease resistance, trial data should be appended. These should specify the method of testing, the reaction of the application variety, and reaction of well-known check varieties grown in the trial (identified by name).

VIRAL DISEASES:

- [ ] Cucumber mosaic
- [ ] Curly top
- [ ] Potato-Y virus
- [ ] Other virus (Specify) _____
- [ ] Tobacco mosaic, Race 0
- [ ] Tobacco mosaic, Race 1
- [ ] Tobacco mosaic, Race 2
- [ ] Tobacco mosaic, Race 2[2]
- [ ] Tomato spotted wilt
- [ ] Tomato yellows

BACTERIAL DISEASES:

- [ ] Bacterial canker (*Corynebacterium michiganense*)
- [ ] Bacterial soft rot (*Erwinia carotovora*)
- [ ] Bacterial speck (*Pseudomonas tomato*)
- [ ] Bacterial spot (*Xanthomonas vesicatorium*)
- [ ] Bacterial wilt, (*Pseudomonas solanacearum*)
- [ ] Other bacterial disease (Specify) _____

FUNGAL DISEASES:

- [ ] Anthracnose (*Colletotrichum* sp.)
- [ ] Brown root rot or corky root, (*Pyrenochaeta lycopersici*)
- [ ] Collar rot or stem canker, (*Alternaria solani*)
- [ ] Early blight defoliation, (*Alternaria solani*)
- [2] Fusarium wilt, Race 1, (*F. oxysporum f. lycopersici*)
- [2] Fusarium wilt, Race 2
- [ ] Fusarium wilt, Race 3
- [ ] Gray leaf spot (*Stemphylium* sp.)
- [ ] Late blight, Race 0, (*Phytophthora infestans*)
- [ ] Late blight, Race 1
- [ ] Leaf mold, Race 1 (*Cacosporium fulvum*)
- [ ] Leaf mold, Race 2
- [ ] Leaf mold, Race 3
- [ ] Leaf mold, other races (Specify) _____
- [ ] Nailhead spot (*Alternaria tomato*)
- [ ] Septoria leafspot (*S. lycopersici*)
- [ ] Target leafspot (*Corynespora cassicola*)
- [2] Verticillium wilt, Race 1 (*V. albo-atrum*)
- [ ] Verticillium wilt, Race 2
- [2] Other fungal disease Alternaria Stem Canker
- [ ] Other fungal disease _____

INSECTS AND PESTS:

- [ ] Colorado potato beetle (*Leptinotarsa decemlineata*)
- [2] Southern root knot nematode (*Meloidogyne incognita*)
- [ ] Spider mites (*Tetranychus* spp.)
- [ ] Sugar beet army worm (*Spodoptera exigua*)
- [ ] Tobacco flea beetle (*Epitrix hirtipennis*)
- [ ] Tomato hornworm (*Manduca quinquemaculata*)
- [ ] Tomato fruitworm (*Heliothis zea*)
- [ ] Whitefly (*Trialeurodes vaporariorum*)
- [ ] Other (Specify) _____

POLLUTANTS:

- [ ] Ozone
- [ ] Sulfur dioxide
- [ ] Other (Specify) _____

Q. CHEMISTRY AND COMPOSITION OF FULL-RIPE FRUITS: Suggested test methods may be found in "Tomato Products" 3rd ed., National Canners Assn. Bull. 27-L. Please identify test methods or give a reference to methods used. Fill in table below with values for the new variety and for at least one well-known check variety or similar type grown in the same trial. Specify names or numbers of check varieties.

	SUBMITTED VARIETY	Check Variety	Check Variety	Check Variety
	NEMA 1200 check			
JUICE BOSTWICK COLD BREAK	27.9			
BRIX (raw puree)	5.7			
PH (raw puree)	4.3			
AGTRON COLOR (raw puree)	26.3			

. PHENOLOGY: Express length of developmental stages either as calendar days or as heat units (growing degree days), in degrees Celsius. If heat units are used, indicate the base temperature used in their calculation here _____ °C. See Cotter or Warnock under "References" for method. Give comparative data for at least one check variety; identify checks by name or by number from table on page 1.

	APPLICATION VARIETY	Check variety	Check variety	Check variety
...ading to 50% flower (1 open flower on 50% ...r plants)				
...to one-over harvest (*If applicable*)				

[4] Fruiting season: 1 = Long ("Marglobe") 2 = Medium ("Nestaver") 3 = Short, concentrated ("VF 145") 4 = Very concentrated ("UC 82")

[1] Relative maturity in areas tested: 1 = Early 2 = Medium early 3 = Medium 4 = Medium late 5 = Late 6 = Variable (If relative maturity is known to differ by location or environment, please explain on separate sheet.)

ADAPTATION: If more than one category applies, list all in rank order.

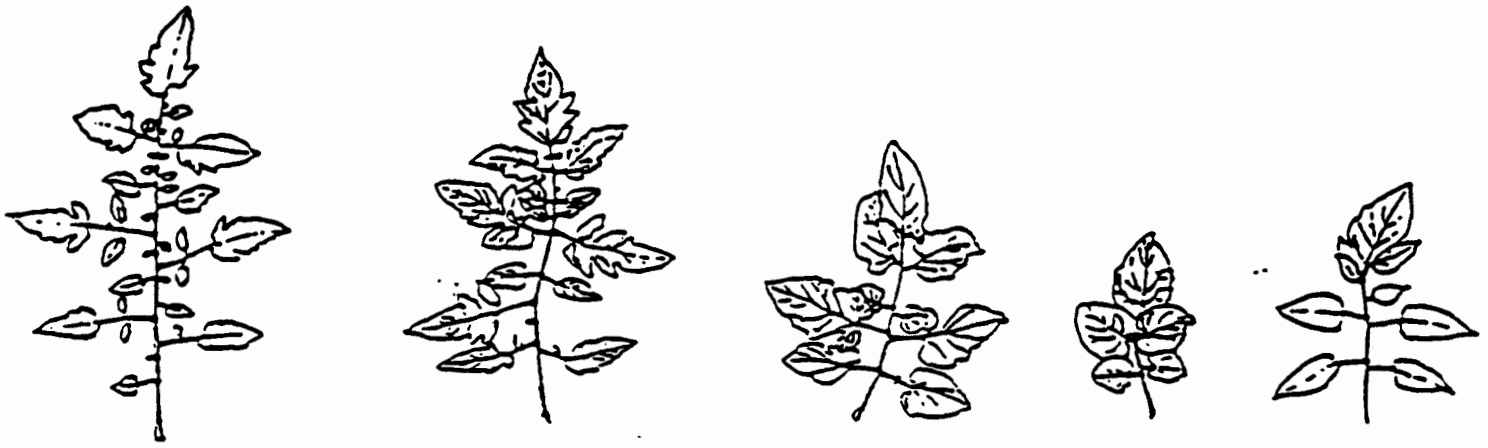
[1] Culture: 1 = Field 2 = Greenhouse

[4] Principal use(s): 1 = Home garden 2 = Fresh market 3 = Whole-pack canning 4 = Concentrated products 5 = Other (Specify) _____

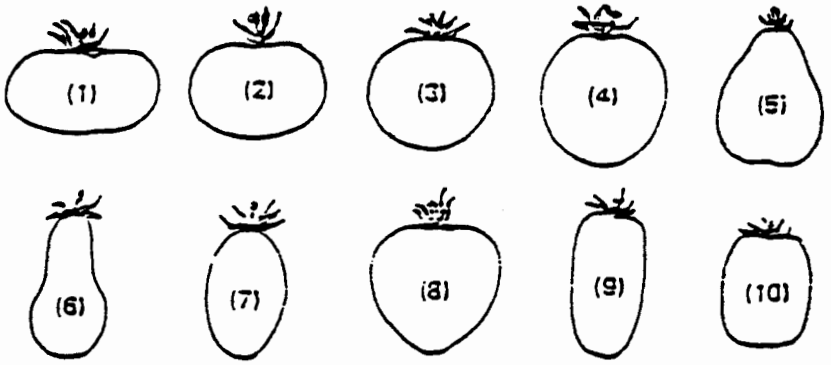
[2] Machine harvest: 1 = Not adapted 2 = Adapted

[10, 11] Regions to which adaptation has been demonstrated:
1 = Northeast 2 = Mid Atlantic 3 = Southeast 4 = Florida
5 = Great Plains 6 = South-central 7 = Intermountain West 8 = Northwest
9 = California: Sacramento and Upper San Joaquin Valley
10 = California: Coastal areas 11 = California: Southern San Joaquin Valley & desert

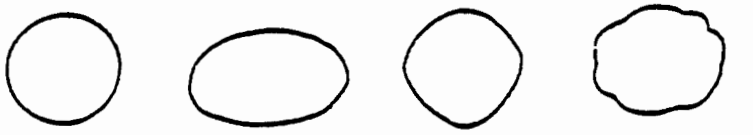
4. LEAF: Morphology:



7. FRUIT: Typical fruit shape:

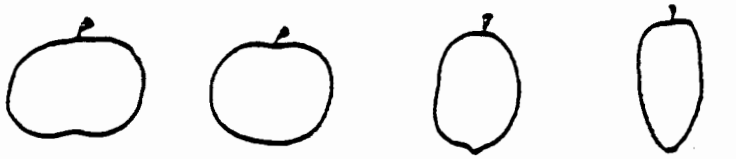


Shape of transverse section:

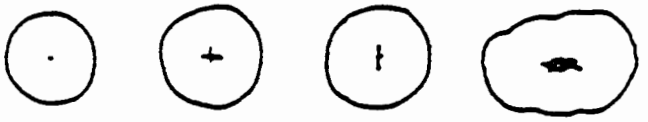


Shape of stem end:

Shape of blossom end:



Shape of pistil scar:



REFERENCES

Anonymous, 1976. All About Tomatoes. Ortho Books, Chevron Chemical Co., San Francisco. In three volumes: Midwest/Northeast Edition, West Edition, and South Edition

Ware, G.W. & J. P. McCollum, 1968. Producing Vegetable Crops. The Interstate Printer & Publishers, Inc., Danville, Illinois. Chapter 30, pp. 451-473. "Tomatoes".

Warnock, S.J. 1978. Using Tomato Heat Units. Leaflet No. 6, Campbell Institute for Agricultural Research, Camden, NJ. 10 p.

Webb, R.E., T. H. Bartsdale, & A. K. Stoner, 1973. "Tomatoes", pp. 344-361. In: Nelson, R.R. (Ed.), Breeding Plants for Disease Resistance. Pennsylvania State University Press, University Park.

Young, P.A. & J.W. MacArthur, 1947. Horticultural characters of tomatoes. Bull. Texas Agric. Exper. Station No. 698.

Hunt - Peto - Zeneca
TG Tomato Field Trial Data Form

Year 1993 APHIS Permit # _____

Location WOODLAND, CA Evaluator _____

NEMA 1200 PGS

Choose responses for the following characters which best fit your variety. Complete this form as fully as possible for best characterization of the variety. When a single quantitative value is requested (e.g., fruit weight), your answer should be the mean of an adequate-sized, unbiased sample of plants. Use leading zeros when necessary (e.g., 0 1 9 or 0 3 1 1, etc.). The applicant variety should be compared with at least one well-known standard check variety of the same type (see list of recommended check varieties below), and grown in the same trials. The characters on this form should be recorded from plants grown under normal conditions of culture for the variety. Indicate by a check whether seed data are from greenhouse _____ or field _____ planted; also direct-seeded _____ or transplanted X ; staked _____ or unstaked _____. Give locations and dates of seeding and transplanting here: _____

SEEDED 4-2-93 TRANSPLANTED 4-29-93

COMPARISONS SHOULD BE MADE TO ONE OR MORE CHECK VARIETIES IN THE FOLLOWING LIST, IF AT ALL POSSIBLE. ENTER THE NUMBER OF THE CHECK IN BOXES WHERE IDENTITY OF CHECK IS REQUESTED.

1 = Ace 55 VF	7 = Homestead 24	13 = Red Rock	19 = VF 134
2 = Campbell 37	8 = Marglobe	14 = Roma VF	20 = US 82
3 = Chico III	9 = Moneta	15 = Rutgers	21 = VF 145 B 7879
4 = Flora Dade	10 = New Yorker	16 = Sunray	22 = Other (Specify) NEMA 1200
5 = Florida MH-1	11 = Ohio MR-13	17 = Tropic	
6 = Heinz 1350	12 = Red Cherry Large	18 = UC 82	

1. SEEDLING:

2 Anthocyanin in hypocotyl at 2-15 cm. seedling: 1 = Absent 2 = Present

1 Habit at 3-4 week old seedling: 1 = Normal 2 = Compact

2. MATURE PLANT (at maximum vegetative development):

0 5 5 Cm. Height

1 Growth: 1 = Indeterminate 2 = Determinate

2 Form: 1 = Lax, open 2 = Normal 3 = Compact 4 = Dwarf 5 = Bracteoid

2 Size of canopy (compared to others of similar type): 1 = Small 2 = Medium 3 = Large

2 Habit: 1 = Sprawling (decumbent) 2 = Semi-erect 3 = Erect ("Dwarf Champion")

3. STEM:

3 Branching: 1 = Sparse ("Brenn's Solid Red", "Firesail") 2 = Intermediate ("Westover") 3 = Profuse ("UC 82")

1 Branching at cotyledonary or first leafy node: 1 = Present 2 = Absent

2 No. of nodes below the first inflorescence: 1 = 1-4 2 = 4-7 3 = 7-10 4 = 10 or more

1 No. of nodes between early (1st - 2nd, 2nd - 3rd) inflorescences.

1 No. of nodes between later-developing inflorescences.

3 Pubescence on younger stems: 1 = Smooth (no long hairs) 2 = Sparsely hairy (scattered long hairs) 3 = Moderately hairy 4 = Densely hairy or woolly

4. LEAF (measure leaf between the 2nd inflorescences):

1 Type: 1 = Tomato 2 = Potato ("Thin-L-Cut")

3 Morphology (choose illustration on pg. 5 of this form that is most similar)

1 Margins of major leaflets: 1 = Nearly entire 2 = Shallowly toothed or scalloped 3 = Deeply toothed or cut, esp. towards base

1 Marginal rolling or wilting: 1 = Absent 2 = Slight 3 = Moderate 4 = Strong

_ Onset of leaflet rolling: 1 = Early-season 2 = Mid-season 3 = Late season

LEAF (mature leaf beneath the 3rd inflorescence – continued):

[2] Surface of major leaflets: 1 = Smooth 2 = Rugose (bumpy or veiny)

[2] Pubescence: 1 = Smooth (no long hairs) 2 = Normal 3 = Hirsute 4 = Woolly

5. INFLORESCENCE (make observations on 3rd inflorescence):

[1] Type: 1 = Simple 2 = Forked (2 major axes) 3 = Compound (much branched)

[0 6] Number of flowers in inflorescence, average

[1] Leafy or "running" inflorescences: 1 = Absent 2 = Occasional 3 = Frequent

6. FLOWER:

[1] Calyx: 1 = Normal, lobes awl-shaped 2 = Macrocalyx, lobes large, leaflike 3 = Fleshy

[1] Calyx-lobes: 1 = Shorter than corolla 2 = Approx. equalling corolla 3 = Distinctly longer than corolla

[1] Corolla color: 1 = Yellow 2 = Old gold 3 = White or tan

[2] Style pubescence: 1 = Absent 2 = Sparse 3 = Dense

[1] Anthers: 1 = All fused into tube 2 = Separating into 2 or more groups at anthesis

[1] Fasciation (1st flower of 2nd or 3rd inflorescence): 1 = Absent 2 = Occasionally present 3 = Frequently present

7. FRUIT (3rd fruit of 2nd or 3rd cluster): *For the first 5 characters below, match your variety with the most similar illustration on pg. 5 of this form.*

[4] Typical fruit shape:

[3] Shape of transverse section:

[2] Shape of blossom end:

[1] Shape of stem end:

[1] Shape of distal scar:

---

[2] Abscission layer: 1 = Present (pedicellate) 2 = Absent (jointless)

[1] Point of detachment of fruit at harvest: 1 = At pedicel joint 2 = At calyx attachment

[1 2] mm length of pedicel (from joint to calyx attachment) (11.9)

[0 5 7]	mm length of mature fruit (stem axis) (56.5)	[0 5 7]	mm length, check var. no. (57.3)	[2 2]
[0 5 8]	mm diameter of fruit at widest point (58.4)	[0 5 7]	mm diameter, check var. no. (56.5)	[2 2]
[0 8 8]	g weight of mature fruit	[0 8 4]	g weight, check var. no.	[2 2]

[2] No. of locules: 1 = Two 2 = Three and four 3 = Five or more

[1] Fruit surface: 1 = Smooth 2 = Slightly rough 3 = Moderately rough or ribbed

[1] Fruit base color (mature-green stage): 1 = Light green ("Lanai", "VF145-F5") 2 = Light gray-green ("Westover") 3 = Apple or medium green ("Heinz 1439 VF") 4 = Yellow green 5 = Dark green

[2] Fruit pattern (mature-green stage): 1 = Uniform green 2 = Green-shouldered 3 = Radial stripes on sides of fruit

[1] Shoulder color if different from base: 1 = Dark green 2 = Gray green 3 = Yellow green

[5] Fruit color, full-ripe: 1 = White 2 = Yellow 3 = Orange 4 = Pink 5 = Red 6 = Brownish 7 = Greenish 8 = Other (Specify) _____

[3] Flesh color, full-ripe: 1 = Yellow 2 = Pink 3 = Red/Crimson 4 = Orange 5 = Other (Specify) _____

[1] Flesh color: 1 = Uniform 2 = With lighter and darker areas in walls

[3] Locular gel color of table-ripe fruit: 1 = Green 2 = Yellow 3 = Red

[2] Ripening: 1 = Blossom-to-stem end 2 = Uniform

7. FRUIT (3rd fruit of 2nd or 3rd cluster): Continued

[2] Ripening: 1 = Inside out 2 = Uniformly 3 = Outside in

[2] Epidermis color: 1 = Colorless 2 = Yellow

[1] Epidermis: 1 = Normal 2 = Easy-peel

[3] Epidermis texture: 1 = Tender 2 = Average 3 = Tough

[3] Thickness of pericarp . . . . . [3] Thickness of pericarp, check var. no. [22]

1 = Under 3 mm 2 = 3-6 mm 3 = 6-9 mm 4 = Over 9 mm

[1] Stem scar size: 1 = Small ('Roma') 2 = Medium ('Rutgers') 3 = Large

[1] Core: 1 = Coreless (absent or smaller than 8x8 mm) 2 = Present

8. RESISTANCE TO FRUIT DISORDERS (Use code: 0 = Unknown, 1 = Susceptible, 2 = Resistant)

[ ] Blossom end rot
[ ] Blotchy ripening
[ ] Bursting
[ ] Catface
[ ] Cracking, concentric
[ ] Cracking, radial
[ ] Fruit pox
[ ] Gold fleck
[ ] Graywall
[ ] Zippering
[ ] Other (Specify) _____

9. DISEASE AND PEST REACTION (Use code: 0 = Not tested, 1 = Susceptible, 2 = Resistant). NOTE: If claim of novelty is based wholly or in substantial part upon disease resistance, trial data should be appended. These should specify the method of testing, the reaction of the application variety, and reaction of well-known check varieties grown in the trial (identified by name).

VIRAL DISEASES:

[ ] Cucumber mosaic
[ ] Curly top
[ ] Potato-Y virus
[ ] Other virus (Specify) _____
[ ] Tobacco mosaic, Race 0
[ ] Tobacco mosaic, Race 1
[ ] Tobacco mosaic, Race 2
[ ] Tobacco mosaic, Race 2[2]
[ ] Tomato spotted wilt
[ ] Tomato yellows

BACTERIAL DISEASES:

[ ] Bacterial canker (*Corynebacterium michiganense*)
[ ] Bacterial soft rot (*Erwinia carotovora*)
[ ] Bacterial speck (*Pseudomonas tomato*)
[ ] Bacterial spot (*Xanthomonas vesicatorium*)
[ ] Bacterial wilt, (*Pseudomonas solanacearum*)
[ ] Other bacterial disease (Specify) _____

FUNGAL DISEASES:

[ ] Anthracnose (*Colletotrichum* spp.)
[ ] Brown root rot or corky root, (*Pyrenochaeta lycopersici*)
[ ] Collar rot or stem canker, (*Alternaria solani*)
[ ] Early blight defoliation, (*Alternaria solani*)
[2] Fusarium wilt, Race 1, (*F. oxysporum f. lycopersici*)
[2] Fusarium wilt, Race 2
[ ] Fusarium wilt, Race 3
[ ] Gray leaf spot (*Stemonylium* spp.)
[ ] Late blight, Race 0, (*Phytophthora infestans*)
[ ] Late blight, Race 1
[ ] Leaf mold, Race 1 (*Cacosporium fulvum*)
[ ] Leaf mold, Race 2
[ ] Leaf mold, Race 3
[ ] Leaf mold, other races (Specify) _____
[ ] Nailhead spot (*Alternaria tomato*)
[ ] Septoria leafspot (*S. lycopersici*)
[ ] Target leafspot (*Corynespora cassiicola*)
[2] Verticillium wilt, Race 1 (*V. albo-atrum*)
[ ] Verticillium wilt, Race 2
[2] Other fungal disease Alternaria Stem Canker
[ ] Other fungal disease _____

INSECTS AND PESTS:

- [ ] Colorado potato beetle (*Leptinotarsa decemlineata*)
- [ ] Southern root knot nematode (*Meloidogyne incognita*)
- [ ] Spider mites (*Tetranychus* spp.)
- [ ] Sugar beet army worm (*Spodoptera exigua*)
- [ ] Tobacco flea beetle (*Epitrix hirtipennis*)
- [ ] Tomato hornworm (*Manduca quinquemaculata*)
- [ ] Tomato fruitworm (*Heliothis zea*)
- [ ] Whitefly (*Trialeurodes vaporariorum*)
- [ ] Other (Specify) _____

POLLUTANTS:

- [ ] Ozone
- [ ] Sulfur dioxide
- [ ] Other (Specify) _____

1. CHEMISTRY AND COMPOSITION OF RIPE FRUITS: Suggested test methods may be found in "Tomato Products," 5th ed., National Canners Assn. Bull. 31-L. Please specify test methods or give a reference to methods used. Fill in table below with values for the new variety and for at least one well-known check variety or similar type grown in the same trial. Specify names or numbers of check varieties.

	SUBMITTED VARIETY	Check Variety NEMA 1200 CONTROL	Check Variety	Check Variety
JUICE BOSTWICK COLD BREAK	21.5	27.9		
BRIX (raw puree)	5.4	5.7		
PH (raw puree)	4.3	4.3		
AGTRON COLOR (raw puree)	25.7	26.3		

PHENOLOGY: Express length of developmental stages either as calendar days or as heat units (growing degree days), in degrees Celsius. If heat units are used, indicate the base temperature used in their calculation here _____ °C. See paper by Warnock under "References" for method. Give comparative data for at least one check variety; identify checks by name or by number from table on page 1.

	APPLICATION VARIETY	Check variety	Check variety	Check variety
...ing to 50% flower (1 open flower on 50% ...plants)				
...s to once-over harvest (if applicable)				

4 Fruiting season: 1 = Long ("Marglobe") 2 = Medium ("Nematex") 3 = Short, concentrated ("VF 145") 4 = Very concentrated ("UC 82")

1 Relative maturity in areas tested: 1 = Early 2 = Medium early 3 = Medium 4 = Medium late 5 = Late 6 = Variable (if relative maturity is known to differ by location or environment, please explain on separate sheet).

ADAPTATION: If more than one category applies, list all in rank order.

1 Culture: 1 = Field 2 = Greenhouse

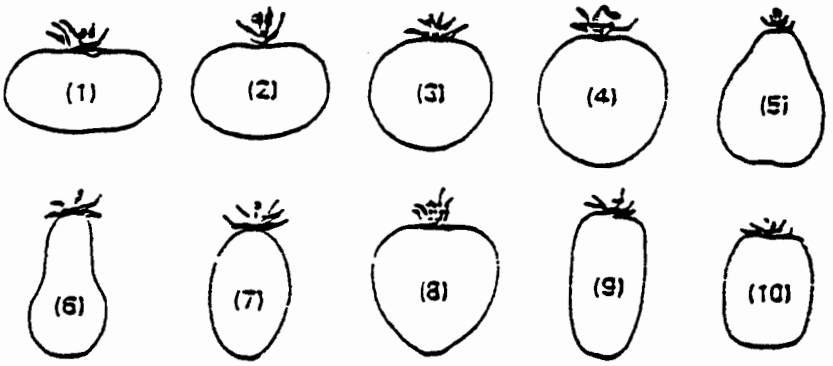
4 Principal use(s): 1 = Home garden 2 = Fresh market 3 = Whole-pack canning 4 = Concentrated products 5 = Other (Specify) _____

2 Machine harvest: 1 = Not adapted 2 = Adapted

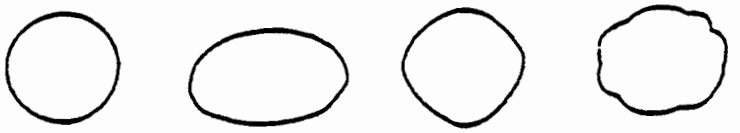
10, 11 Regions to which adaptation has been demonstrated:
1 = Northeast 2 = Mid Atlantic 3 = Southeast 4 = Florida
5 = Great Plains 6 = South-central 7 = Intermountain West 8 = Northwest
9 = California: Sacramento and Upper San Joaquin Valley
10 = California: Coastal areas 11 = California: Southern San Joaquin Valley & desert

4. LEAF: Morphology:

7. FRUIT: Typical fruit shape:

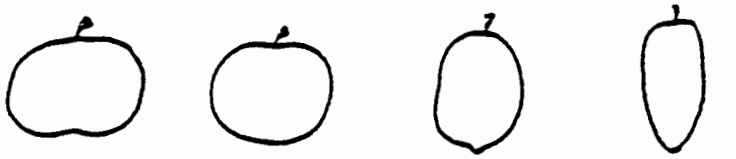


Shape of transverse section:

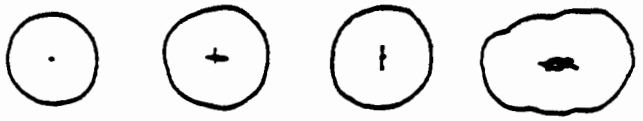


Shape of stem end:

Shape of blossom end:



Shape of pistil scar:



REFERENCES

Anonymous, 1976. All About Tomatoes. Orthe Books, Chevron Chemical Co., San Francisco. In three volumes: Midwest/Northeast Edition, West Edition, and South Edition

Ware, G.W. & J. P. McCollum, 1968. Producing Vegetable Crops. The Interstate Printer & Publishers, Inc., Danville, Illinois. Chapter 30, pp. 451-472. "Tomatoes".

Warnock, S.J. 1978. Using Tomato Heat Units. Leaflet No. 5, Campbell Institute for Agricultural Research, Camden, N.J. 10 p.

Webb, R.E., T. H. Barksdale, & A. K. Stoner, 1973. "Tomatoes", pp. 344-381. In: Nelson, R.R. (Ed.), Breeding Plants for Disease Resistance. Pennsylvania State University Press, University Park.

Young, P.A. & J.W. MacArthur, 1947. Horticultural characters of tomatoes. Bull. Texas Agric. Exper. Station No. 698.

PETOSEED

Woodland Research Station

37437 State Highway 16 • Woodland, CA 95695
Telephone: (916) 666-0931 • Fax: (916) 668-0219

November 11, 1993

Dr. Arnold Foudin
Biological Assessment Support Staff
Plant Protection and Quarantine
APHIS - USDA
Federal Building
6506 Belcrest Road, Room 600A
Hyattsville, MD 20782

Dear Dr. Foudin:

Enclosed you will find the Summary/Final Report for the 1992 Petoseed Transgenic Tomato Field Trial 92-049-03.

The Petoseed Transgenic Tomato Field Trial grown under Permit Number 92-049-03 during the summer of 1992 at Woodland, California included tomatoes carrying PGA, PGS, PEA and EFEA constructs as described in the permit application. The objectives of the trial were to evaluate the field performance of the transgenic plants, to determine the enzyme expression levels of inbreds and hybrids and to measure the effect of different enzyme expression levels on select fruit quality characteristics in fresh market and processing tomatoes. The trial was conducted as described in the permit application following all procedures and safeguards stated and according to the standard and supplemental permit conditions. All pollen and seed from the transgenic plants were handled to insure that no unintended dispersal of the introduced genes occurred. There were no unusual phenotypes or plants with altered survival characteristics in the trial beyond the normal range of variation and off types that spontaneously occurs in tomato fields. All volunteer tomato plants on the site were controlled throughout the winter and early spring on fallow ground and throughout the next growing season within a melon crop.

Field Performance Observations

The transgenic plants were evaluated for plant habit, maturity, fruit set, size, shape and color. For horticultural features and field performance the transgenic plants were just like the corresponding unmodified check inbred lines.

Gene Copy Number and Stability

The transgenic T_3 and T_2 lines that went to the field trial were derived from plants determined to be single copy by T_1 and T_2 segregation analysis and Southern blot analysis. T_3 lines were also determined to be homozygous for the introduced gene before going to the field. The stability of transmission through seed generations for the gene was checked by sampling for PCR analysis in the T_3 PGA lines. Up to 80 plants per line were checked for the presence of the construct. The PGA construct was detected in all plants of the advanced PGA T_3 lines. PCR analysis was also used to identify homozygous T_2 PGS lines and plants carrying the PEA and EFEA constructs from segregating lines in the observation trial.

Gene Expression

Both PG and PE enzyme activity analyses were carried out on fruit samples collected from the trial. For the PG expression levels, inbred PGA and PGS lines were identified that had 0% to 5%, 0% to 15%, 0% to 20%, and 0% to 60% of normal enzyme activity levels in the fruit. PGA and PGS hybrids were identified that had 0% to 5% and 20% plus normal levels. The data demonstrated dominant gene action for the introduced gene in some of the T_3 PGA and PGS lines, and incomplete dominant gene action for others. The PE expression levels were checked in only a few T_2 lines and ranged from 10% to 30% of normal in PCR positive plants.

Fruit Quality Characteristics

Juice bostwick flow was measured from low PG fruits from the processing tomato transgenic lines and hybrids. In summary, the data showed that 0% to 5% PG activity gives a significant increase in viscosity across genetic backgrounds. Firmness determination on low PG fresh market tomato fruit showed no difference compared with unmodified fruit and a number of sensory analyses conducted on low PG fresh market tomatoes gave inconclusive results.

Please contact us if you have any questions regarding the trial.

Sincerely,

Jeffrey B. McElroy, Ph.D.
Senior Plant Breeder

/vem

PETOSEED

Woodland Research Station

37437 State Highway 16 • Woodland, CA 95695
Telephone: (916) 666-0931 • Fax: (916) 668-0219

June 29, 1994

Dr. Arnold Foudin
Biological Assessment Support Staff
Plant Protection and Quarantine
APHIS - USDA
Federal Building
6506 Belcrest Road. Rm. 600A
Hyattsville, MD 20782

Dear Dr. Foudin:

Enclosed you will find the Summary/Final Report for the 1993 Petoseed Transgenic Tomato Field Trial 92-352-01.

The Petoseed Transgenic Tomato Field Trial grown under Permit Number 92-352-01 during the summer of 1993 at Woodland, California included tomatoes carrying PGA, PGS, PEA, EFEA, modified invertase and PGES double construct constructs as described in the permit application. The objectives of the trial were to evaluate the field performance of the transgenic plants, to determine the enzyme expression levels of inbreds and hybrids and to measure the effect of different enzyme expression levels on select fruit quality characteristics in fresh market and processing tomatoes. The trial was conducted as described in the permit application following all procedures and safeguards stated and according to the standard and supplemental permit conditions. All pollen and seed from the transgenic plants were handled to insure that no unintended dispersal of the introduced genes occurred. There were no unusual phenotypes or plants with altered survival characteristics in the trial beyond the normal range of variation and off types that spontaneously occurs in tomato fields. All volunteer tomato plants on the site were controlled throughout the winter and early spring on fallow ground and throughout the next growing season within a melon crop.

Field Performance Observations

The transgenic plants were evaluated for plant habit, maturity, fruit set, size, shape and color. For horticultural features and field performance the transgenic plants were just like the corresponding unmodified check inbred lines.

Gene Copy Number and Stability

The transgenic T_3 and T_2 lines that went to the field trial were derived from plants determined to be single copy by T_1 and T_2 segregation analysis and Southern blot analysis. T_3 lines were also determined to be homozygous for the introduced gene before going to the field. PCR analysis was used to identify homozygous T_2 lines containing the modified invertase construct.

Gene Expression

Both PG and PE enzyme activity analyses were carried out on fruit samples collected from the trial. For the PG expression levels, inbred PGA and PGS lines were identified that had 0-5% normal enzyme activity levels in the fruit. PGA and PGS hybrids were identified that had 0-5% and others that had 20% and higher levels of PG. The data demonstrated dominant gene action for the introduced gene in some of the PGA and PGS hybrids, and

incomplete dominant gene action for others. The PE expression levels were checked in two T_2 lines and ranged from 10% to 30% of normal in PCR positive plants. PE hybrids had similar expression levels, thus demonstrating a dominant gene action. Plants with modified invertase gene showed Brix readings ranging from control levels up to twice that of control. EFEA plants were primary transgenics, and were planted for seed increase and selection for agronomic characteristics. The plants transformed with PGES double construct expressed 0-10% PG and 20-30% PE.

Fruit Quality Characteristics

Juice bostwick flow was measured from low PG fruits from the processing tomato transgenic lines and hybrids. In summary, the data showed that 0% to 5% PG activity gives a significant increase in viscosity across genetic backgrounds. Limited data from lines transformed with PE showed some elevation in soluble solids. Firmness determination on low PG fresh market tomato fruit showed no difference compared with unmodified fruit and a number rove sensory analyses conducted on low PG fresh market tomatoes gave inconclusive results.

Please contact us if you have any questions regarding the trial.

Sincerely,

Ed Green

Charles E. Green, Ph.D.
Vice President New Technology

/vem

No permit shall be issued until this application has been approved.

OMB NO. 0579-0015

U.S. DEPARTMENT OF AGRICULTURE
BIOTECHNOLOGY, BIOLOGICS, AND ENVIRONMENTAL PROTECTION

APPLICATION FOR PERMIT OR COURTESY PERMIT UNDER 7 CFR 340

(Genetically Engineered Organisms or Products)

INSTRUCTIONS: Complete this form and enclose the supporting materials listed on the reverse side. See page 3 for detailed instructions.

1. NAME AND ADDRESS OF APPLICANT

C.E. GREEN, PhD.
PETOSEED RESEARCH CENTER
37437 STATE HWY 16, WOODLAND CA 95695
Area Code ( )

2. PERMIT REQUESTED ("X" one)

- [ ] Limited - Interstate Movement
- [ ] Limited - Importation
- [X] Release into the Environment
- [ ] Courtesy Permit

3. THIS REQUEST IS ("X" one)

- [X] New
- [ ] Renewal
- [ ] Supplemental

4. TELEPHONE NUMBER

916-666-0931

5. MEANS OF MOVEMENT

- [ ] Mail
- [ ] Baggage or Handcarried
- [ ] Common Carrier
- By whom

SEED MOVEMENT PERMIT ATTACHED

6. GIVE THE FOLLOWING (if available) (if more space is needed, attach additional sheet)

	Scientific Name	Common Name	Trade Name	Other Designation
a. Donor Organism	LYCOPERSICON ESCULENTUM	TOMATO		
b. Recipient Organism	LYCOPERSICON ESCULENTUM	TOMATO		
c. Vector or Vector Agent	AGROBACTERIUM TOMEFACIENS AND Ti PLASMID pBIN 19			
d. Regulated Organism or Product	TOMATO EXPRESSING ANTISENSE AND SENSE PG AND ANTISENSE PE GENES AND ANTISENSE EFE (pTOM13).			
e. If product, list names of constituents:				

7. QUANTITY OF REGULATED ARTICLE TO BE INTRODUCED AND PROPOSED SCHEDULE AND NUMBER OF INTRODUCTIONS

NO MORE THAN 8,000 PLANTS SCHEDULED FOR FIELD PLANTING ON OR ABOUT 4/15/92

8. DATE (or inclusive dates of period) **OF IMPORTATION, INTERSTATE MOVEMENT, OR RELEASE**

ON OR ABOUT APRIL 15, 1992

9. COUNTRY OR POINT OF ORIGIN OF THE REGULATED ARTICLE

PETOSEED RESEARCH CENTER WOODLAND, CA AND PETOSEED COMPANY, COLINA, CHILE

10. PORT OF ARRIVAL, DESTINATION OF MOVEMENT, OR SPECIFIC LOCATION OF RELEASE

PETOSEED RESEARCH CENTER
WOODLAND, CA USA

11. ANY BIOLOGICAL MATERIAL (e.g., culture medium, or host material) **ACCOMPANYING THE REGULATED ARTICLE DURING MOVEMENT**

N/A

12. APPLICANTS FOR A COURTESY PERMIT - STATE WHY YOU BELIEVE THE ORGANISM OR PRODUCT DOES NOT COME WITHIN THE DEFINITION OF A REGULATED ARTICLE

N/A

13. SEE REVERSE SIDE

I hereby certify that the information in this application and all attachments is complete and accurate to the best of my knowledge and belief.

False Statement: Falsification of any item on this application may result in a fine of not more than $10,000 or imprisonment for not more than 5 years or both. (18 U.S.C. 1001)

14. SIGNATURE OF RESPONSIBLE PERSON	15. PRINTED NAME AND TITLE	16. DATE
C. E. Green	C.E. GREEN, PhD. VICE PRESIDENT, NEW TECHNOLOGY	2/12/92

FOR APHIS USE ONLY

State Notification Sent	State Review Returned	Permit Issued
March 16, 1992	March 19, 1992	[X] Yes [ ] No

Date of Determination	Permit No.	No. of Permit Labels Issued	Supplemental Conditions Enclosed
April 9, 1992	92-049-03	N/A	[X] Yes [ ] No

Signature of APHIS Official	Date	Expiration Date
James Lhute for Arnold Foudin, Deputy Director, BBEP-BP	April 15, 1992	April 15, 1993

PETOSEED

Woodland Research Station

37437 State Highway 16 • Woodland, CA 95695
Telephone: (916) 666-0931 • Fax: (916) 668-0219

September 27, 1994

Mr. Michael A. Lidsky
Deputy Director, BBEP, APHIS, USDA
6506 Belcrest Road
Federal Building
Hyattsville, MD 20782

Dear Mr. Lidsky:

I have been employed by Petoseed for twenty years and have accumulated extensive experience as a Research Assistant (14 years) working for fresh market and processing tomato breeders. Overlapping with this, I have seventeen years experience in hybrid seed production, particularly tomatoes. I currently work in the Foundation Seed/Stock Seed Department. My current responsibilities are to insure that inbred lines used for hybrid production are "true-to-type", have normal growth, development and seed set and that each line is highly pure.

During the 1992/1993 crop year I was in Santiago, Chile at Petoseed's production farm for virtually the entire growing season. I arrived in mid-November and was there until mid-March. Part of my responsibilities during that trip was to observe and evaluate the performance of the T7 transgenic lines. As the season progressed I was responsible for and personally collected and extracted fruit samples from over 700 individual plants in the transgenic hybrid and T7 inbred growing block. The pericarp tissue samples collected were shipped to Woodland, California for PG enzyme analysis in the laboratory. I was also involved in the seed harvest for the F_1 hybrids with the T7 transgenic line.

During the growing season no difference from non-transgenic control T7 inbred or hybrids were observed or noted in the transgenic lines with respect to growth habit, plant morphology, flowering, fruit-set, fruit development and seed set.

In conclusion, I did not observe any distinguishable differences between the T7 transgenic lines and hybrids and traditionally breed tomatoes.

Sincerely,

Cathy White

Cathy White
Crop Specialist

HUNT
WESSON, INC.
1645 West Valencia Drive
Fullerton, CA 92633-3899
714 680-1000
Writer's Direct Dial Number
714-680-2811

July 1, 1994

Mr. Michael A. Lidsky
Deputy Director, BBEP, APHIS, USDA
6506 Belcrest Road
Federal Building
Hyattsville MD 20782

Dear Mr. Lidsky:

Hunt-Wesson is conducting field trials of tomatoes modified to suppress polygalacturonase activity (T-7 hybrids) from Zeneca and Petoseed. T-7 hybrids are the subject of a pending petition for release from regulation.

Our testing began in 1992 with plots at the Petoseed facility and continued with trials in our own contract fields in California at 5 locations during 1993 and 2 locations during 1994. These trials, and ongoing Petoseed plots, have been evaluated by Hunt-Wesson field and research personnel on numerous occasions. 1993 trial sites are being monitored regularly during 1994. Trial fields were worked by growers using ordinary practices.

We observed no characteristics of the modified tomatoes to suggest that they would pose any problem to agriculture, the environment or consumers. The T-7 hybrids were comparable to traditional tomatoes with regard to horticultural attributes such as; germination, vegetative growth, fruit growth, fruit ripening, etc. Nothing atypical was seen in disease and pest susceptibility or carryover of volunteer plants. Nutritional value and processing characteristics of T-7 and traditional tomatoes were well within expectations for equivalence. There were no reports from the growers of any unusual aspects or problems with the trial plants.

We recommend that the T-7 hybrids be released from regulation. Testing has addressed fundamental issues of safety, agricultural fitness, and nutrition. It is now appropriate to test the horticultural and processing characteristics of these tomatoes at large scale to validate them for future commercial use.

Sincerely,

Alan L. Boyer
Director,
Agricultural
Operations

T. Casey Garvey, PhD.
Manager,
Agricultural
Research

Robert Sacher, Ph.D.
Sr. Scientist,
Biotechnology
Coordinator

TSL
TIMOTHY, STEWART & LEKOS
Seed Company
Post Office Box 359 • Woodland, California 95776
Office (916) 666-1239 • Fax (916) 666-2910

June 20, 1994

Ms. Anne Mueller
Zeneca Plant Science
P.O. Box 751
Wilmington, DE 19897

Dear Ms. Mueller:

I am an agribusiness manager with over 20 years of experience in the processing tomato industry. During my career I have spent a significant amount of time in the area of variety evaluation and new product development. I have observed several Zeneca trial sites in California during 1993 which included genetically transformed tomato varieties.

I can confirm that I have observed no agronomic differences between the transformed tomato varieties and their non-transformed variety counterparts. This includes evaluation of vine size and habit; fruit shape, size, color (internal and external) and fruit texture. Additionally no differences in fruit defects were observed. Each of these Zeneca trials were statistically randomized and grown under normal, standard field conventions.

California climatic conditions were also normal during this period of evaluation and observation. Temperatures ranged between 90°F and 100°F, rainfall was less than 0.5 inches.

I am pleased to confirm these agronomic and horticultural results. It was impossible to distinguish any difference whatsoever between traditionally breed non-transformed tomato varieties and their genetically transformed complements.

Sincerely yours,

Dave Storz
Business Manager

Tracy
(209) 832-8894 • Fax (209) 832-2216

Visalia
(209) 739-7333 • Fax (209) 739-7165

MACLAY BURT ASSOCIATES
[illegible]

13 June 1994

Ms. Anne Mueller
Zeneca Plant Science
1800 Concord Pike
Wilmington, DE 19897

Dear Ms. Mueller:

As an agribusiness consultant with extensive processing tomato experience and also as a photographer, I observed the Zeneca tomato trials for three days in the Fresno area in July, 1993. Several trials sites were visited.

I found no observable differences whatsoever between the several comparisons of "standard" and genetically transformed varieties. This included size, shape, color, internal color and texture and the number of processing inspection defects. All trials were statistically randomized and grown under standard field trials protocol.

There were no observable differences in tomato plant size, shape, vigor, color or harvest timing.

Weather was hot (up to 100F), typical of the area for that time. I judged the disease and insect pressures on the crop to be normal for the time and location.

In short, I found it impossible to detect any differences between the standard and transformed varieties.

Very truly yours,

Maclay Burt Associates

Maclay Burt

by: Maclay Burt

THE MORNING STAR COMPANY
724 MAIN STREET
WOODLAND, CALIFORNIA 95695
Phone 916/666-6600 • Fax 916/666-6690

June 15, 1994

Dr. Ed Green
Vice President, New Technology
Petoseed
37437 State Hwy 16
Woodland, CA 95695

Dear Dr. Green

During the summer tomato season of 1993, I was able to observe some of the transgenetic tomato material in California. I was not with The Morning Star Company during this time, but was with another major tomato processor. I actually observed the material in the Imperial Valley in June, and the Sacramento Valley in September. The ability to observe these various trials gave me a clearer understand of the potential impact of this new technology.

Most of the evaluations I preformed were of horticultural tomato characteristics. I was unable to do many quality characteristics of the tomato varieties, but was able to obtain a small amount of quality data. From a horticultural standpoint I could not tell any significant difference in the growing aspect of the control varieties verus the transgenetic material. Fruit shape, plant size, plant vigor all seemed consistent between the trialed materials. The presence of any diseases seemed absent in the material. The color, both external and internal, were the same from an observational standpoint. The conclusion to my observations were that there is no noticeable difference in a variety that has been altered against the control.

I feel that this new technology has the ability to keep the consumer supplied with a high quality, low cost supply of tomato products in the future. The potential ability to enhance disease and insect resistance also could have a positive effect on pesticide uses in the future.

This new technology has great interest to me and feel it is the next step in moving the tomato industry into the future.

Sincerely,

Jerald E. Gilbert

Analytical Procedures Used to Determin Contents of Important Nutrients in T7 Transgenic Tomato Hybrids and Controls

Ascorbic Acid	Ascorbic acid content is measured by visual titration of an acidic (Titration) ascorbate solution with the dye, 2,6 dichloroindophenol. The dye has been standardized so that the titration volume is directly proportional to ascorbic acid content.
Ash	% ash is the mineral fraction which remains after a high heat treatment which burns off the organic fraction. The sample is weighed before and after the operation to obtain the value.
Color, Hunter	This method determines color in tomato products using a Hunter tristimulus reflectance colorimeter. Color is reported in units of tristimulus Hunter L, a and b.
Fatty Acid	This procedure determines the types and amount of fatty acids Composition present in the triglycerides of an oil. It involves the esterification (GLC) of the fatty acids with BF_3 - methanol reagent, followed by the capillary GC analysis of the prepared fatty acid methylesters (FAME's). The fatty acids are obtained by the complete saponification of the triglycerides.
Fat (Soxhlet)	This method determines the percentage of ether extractable matter in food products. It involves a continuous solid liquid extraction in a Soxhlet apparatus. The extracted material is quantitated gravimetrically after solvent evaporations and reports. The ether extractable material is considered crude fat.
Mineral Elements	This method determines calcium, magnesium, sodium, potassium, (ICP) tin, iron, sulfur, phosphorus, silicon, lead, cadmium, copper and nickel in tomato products by inductively coupled plasm (ICP) spectroanalysis.
Pectin %	This method determines the amount of total pectin in a tomato product. The pectin is divided into three fractions by progressive extractions with distilled water, 0.4% sodium hexametaphosphate, 0.05 N sodium hydroxide. The amount of pectin is expressed as percent anhydrous galacturonic acid (AGA).
Protein	This method determines the percent crude protein in food products. The sample is digested, the nitrogen distilled off as ammonia, and the ammonia collected and back titrated to determine the nitrogen content of the sample. Percent nitrogen is converted to percent protein using an appropriate conversion factor.
Sugars (HPLC)	This method is based on HPLC separation of fructose, glucose and sucrose and their detection and quantitation with a refractive index (RI) detector.
Vitamin A	Carotenoids are extracted with a suitable solvent system, separated by chromatography on a MgO-Hyflo Super Cel and quantitatively determined by their light absorption at 436 nm.