PATENT ASSIGNMENT

Electronic Version v1.1
Stylesheet Version v1.1

SUBMISSION TYPE:	NEW ASSIGNMENT
NATURE OF CONVEYANCE:	Release of Security Interest

CONVEYING PARTY DATA

Name	Execution Date
Harris Trust and Savings Bank	09/29/2003

RECEIVING PARTY DATA

Name:	Seminis, Inc.
Street Address:	2700 Camino del Sol
City:	Oxnard
State/Country:	CALIFORNIA
Postal Code:	93030-7967

Name:	Seminis Vegetable Seeds, Inc.
Street Address:	2700 Camino del Sol
City:	Oxnard
State/Country:	CALIFORNIA
Postal Code:	93030-7967

PROPERTY NUMBERS Total: 13

Property Type	Number
Application Number:	09555820
Patent Number:	6087162
Patent Number:	5623066
Patent Number:	5677157
Patent Number:	5973232
Patent Number:	6291743
Patent Number:	6127605
Patent Number:	6143562
Patent Number:	6340785
Patent Number:	5998699

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Patent Number:	6002072
Patent Number:	6160201
Patent Number:	6060648

CORRESPONDENCE DATA

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Total Attachments: 104
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RELEASE AND REASSIGNMENT OF PATENTS

THIS RELEASE AND REASSIGNMENT dated September 29, 2003, by Harris Trust and Savings Bank, as agent, as hereinafter described;

WITNESSETH:

WHEREAS, Harris Trust and Savings Bank, an Illinois banking corporation, as agent, with its mailing address at 111 West Monroe Street, Chicago, Illinois 60603 (Harris Trust and Savings Bank in its capacity as such Agent, is referred to herein as the *"Secured Party"*) and Seminis, Inc., a Delaware corporation , and Seminis Vegetable Seeds, Inc., a California corporation, each with its mailing address at 2700 Camino del Sol, Oxnard, California 93030-7967 (individually a *"Debtor"*) and collectively the *"Debtors"*) are parties to a certain Security Agreement Re: Intellectual Property dated as of June 28, 1999 which was recorded in the United States Patent and Trademark Office on August 24, 1999, at Reel 010175, Frame 0673 (the *"Assignment"*), pursuant to which the Debtors granted a security interest in and collateral assignment of certain patents listed on Schedule A attached hereto and certain other property (collectively the *"Patents"*); and

WHEREAS, the Debtors have requested that the Secured Party release its security interests in the Patents and reassign the same to the Debtors;

NOW, THEREFORE, for good and valuable consideration, receipt and sufficiency of which are hereby acknowledged:

The Secured Party hereby releases its security interests in and collateral assignment of, and reassigns, grants and conveys to the Debtors without any representation, warranty, recourse or undertaking by the Secured Party, all of its right, title and interest, if any, in and to:

(i) *Patents*. Patents, whether now owned or hereafter acquired, or in which such Debtor now has or hereafter acquires any rights (the term *"Patents"* means and includes (i) all letters patent of the United States of America or any other country or any political subdivision thereof, all registrations and recordings thereof, and all applications for letters patent of the United States of America or any other country or any political subdivision thereof, including, without limitation, registrations, recordings and applications in the United States Patent and Trademark Office or in any similar office or agency of the United States of America, any state thereof or any other country or any political subdivision thereof and (ii) all reissues, continuations, continuations-in-part or extensions thereof), including, without limitation, each Patent listed on Schedule A hereto, and all of the inventions now or hereafter described and claimed in such Debtor's Patents;

(ii) *Patent Licenses*. Patent Licenses, whether now owned or hereafter acquired, or in which such Debtor now has or hereafter acquires any rights (the term *"Patent Licenses"* means and includes any written agreement granting to any person any right to exploit, use or practice any invention on which a Patent is owned by another person), including, without limitation, each Patent License listed on Schedule A hereto, and all royalties and other sums due or to become due under or in respect of such

Debtor's Patent Licenses, together with the right to sue for and collect all such royalties and other sums;

(iii) *General Intangibles and Records and Cabinets.* General intangibles relating to any of the above-described property and supporting evidence and documents relating to any of the above-described property, including, without limitation, written applications, correspondence, delivery receipts and notes, together with all books of account, ledgers and cabinets in which the same are reflected or maintained, all whether now existing or hereafter arising;

(iv) *Accessions and Additions.* All accessions and additions to, and substitutions and replacements of, any and all of the foregoing, whether now existing or hereafter arising; and

(v) *Proceeds and Products.* All proceeds and products of the foregoing and all insurance of the foregoing and proceeds thereof, whether now existing or hereafter arising, including, without limitation, (i) any claim of such Debtor against third parties for damages by reason of past, present or future infringement of any Patent or any Patent licensed under any Patent License, and (ii) any claim by such Debtor against third parties for damages by reason of past, present or future misappropriation or wrongful use or disclosure of any trade secret or other property or right described above or of any such trade secret or other property or right licensed under any license agreement described above, and together with the right to sue for and collect the damages described in the immediately preceding clauses (i) and (ii);

IN WITNESS WHEREOF, the Secured Party has caused this Release and Reassignment to be duly executed by its duly authorized officer as of the day and year first above written.

HARRIS TRUST AND SAVINGS BANK, as Agent as aforesaid

By _____
Name: Sandra J. Sanders
Title: Vice President

SVS3801P0010US

For: Genetic Factor Responsible For A Defective Endosperm Phenotype In Seeds, Plants Comprising Said Factor And Their Use In Hybridization Processes

SUMMARY OF THE INVENTION

This invention relates to a genetic male gametophytic factor responsible for a defective endosperm phenotype in seeds. This invention also relates to plants comprising the said factor, especially those obtained from such seeds and the use of such plants in processes for obtaining hybrid seeds and hybrid plants.

This invention relies on the identification made by the inventors of a genetic male gametophytic factor in plants, said factor being susceptible to correspond to one or several nucleotide sequence(s), and more particularly to one or several gene(s), and said factor being responsible for the defective endosperm phenotype of seeds borne by the fruits resulting from fertilization by the pollen of those plants.

Thus this invention provides for the first time processes for isolating this genetic male gametophytic factor in plants, or in parts of plants, and controlling its expression, as well as for isolating plants, or parts of plants, carrying such genetic male gametophytic factor, and more particularly seeds characterized by said defective endosperm phenotype.

This invention also provides processes for transferring this genetic male gametophytic factor from plants into other plants.

This invention also provides new tools for the study of seed maturation mechanism, and consequently for the study of seed quality.

This invention also provides new tools for the study of artificial seed production where seed maturation mechanism is the major limiting factor for successful technology development.

This invention also provides new process for obtaining hybrid seeds and plants, carrying predetermined characteristics, the hybridization technique being based on the use of the genetic male gametophytic factor, which use is comparable to the one of a male sterility

system (such as described in the French patent n° 2 542 569, or in the UK patent application n° 2 211 205). This hybridization technique can replace existing techniques like emasculation, chemical treatments, cytoplasmic or genetic male sterilities, self incompatability.

Thus this invention relates to a genetic male gametophytic factor derived from non-endospermic seeded plants, which genetic factor is capable, when expressed, of conferring a defective endosperm (De) phenotype to seeds, these defective endosperm seeds, also called deficient seeds when extracted from mature fruits, being unable to germinate on their own in soil, or any classical seed germination substrate used in agriculture and horticulture for plant production or in laboratories for seed germination tests (such as substrates described in Seed Science and Technology, Proceedings of the International Seed Testing Association, International Rules for Seed Testing, 1993, Annex to Chapter 5, p.148-150).

Title: Transgenic Plants Resistant to Geminivirus Infection
Reference No.: SVS3801P0020

COUNTRY	SERIAL NUMBER	FILING DATE	PATENT NUMBER	ISSUE DATE
United States	08/643,779	05/06/96	Pending	
PCT	US97/07563	05/05/97	Pending	
PCT	US97/07817	05/06/97	Pending	

SUMMARY OF THE INVENTION

This invention involves transgenic plants which are resistant to geminivirus infection, such as, but not limited to, infection by tomato yellow leaf curl virus (TYLCV). These transgenic plants contain in their chromosomal DNA, geminivirus DNA. The geminivirus DNA encodes at least one of the six open reading frames. For example, plants resistant to infection by TYLCV would contain TYLCV DNA in their chromosomal DNA. The TYLCV DNA, may be any portion of the viral genome, such as, but not limited to, the C1 and C4 open reading frames and portions of the C2 and C3 open reading frames. This invention also includes methods for making plants resistant to a geminivirus infection.

This invention involves a chimeric plant gene that contains two or three elements in sequence. The first element, is a promoter DNA segment, which is optional, but, if present, functions in plant cells. The second element is a DNA sequence encoding at least one open reading frame of a geminivirus. The third element of the chimeric gene is a 3' nontranslated termination segment. The promoter DNA segment, if present, and the 3' nontranslated termination segment are operatively linked to the DNA sequence.

The promoter DNA segment, if present, may be a constitutive promoter such as the cauliflower mosaic virus 35S promoter, the octopine synthase promoter, the nopaline synthase promoter and the mannopine synthase promoter with octopine synthase activators. Other promoters which function in plant cells can be used as well.

The DNA sequence encodes at least one open reading frame of a geminivirus. If the geminivirus is TYLCV, it is desired that the DNA sequence encode the C1 and C4 open reading frame and portions of the C2 and C3 open reading frames.

The 3' non-translated termination segment may be the 3' non-translated termination segment of the nopaline synthase gene (NOS-T). However, those skilled in the art will recognize that other terminators can be used.

This invention also involves a cassette containing the chimeric plant gene described above as well as a heterologous DNA segment containing said cassette. Plants transformed with said heterologous DNA segment are also contemplated.

Additionally, this invention involves a method of producing plants resistant to infection by a geminivirus, such as, but not limited to infection by tomato yellow leaf curl virus. The method involves first constructing a heterologous DNA segment comprising at least one cassette. The one cassette that must be present is referred to as an "effect" cassette. The effect cassette confers geminivirus resistance to a plant and contains a chimeric gene capable of expression in a plant cell. The chimeric gene contains two or three elements. The first element is a promoter DNA segment, which is optional, but, if present, functions in plant cells. The second element is a DNA sequence that encodes at least one of the six reading frames of geminivirus. For example, if the geminivirus is TYLCV, it is desired that the DNA sequence encode the C1 and C4 open reading frames and portions of the C2 and C3 open reading frames. The third element is a 3' non-translated termination segment. The promoter DNA segment, if present, and the 3' non-translated termination segment are operatively linked to the geminivirus DNA sequence.

The promoter DNA segment, if present, may be a constitutive promoter such as the cauliflower mosaic virus 35S promoter, the octopine synthase promoter, the nopaline synthase

promoter and the mannopine synthase promoter with octopine synthase activators. Other promoters which function in plant cells can be used as well.

The 3' non-translated termination segment may be the 3' non-translated termination segment of the nopaline synthase gene (NOS-T). However, those skilled in the art will recognize that other terminators can be used.

Additionally, the heterologous DNA segment may contain two cassettes. For example, the heterologous DNA segment may contain two cassettes. As discussed earlier, the first cassette is the "effect"cassette that contains a chimeric gene.

The second cassette may be a "selectable marker" cassette that contains a chimeric gene capable of expression in a plant cell. The chimeric gene contains three elements. The first element is a second promoter DNA segment that functions in plant cells. The second element is a DNA sequence that encodes for the expression of a protein which allows for selection of plant cells containing said cassette. The protein may encode antibiotic or herbicide resistance. For example, the protein may encode the enzyme neomycin phosphotransferase II. The third element is a second 3' non-translated termination segment.

The promoter of the effect cassette, if present, and the promoter of the selectable marker cassette may be the same or different. In addition, these promoters may be constitutive promoters such as the cauliflower mosaic virus 35S promoter, the octopine synthase promoter, the nopaline synthase promoter and the mannopine synthase promoter with octopine synthase activators. Other promoters which function in plant cells can be used as well.

The 3' non-translated termination segment of the effect and selectable marker cassettes may be the same or different. The 3' non-translated termination segment may be the 3' non-

translated termination segment of the nopaline synthase gene (NOS-T). However, those skilled in the art will recognize that other terminators can be used.

The effect cassette as well as any other cassettes, such as a selectable marker cassette, are linked together in the heterologous DNA segment. Plant cells are then transformed with this heterologous DNA segment. Transgenic plant cells containing this heterologous DNA segment are selected from non-transgenic plant cells that do not contain this heterologous DNA segment and then regenerated into transgenic plants which are resistant to geminivirus infection.

This invention also involves plants produced by the above described methods and seed produced by these plants.

Title: Carbon Based Process for the Selection of Transgenic Plant Cells
Reference No.: SVS3801P0030

COUNTRY	SERIAL NUMBER	FILING DATE	PATENT NUMBER	ISSUE DATE	EXPIRATION DATE
United States	09/076359	05/12/98	Pending		
United States	08/930,186	10/03/97	Pending		
Europe	969125780	04/05/96	Pending		
Australia	5535196	04/05/96	Pending		
China	961945400	04/05/96	Pending		
Mexico	977666	04/05/96	Pending		

SUMMARY OF THE INVENTION

This invention contemplates a process for selectively growing transformed plant cells cultured under heterotrophic conditions. Also contemplated is a process for selectively increasing the number of transformed plant cells regenerated from a mixture of transformed and non-transformed plant cells cultured under heterotrophic culture conditions. Still further contemplated is a method for increasing the number of transformed plant cells regenerated from a mixture of transformed and non-transformed plant cells under delayed heterotrophic culture conditions. Still even further contemplated are transformed plants whose genome contains an identifiable heterologous, exogenously supplied DNA segment that contains at least one expression cassette. A kit useful for transforming plant cells is also contemplated.

Thus, in one embodiment, a selection process for transformed plant cells is contemplated. In accordance with this process,

(a) a mixture of transformed and non-transformed plant cells is cultured under heterotrophic culture conditions in a culture medium that contains minimal nutrients required for growth and proliferation by those plant cells except for a source of carbon that is utilized to support that growth and proliferation. The source of carbon utilized is replaced by an encrypted or latent (growth-limiting) carbon source that does not support growth and proliferation by the non-transformed cells. The transformed cells of the mixture contain a genomic heterologous DNA segment that contains at least two expression cassettes,

The first expression cassette contains a heterologous DNA selectable marker segment that includes (i) a first heterologous gene that encodes a heterologous enzyme that on expression converts the encrypted carbon source into a carbon source that supports growth and proliferation by the transformed plant cells under heterotrophic culture conditions. The first gene is operatively linked to (ii) a first promoter DNA segment that controls expression of the heterologous gene, and (iii) a termination DNA segment. The second expression

cassette contains (i) a second gene that is expressed in a transformed plant, and that gene is operatively linked to (ii) a second promoter DNA segment that controls expression of that second gene and (iii) a termination DNA segment.

(b) , The heterotrophic culture conditions are maintained for a time period sufficient for the transformed plant cells to express the heterologous enzyme, proliferate and grow. Inasmuch as the non-transformed plant cells cannot utilize the encrypted or latent carbon source, those cells do not grow and proliferate. The transformed cells that do grow and proliferate can thereby be selected from the non-transformed cells.

A particularly preferred first gene encodes the enzyme phosphomannose isomerase (*pmi*; EC 5.3.1.8) that converts non-utilizable mannose-6-phosphate into fructose-6-phosphate that can be used by plant cells as a carbon source to support cell growth and proliferation. The *pmi* gene is also known as manA, and this gene is often referred to herein as *pmi/manA*. The encrypted (growth-limiting) carbon source useful with this first gene is mannose. Another preferred useful gene encodes mannitol-1-oxidoreductase that converts mannitol into mannose, and here, mannitol is the encrypted (growth-limiting) carbon source. This second gene and its encrypted carbon source are used in plant cells that have previously been transformed with a *pmi/manA* gene. Another preferred first gene encodes human L-iditol dehydrogenase (EC 1.1.1.14) that converts sorbitol into fructose, so that sorbitol is used as the encrypted (growth-limiting) carbon source. Similar aldehyde reductase enzyme genes can also be used.

The proliferating cells so produced and selected can thereafter be harvested or regenerated by culture in appropriate media into mature plants via meristematic tissue or embryos, or via callus tissue conversion into meristematic tissue or embryos. Thus, the selected proliferating cells are preferably collected thereafter regenerated into mature plants that grow autotrophically. The above process therefore more preferably utilizes the added steps of:

(c) recovering the selected proliferating cells; and/or

(d) regenerating plants from those proliferating cells.

The promoter of the first expression cassette is repressed by a product of the normal autotrophic metabolism of the transgenic plant, which product is also present in a non-transgenic plant. Exemplary preferred promoters include the cucumber isocitrate lyase promoter and the rice α-amylase Amy3A promoter.

The second gene and the genes in the subsequent cassettes can be any gene desired to be expressed in a plant, and its promoter and termination DNA segments can be any desired promoter and terminator that operate in plants.

In a second embodiment, a selection process for increasing the number of transformed plant cells from a mixture of transformed and non-transformed plant cells cultured under heterotrophic culture conditions is contemplated. In accordance with this process,

(a) a mixture of transformed and non-transformed plant cells is cultured under heterotrophic culture conditions in a culture medium that contains minimal nutrients required for proliferation and growth by non-transformed plant cells except for a source of carbon that supports growth and proliferation and about 1.5 to 3 times the standard amount of phosphorus. The source of carbon utilized is replaced by an encrypted carbon source that does not support growth and proliferation of said non-transformed cells. The transformed cells of the mixture have a heterologous DNA segment inserted into their genome that contains at least one expression cassette.

At least one expression cassette contains a heterologous DNA selectable marker segment that includes (i) a first heterologous gene that encodes a heterologous enzyme that on expression converts the encrypted carbon source into a carbon source that supports growth and proliferation by the transformed plant cells under heterotrophic culture conditions. The first gene is operatively linked to (ii) a first promoter DNA segment that controls expression of the heterologous gene, and (iii) a termination DNA segment.

(b) The heterotrophic culture conditions are maintained for a time period sufficient for the transformed plant cells to express the heterologous enzyme, proliferate and grow.

In a third embodiment, a selection process for increasing the number of transformed plant cells from a mixture of transformed and non-transformed plant cells cultured under delayed selective culture conditions is contemplated. In accordance with this process,

(a) a mixture of transformed and non-transformed plant cells is cultured for up to two weeks in a first culture medium that contains the minimal nutrients required for proliferation and growth by both, transformed and non-transformed plant cells including a source of carbon that supports growth and proliferation of both the transformed and non-transformed plant cells. The transformed plant cells have a heterologous genomic DNA segment that contains at least one expression cassette.

At least one expression cassette contains a heterologous DNA selectable marker segment that includes (i) a first heterologous gene that encodes a heterologous enzyme that on expression converts an encrypted carbon source into a carbon source that supports growth and proliferation of said transformed plant cells under heterotrophic culture conditions, said first gene being operatively linked to (ii) a first promoter DNA segment that controls expression of said heterologous gene, and (iii) a termination DNA segment.

(b) After sufficient time in the first medium, the transformed and non-transformed plant cells are removed from the first culture medium.

(c) The transformed and non-transformed pant cells are then placed under heterotrophic culture conditions in a second culture medium that contains the minimal nutrients required for proliferation and growth of the non-transformed plant cells except for an encrypted carbon source that does not support growth and proliferation of said non-transformed plant cells and 1.5 to 3 times the standard amount of phosphorous.

(d) The heterotrophic culture conditions are maintained for a time period sufficient for said transformed plant cells to express said heterologous enzyme, proliferate and grow.

A transgenic plant whose genome comprises a heterologous DNA segment that contains at least two expression cassettes is further contemplated.

The first expression cassette contains a heterologous DNA selectable marker segment that includes (i) a first heterologous gene that encodes a heterologous enzyme that on expression during heterotrophic culture of cells from the transformed plant converts an encrypted carbon source that does not support growth and proliferation of non-transformed plant cells of the same type into a carbon source that supports growth and proliferation of those transformed cells. That first gene is operatively linked to (ii) a promoter DNA segment

that controls expression of the heterologous gene and (iii) a termination DNA segment. The second expression cassette contains (i) a second gene that is expressed in the transformed plant that is operatively linked to (ii) a second promoter DNA segment that controls expression of the second gene and (iii) a termination DNA segment.

The before-noted preferences are also followed for the first gene and its promoter in the transgenic plant. The second gene and its promoter are also as discussed before.

A kit for forming transformed plant cells is also contemplated. That kit comprises:

(a) a first package containing a DNA segment for transforming plant cells that contains an expression cassette operatively linked to a linker segment containing at least one restriction endonuclease site. The expression cassette contains a heterologous DNA selectable marker segment that includes (i) a first heterologous gene that encodes a heterologous enzyme that on expression during heterotrophic culture of transformed plant cells converts an encrypted carbon source that does not support growth and proliferation of non-transformed plant cells into a carbon source that supports growth and proliferation the transformed cells. The first heterologous gene is operatively linked to (ii) a promoter DNA segment that controls expression of the heterologous gene and (iii) a termination DNA segment.

(b) a second package is also present that contains minimal nutrients required for growth and proliferation of plant cells during heterotrophic culture except for a source of carbon and about 1.5 to 3 times the standard amount of phosphorous. That source of carbon is replaced by an encrypted carbon source that does not support growth and proliferation of non-transformed plant cells but does support growth and proliferation of a transformed plant cell whose genome contains the DNA segment of the first package. Instructions for use of the kit components are also preferably provided.

This invention has several benefits and advantages.

One benefit of the invention is that its selective growth process does not rely upon potentially harmful antibiotics, herbicides or other possibly toxic materials.

One advantage of the invention is that its encrypted (growth-limiting) carbon source can be and preferably is a carbohydrate.

Another benefit of this invention is that expression of the selectable marker can be repressed in the regenerated plant under autotrophic growth conditions.

Another advantage of this invention is that selectable marker gene can be used with any second expressed gene.

Still another benefit of this invention is that the kit provides a ready means for inserting a second expression cassette into a plant transforming vector and an appropriate selection medium for the enhanced transformation and selected growth of transformed plant cells.

Still another advantage is that successive transformations can be made in which one encrypted (growth-limiting) carbohydrate can be converted by a second selectable marker gene into another encrypted (growth-limiting) carbohydrate that is converted into a useful carbon source by a first selectable marker gene also present in the transformed cells.

SUMMARY OF THE INVENTION

This invention contemplates a process for selectively growing transformed pant cells cultured under heterotrophic conditions. Also contemplated is a process for selectively increasing the number of transformed plant cells regenerated from a mixture of transformed and non-transformed plant cells cultured under heterotrophic culture conditions. Still further contemplated is a method for increasing the number of transformed plant cells regenerated from a mixture of transformed and non-transformed plant cells under delayed heterotrophic culture conditions. Still even further contemplated are transformed plants whose genome contains an identifiable heterologous, exogenously supplied DNA segment that contains at least one expression cassette. A kit useful for transforming plant cells is also contemplated.

Thus, in one embodiment, a selection process for transformed plant cells is contemplated. In accordance with this process,

(a) a mixture of transformed and non-transformed plant cells is cultured under heterotrophic culture conditions in a culture medium that contains minimal nutrients required for growth and proliferation by those plant cells except for a source of carbon that is utilized to support that growth and proliferation. The source of carbon utilized is replaced by an encrypted or latent (growth-limiting) carbon source that does not support growth and proliferation by the non-transformed cells. The transformed cells of the mixture contain a genomic heterologous DNA segment that contains at least two expression cassettes,

The first expression cassette contains a heterologous DNA selectable marker segment that includes (i) a first heterologous gene that encodes a heterologous enzyme that on expression converts the encrypted carbon source into a carbon source that supports growth and proliferation by the transformed pant cells under heterotrophic culture conditions. The

first gene is operatively linked to (ii) a first promoter DNA segment that controls expression of the heterologous gene, and (iii) a termination DNA segment. The second expression cassette contains (i) a second gene that is expressed in a transformed plant, and that gene is operatively linked to (ii) a second promoter DNA segment that controls expression of that second gene and(iii) a termination DNA segment.

(b) The heterotrophic culture conditions are maintained for a time period sufficient for the transformed plant cells to express the heterologous enzyme, proliferate and grow. Inasmuch as the non-transformed plant cells cannot utilize the encrypted or latent carbon source, those cells do not grow and proliferate. The transformed cells that do grow and proliferate can thereby be selected from the non-transformed cells.

A particularly preferred first gene encodes the enzyme phosphomannose isomerase (pmi; EC 5.3.1.8) that converts non-utilizable mannose-6-phosphate into fructose-6-phosphate that can be used by plant cells as a carbon source to support cell growth and proliferation. The pmi gene is also known as manA, and this gene is often referred to herein as pmi/manA. The encrypted (growth-limiting) carbon source useful with this first gene is mannose. Another preferred useful gene encodes mannitol-1-oxidoreductase that converts mannitol into mannose, and here, mannitol is the encrypted (growth-limiting) carbon source. This second gene and its encrypted carbon source are used in plant cells that have previously been transformed with a pmi/manA gene. Another preferred first gene encodes human L-iditol dehydrogenase (EC 1.1.1.14) that converts sorbitol into fructose, so that sorbitol is used as the encrypted (growth-limiting) carbon source. Similar aldehyde reductase enzyme genes can also be used.

The proliferating cells so produced and selected can thereafter be harvested or regenerated by culture in appropriate media into mature plants via meristematic tissue or embryos, or via callus tissue conversion into meristematic tissue or embryos. Thus, the selected proliferating cells are preferably collected and thereafter regenerated into mature plants that grow autotrophically. The above process therefore more preferably utilizes the added steps of:

(c) recovering the selected proliferating cells; and/or

(d) regenerating plants from those proliferating cells.

The promoter of the first expression cassette is repressed by a product of the normal autotrophic metabolism of the transgenic plant, which product is also present in a non-transgenic plant. Exemplary preferred promoters include the cucumber isocitrate lyase promoter and the rice α-amylase Amy3A promoter.

The second gene and the genes in the subsequent cassettes can be any gene desired to be expressed in a plant, and its promoter and termination DNA segments can be any desired promoter and terminator that operate in plants.

In a second embodiment, a selection process for increasing the number of transformed plant cells from a mixture of transformed and non-transformed plant cells cultured under heterotrophic culture conditions is contemplated. In accordance with this process,

(a) a mixture of transformed and non-transformed plant cells is cultured under heterotrophic culture conditions in a culture medium that contains minimal nutrients required for proliferation and growth by non-transformed plant cells except for a source of carbon that supports growth and proliferation and about 1.5 to 3 times the standard amount of phosphorus. The source of carbon utilized is replaced by an encrypted carbon source that does not support growth and proliferation of said non-transformed cells. The transformed cells of the mixture have a heterologous DNA segment inserted into their genome that contains at least one expression cassette.

At least one expression cassette contains a heterologous DNA selectable marker segment that includes (i) a first heterologous gene that encodes a heterologous enzyme that on expression converts the encrypted carbon source into a carbon source that supports growth and proliferation by the transformed plant cells under heterotrophic culture conditions. The first gene is operatively linked to (ii) a first promoter DNA segment that controls expression of the heterologous gene, and (iii) a termination DNA segment.

(b) the heterotrophic culture conditions are maintained for a time period sufficient for the transformed plant cells to express the heterologous enzyme, proliferate and grow.

In a third embodiment, a selection process for increasing the number of transformed plant cells from a mixture of transformed and non-transformed plant cells cultured under delayed selective culture conditions is contemplated. In accordance with this process,

(a) , a mixture of transformed and non-transformed plant cells is cultured for up to two weeks in a first culture medium that contains the minimal nutrients required for proliferation and growth by both, transformed and non-transformed plant cells including a source of carbon that supports growth and proliferation of both the transformed and non-transformed plant cells. The transformed plant cells have a heterologous genomic DNA segment that contains at least one expression cassette.

At least one expression cassette contains a heterologous DNA selectable marker segment that includes (i) a first heterologous gene that encodes a heterologous enzyme that on expression converts an encyrpted carbon source into a carbon source that supports growth and proliferation of said transformed plant cells under heterotrophic culture conditions, said first gene being operatively linked to (ii) a first promoter DNA segment that controls expression of said heterologous gene, and (iii) a termination DNA segment.

(b) After sufficient time in the first medium, the transformed and non-transformed plant cells are removed from the first culture medium.

(c) The transformed and non-transformed plant cells are then placed under heterotrophic culture conditions in a second culture medium that contains the minimal nutrients required for proliferation and growth of the non-transformed plant cells except for an encrypted carbon source that does not support growth and proliferation of said non-transformed plant cells and 1.5 to 3 times the standard amount of phosphorous.

(d) The heterotrophic culture conditions are maintained for a time period sufficient for said transformed plant cells to express said heterologous enzyme, proliferate and grow.

A transgenic plant whose genome comprises a heterologous DNA segment that contains at least two expression cassettes is further contemplated.

The first expression cassette contains a heterologous DNA selectable marker segment that includes (i) a first heterologous gene that encodes a heterologous enzyme that on expression during heterotrophic culture of cells from the transformed plant converts an

encrypted carbon source that does not support growth and proliferation of non-transformed plant cells of the same type into a carbon source that supports growth and proliferation of those transformed cells. That first gene is operatively linked to (ii) a promoter DNA segment that controls expression of the heterologous gene and (iii) a termination DNA segment. The second expression cassette contains (i) a second gene that is expressed in the transformed plant that is operatively linked to (ii) a second promoter DNA segment that controls expression of the second gene and (iii) a termination DNA segment.

The before-noted preferences are also followed for the first gene and its promoter in the transgenic plant. The second gene and its promoter are also as discussed before.

A kit for forming transformed pant cells is also contemplated. That kit comprises:

(a) a first package containing a DNA segment for transforming plant cells that contains an expression cassette operatively linked to a linker segment containing at least one restriction endonuclease site. The expression cassette contains a heterologous DNA selectable marker segment that includes (i) a first heterologous gene that encodes a heterologous enzyme that on expression during heterotrophic culture of transformed plant cells converts an encrypted carbon source that does not support growth and proliferation of non-transformed plant cells into a carbon source that supports growth and proliferation of non-transformed plant cells into a carbon source that supports growth and proliferation the transformed cells. The first heterologous gene is operatively linked to (ii) a promoter DNA segment that controls expression of the heterologous gene and (iii) a termination DNA segment.

(b) a second package is also present that contains minimal nutrients required for growth and proliferation of plant cells during heterotrophic culture except for a source of carbon and about 1.5 to 3 times the standard amount of phosphorous. That source of carbon is replaced by an encrypted carbon source that does not support growth and proliferation of non-transformed plant cells but does support growth and proliferation of a transformed plant cell whose genome contains the DNA segment of the first package. Instructions for use of the kit components are also preferably provided.

This invention has several benefits and advantages.

One benefit of this invention is that its selective growth process does not rely upon potentially harmful antibiotics, herbicides or other possibly toxic materials.

One advantage of this invention is that its encrypted (growth-limiting) carbon source can be and preferably is a carbohydrate.

Another benefit of this invention is that expression of the selectable marker can be repressed in the regenerated plant under autotrophic growth conditions.

Another advantage of this invention is that the selectable marker gene can be used with any second expressed gene.

Still another benefit of this invention is that the kit provides a ready means for inserting a second expression cassette into a plant transforming vector and an appropriate selection medium for the enhanced transformation and selected growth of transformed plant cells.

Still another advantage is that successive transformations can be made in which one encrypted (growth-limiting) carbohydrate can be converted by a second selectable marker gene into another encrypted (growth-limiting) carbohydrate that is converted into a useful carbon source by a first selectable marker gene also present in the transformed cells.

SVS3801P0040US

For: A Method of Visually Selecting Transformed Plant Cells or Tissue by Carotenoid Pigmentation

SUMMARY OF THE INVENTION

This invention involves a method for visually identifying and subsequently regenerating transgenic plants. This invention also provides a method for the visual identification of proprietary transgenic germplasm.

The method for visually identifying transgenic plant cells or tissues involves culturing non-transgenic (or non-transformed) and transgenic plant cells in a culture medium. The transgenic plant cells or tissues contain a heterologous, recombinant chimeric DNA segment which contains at least one expression cassette. An example of the plant cells or tissues that can be used in this method include but are not limited to tomato, curcurbits, pepper, lettuce and carrots.

At least one expression cassette must contain a promoter DNA segment which functions in specific plant cells to cause the production of an RNA sequence from the DNA segment described as the second component. The second component is a DNA segment which contains a plastid targeting signal fused to the amino terminal end of the coding region of the phytoene synthase gene from the *Erwinia* group of genes, which when expressed results in the production of a carotenoid. The preferred gene is the phytoene synthase gene from *Erwinia herbicola*.

The DNA segment containing the plastid targeting signal and phytoene synthase gene produces mRNA which encodes a chimeric polypeptide. The chimeric polypeptide is produced in the cytoplasm and then transported to the plastids of the plant cells by the plastid targeting signal contained in the DNA segment.

Title: A Method for Visually Selecting Transgenic Plant Cells or Tissues by Carotenoid Pigmentation
Reference No.: SVS3801P040

COUNTRY	SERIAL NUMBER	FILING DATE	PATENT NUMBER	ISSUE DATE
United States	08/543,608	10/16/95	Pending	
Europe		03/29/96	Pending	

The third component of the expression cassette is a 3' non-translated DNA segment. This segment contains sequences that in plant cells or tissues result in the termination of transcription and additional sequences that when transcribed into RNA result in the addition of a polyadenylate tract of residues to the 3' end of the RNA, which encodes the chimeric polypeptide.

The transgenic plant cells or tissues may also contain a heterologous, recombinant chimeric DNA segment which contains additional expression cassettes. The first expression cassette is the same as the expression cassette described above. It contains a suitable promoter DNA segment, a DNA segment containing a plastid targeting signal fused to the amino-terminal end of the coding region of the phytoene synthase gene from the *Erwinia* group of genes which when expressed results in the production of a carotenoid, and a 3' non-translated termination segment.

The second and subsequent expression cassettes will each contain a promoter segment that controls the expression of a DNA segment which encodes a second gene that is expressed in the transformed plant, and a 3' non-translated segment. The second and subsequent genes may be any DNA sequence that one wishes to express in plants.

The transgenic and non-transgenic plant cells or tissues are grown for a sufficient period of time in culture to allow the transgenic plant cells or tissues to express the phytoene synthase gene, and to accumulate a colored carotenoid product. Transgenic plant cells are identified from the non-transgenic plant cells by the appearance of orange or red color due to carotenoid pigmentation. Once the transgenic plant cells or tissues are identified, the transgenic plant cells are recovered and regenerated into plants.

The recombinant chimeric DNA segment described above can be inserted into a vector for use in the method of this invention. Any vector can be used in this invention; however, the

preferred vectors are those referred to as binary vectors. The DNA of interest can be delivered from the vector plasmid to the plant via *Agrobacterium*-mediated gene transfer.

In addition, the recombinant chimeric DNA segment can be introduced into the plant cells or tissues by a variety of other techniques which are well known to those skilled in the art such as electroporation, microinjection and microprojectile bombardment.

This invention also encompasses transgenic plants which contain the expression cassettes described above as well as seed generated from said transgenic plants.

This invention also involves a method for the visual identification of proprietary transgenic germplasm. The method involves culturing an explant (e.g. leaf, cotyledon, root or stem fragments) on a culture medium that promotes formation of callus tissue. The proprietary transgenic plants contain a heterologous, recombinant chimeric DNA segment which contains at least one expression cassette. An example of the plants that can be used in the method include but are not limited to tomato, curcubits, pepper, lettuce and carrots.

At least one expression cassette contains a promoter DNA segment which functions in specific plant cells or tissues to cause the production of an RNA sequence from the DNA segment described as the second component. The second component is a DNA segment which contains a plastid targeting signal fused to the amino-terminal end of the coding region of the phytoene synthase gene from the *Erwinia* group of genes, which when expressed results in the production of a carotenoid. The preferred gene is the phytoene synthase gene from *Erwinia herbicola*.

The DNA segment containing the plastid targeting signal and phytoene synthase gene produces RNA which encodes a chimeric polypeptide. The chimeric polypeptide is produced in

the cytoplasm and then transported to the plastids of the plant cells by the plastid targeting signal contained in the DNA segment.

The third component of the expression cassette is a 3' non-translated DNA segment. This segment contains sequences that in plant cells result in the termination of transcription and additional sequences that when transcribed into RNA result in the addition of a polyadenylate tract of residues to the 3' end of the RNA, which encodes the chimeric polypeptide.

The proprietary transgenic plants may also contain a heterologous, recombinant chimeric DNA segment which contains additional expression cassettes. The first expression cassette is the same as the first expression cassette described above. It contains a suitable promoter DNA segment, a DNA segment containing a plastid targeting signal fused to the amino-terminal end of the coding region of the phytoene synthase gene from the *Erwinia* group of genes which when expressed results in the production of a carotenoid, and a 3' non-translated termination segment.

The second and subsequent expression cassettes will each contain a promoter segment that controls the expression of a DNA segment, which encodes a second gene that is expressed in the transformed plant, and a 3' non-translated segment. The second and subsequent genes may be any DNA sequence that one wishes to express in plants.

To identify proprietary transgenic germplasm, the explant (e.g. leaf, cotyledon, root or stem fragments) is cultured for a sufficient period of time under conditions that allow for the creation of callus, and for the calli cells to express the phytoene synthase gene, and to accumulate a colored carotenoid product. Transgenic plants are identified by the appearance of an orange to red colored callus.

Finally, this invention involves a plasmid designated as pETO203 having American Type Culture Collection accesion number 97282.

Title: Cytoplasmic Male Sterile *Brassica Oleracea* Plants which Contain the Polima CMS Cytoplasm and are Male Sterile at High and Low Temperatures
Reference No.: SVS3801P0050

COUNTRY	SERIAL NUMBER	FILING DATE	PATENT NUMBER	ISSUE DATE	EXPIRATION DATE
United States	09/029,709	09/11/95	Pending		
Europe	959428285	09/11/95	Pending		
Australia	4404496	09/11/95	Pending		
Brazil	PCT/US95/11497	09/11/95	Pending		
Canada	2231423	09/11/95	Pending		
China	95197954	09/11/95	Pending		
Czech Republic	PCT/US95/11497	09/11/95	Pending		
Hungary	PCT/US95/11497	09/11/95	Pending		
Japan	PCT/US95/11497	09/11/95	Pending		
Norway	981050	09/11/95	Pending		
New Zealand	298533	09/11/95	Pending		
Poland	PCT/US95/11497	09/11/95	Pending		
Romania	PCT/US95/11497	09/11/95	Pending		
Russian Federation	98106843	09/11/95	Pending		
Ukraine	PCT/US95/11497	09/11/95	Pending		
South Korea	98701783	09/11/95	Pending		
Macedonia	PCT/US95/11497	09/11/95	Pending		

SVS3801P0050US

For: Cytoplasmic Male Sterile *Brassica Oleracea* Plants which Contain the Polima CMS Cytoplasm and Are Male Sterile at High and Low Temperatures

SUMMARY OF THE INVENTION

In an effort to increase the productivity of plants and food crops, plant breeders generally develop cultivars that contain certain desirable characteristics such as increased height, growth rate, higher yields, etc. One of the ways in which this may be accomplished is by infusing desirable characteristics into a plant to form a superior plant line. Superior lines are then combined to form an F_1 hybrid that contains the desirable characteristics. Such superior hybrids can be developed in numerous ways.

One popular way of producing superior hybrids is by using male sterility in one of the plants for which hybridization is desired. Male sterile lines allow the breeder to produce hybrid seed more economically by controlling cross-fertilization in the flower of a plant. Cross-fertilization can be controlled by preventing the female parent from self fertilizing. Self-fertilization is eliminated by making the plant male sterile. If the plant is male sterile, then no pollen can be produced for fertilization. Once rendered male sterile, the plant may then be hybridized with a gene donor plant possessing the desired characteristics.

One way to effectuate male sterility is through the use of cytoplasmic male sterility. Present belief is that genetic factors controlling cytoplasmic male sterility (CMS) are found in the cytoplasm, particularly in the genes of the mitochondrial DNA.

Three of the most common cytoplasmic male sterilities in the Brassica species are:

1) Ogura male sterile cytoplasm of *Raphanus sativus*;
2) Polima male sterile cytoplasm of *Brassica napus*; and
3) Nap male sterile cytoplasm of *Brassica napus*.

In *Brassica*, cytoplasmic male sterility can be transmitted by crossing. The female (egg) parent contributes the cytoplasm, therefore, crossing to CMS females produces CMS progeny. The nuclear genes however are heterozygous. Therefore, six to eight generations

of "backcrossing" are necessary to produce a CMS line breeding homozygous for nuclear characters. As an alternative, cytoplasmic male sterile lines can also be produced by protoplast fusion. In protoplast fusion, a protoplast from a plant having commercially desirable traits is combined with a protoplast of a CMS line is either removed or inactivated prior to fusion so it donates only the cytoplasm. The resulting cytoplasmic hybrid (or cybrid) possesses the CMS cytoplasm and is male sterile. For example, U.S. Patent 5,254,802 discloses *B. oleracea* plants that contain the Ogura CMS cytoplasm. These plants were obtained by protoplast fusion.

Polima CMS cytoplasm has been used to produce CMS in varieties such as winter-type oil seed rape (*Brassica napus*) (See Barsby et al., Plant Science, 53: 243-248 (1987)). However, one significant problem with the expression of cytoplasmic male sterility by the polima CMS cytoplasm is that the polima cytoplasm is influenced by environmental conditions. Fan, Z et al. Can. J. Plant Sci. 66:221-227 (1985). More specifically, male sterile plants containing polima CMS cytoplasm are known to become fertile under high temperatures in the field. Id. See also Fu, T.D., Encarpia Cruciferea Newsletter 6: 6-7 (1981).

This invention involves *Brassica oleracea* plants that contain Polima CMS cytoplasm which remain male sterile at high and low temperatures and exhibit good female fertility. The *Brassica oleracea* plants of this invention can be produced by traditional breeding methods. Different *Brassica* types can then be developed by further crossing or backcrossings or by protoplast fusion.

To obtain the *Brassica oleracea* plants of this invention by traditional breeding techniques, an interspecific cross was made between *Brassica campestris* cultivar 87110 and *Brassica oleracea* cultivar 87101. The seeds resulting from the cross are collected, planted and regenerated. The resulting plants are *Brassica napus* and contain a haploid set of chromosomes. The chromosomal content of said *Brassica napus* must be doubled by treating the plants with colchicine.

A second interspecific cross is performed by crossing *Brassica napus* cultivar 87118 with *Brassica oleracea* cultivar 87101. The seeds resulting from the cross are collected,

planted and regenerated. As in the previous cross, the resulting plants are *Brassica napus* and contain a haploid set of chromosomes. The plants are treated with colchicine to double their chromosome content.

The *Brassica napus* plants produced as a result of the second interspecific cross are next crossed with a *Brassica cultivar* 87102, which contains polima CMS cytoplasm and is male sterile. The seeds resulting form the cross are collected, planted and regenerated. The regenerated plants are *Brassica napus* and contained the polima CMS cytoplasm.

The resulting plants are subsequently crossed with the *Brassica napus* plants produced as a result of the first interspecific cross. The seeds resulting from the cross are collected, planted and regenerated. The regenerated plants are *Brassica napus*, contain the Polima CMS cytoplasm and are male sterile.

The resulting plants then crossed with a normal *Brassica oleracea*. As a result of the cross, siliques are produced, collected and examined for seeds. The seeds are collected for embryo rescue, because typically, embryos produced from such interspecific hybridization abort prior to maturation. However, by employing embryo rescue techniques, interspecific hybrid plants can be produced. The resulting F_1 plants contain the polima CMS cytoplasm from the female Brassica napus, however; the nuclear DNA content is a combination of the *Brassica napus* (N=19) and the *Brassica oleracea* (N=9).

The resulting plants are then backcrossed with a *Brassica oleracea*. Siliques are again produced, collected and examined for seeds. The seeds are collected for embryo rescue. The embryos are then regenerated as in the previous cross. The resulting plants are intermediate for chromosome number and contain the polima CMS cytoplasm. The nuclear content of the plants is a combination of *Brassica napus* and *Brassica oleracea*.

The resulting plants are then backcrossed with Brassica oleracea. Siliques are again produced, collected and examined for seeds. The seeds are sown. The resulting plants are Brassica oleracea which are male sterile and contain the polima CMS cytoplasm.

Optionally, the male sterile *Brassica oleracea* plants may be further crossed or backcrossed to produce different *Brassica* types. Siliques will again be produced, collected

and examined for seeds. The seeds are sown. The resulting plants are *Brassica oleracea* which contain the polima cytoplasm and are male sterile.

Different *Brassica* types can also be produced by protoplast fusion. A protoplast from a male sterile Brassica oleracea containing the polima CMS cytoplasm and inactivated nuclei is fused with a protoplast of a *Brassica* having commercially desirable characteristics. After the fusion, the allogenic cells are regenerated into CMS *Brassica* plants. The resulting plants are male sterile and contain the polima cytoplasm. The regenerated CMS *Brassica* plants contain the polima cytoplasm and can be employed in crossings with other *Brassica* types containing commercially desirable characteristics.

SVS3801P0115US

For: Potyvirus Coat Protein Genes and Plants Transformed Therewith

SUMMARY OF THE INVENTION

This invention relates to the coat protein genes of Papaya Ringspot Virus Strain papaya ringspot (PRV-p), Watermelon Mosaic Virus II (WMVII) , and Zucchini Yellow Mosaic Virus (ZYMV).

This invention relates to a recombinant DNA molecule which encodes a potyvirus coat protein. This invention relates to a recombinant DNA molecule comprising a potyvirus coat protein gene operably linked to genetic regulatory sequences necessary for gene expression.

This invention relates to expression vectors which contain a coat protein gene for potyviruses, and, additionally, the necessary genetic regulatory sequences needed for expression of a gene transferred into a plant. This invention also relates to bacterial or plant cells which are transformed with an expression vector containing the coat protein genes. Furthermore, this invention relates to transgenic plants which are produced from plant cells transformed with an expression vector containing the coat protein gene from potyviruses. In addition, this invention relates to a process of producing transgenic plants which have increased resistance to viral infection.

Title: *Lycopersicon Pimpinellifolium* as a Source of Resistance to the Plant Pathogen *Phytophthora Infestans*
Reference No.: SVS3801P060

COUNTRY	SERIAL NUMBER	FILING DATE	PATENT NUMBER	ISSUE DATE
United States	08/621,352	5/22/98	Issue Fee Paid	
Europe			Pending	
Australia			Pending	
Brazil			Pending	
Canada			Pending	
China			Pending	
Israel			Pending	
Japan			Pending	
South Korea			Pending	

SVS3801P0060US

For: *Lycopersicon Pimpinellifolium* As A source Of Resistance To The Plant Pathogen *Phytophthora Infestans*

SUMMARY OF THE INVENTION

This invention involves a method for producing tomato plants (*Lycopersicon esculentum*) which are resistant to the tomato strain of P. *infestans* races 0 and 1. These plants are produced by crossing a *Lycopersicon pimpinellifolium* plant which was discovered to contain a new allele(s) which confers resistance to P. *infestans* races 0 and 1 with a *Lycopersicon esculentum*. After the cross is made, the seed is collected and regenerated into plants. The resulting plants are evaluated for resistance to the tomato strain of P. *infestans* races 0 and 1. Plants that demonstrate resistance are identified and selected. These selected resistant plants are backcrossed with other *Lycopersicon esculentum* lines displaying desirable phenotypes to obtain commercially acceptable varieties which are resistant to the tomato strain of P. *infestans* races 0 and 1.

The *Lycopersicon pimpinellifolium* selection which was discovered to have novel resistance to P. *infestans* races 0 and 1 and subsequently used in crosses with L. *esculentum* is designated as LA 2533, which has also been referred to by the inventors as Hope 84.

The plants of this invention can also be produced by protoplast fusion. To produce plants by protoplast fusion, a protoplast from a *Lycopersicon pimpinellifolium* plant which is resistant to the tomato strain of P. *infestans* races 0 and 1 is obtained along with a protoplast from a *Lycopersicon esculentum*. The protoplasts are then fused using standard protoplast fusion procedures which are well known in the art. The resulting allogenic cells are obtained and regenerated into plants which are evaluated for resistance to the tomato strain of P. *infestans* races 0 and 1. Resistant plants are identified and selected.

The *Lycopersicon esculentum* plants produced according to the method of this invention are resistant to the tomato strain of P. *infestans*, races 0 and 1 and remain resistant to P. *infestans* races 1 in the field when the disease pressure is high.

This invention also involves tomato plants which contain an allele(s) which confers resistance to the tomato strain of P. *infestans* races 0 and 1 and seed produced by said tomato plants.

Title: Transgenic Plants Expressing DNA Construct Containing A Plurality of Genes to Impart Virus Resistance
Reference No.: SVS3801P0080

COUNTRY	SERIAL NUMBER	FILING DATE	PATENT NUMBER	ISSUE DATE	EXPIRATION DATE
United States	08/860,379	06/25/97	Pending		
Chile	207495	12/29/95	Pending		
Spain	109495	12/30/95	Pending		
Israel	116114	11/23/95	Pending		
India	1554/CAL/95	11/30/95	Pending		
Thailand	029071	12/04/95	Pending		
Europe	959228750	06/07/95	Pending		
Australia	2761395	06/07/95	Pending		
China	951972073	06/07/95	Pending		
Mexico	974794	06/07/95	Pending		

SVS3801P0081US

For: Transgenic Plants Expressing DNA Construct Containing A Plurality Of Genes To Impart Virus Resistance

SUMMARY OF THE INVENTION

This invention provides a recombinant chimeric DNA molecule comprising a plurality of DNA sequences each of which comprises a promoter operably linked to a DNA sequence which encodes a virus-associated protein, such as a coat protein (cp), a protease, or a replicase, wherein said DNA sequences are expressed in virus-susceptible plant cells transformed with said recombinant DNA molecule to impart resistance to infection by each of said viruses. Preferably, the DNA sequences are linked in tandem, i.e., exist in head to tail orientation relative to one another. Also, preferably substantially equal levels of resistance to infection by each of said viruses occurs in plant cells transformed with said plurality of DNA sequences.

Preferably, each DNA sequence is also linked to a 3' nontranslated DNA sequence which functions in plant cells to cause the termination of transcription and the addition of polyadenylated ribonucleotides to the 3' end of the transcribed mRNA sequences. Preferably, the virus is a plant-associated virus, such as potyvirus.

Thus, this DNA molecule can be employed as a chimeric recombinant "expression construct", or "expression cassette" to prepare transgenic plants that exhibit increased resistance to infection by at least two plant viruses, such as potyviruses. These cassettes also preferably comprise at least one selectable marker gene or reporter gene which is stably integrated into the genome of the transformed plant cells in association with the viral genes. The selectable marker and/or reporter genes facilitate identification of transformed plant cells and plants. Preferably, the virus gene array is flanked by two ore more selectable marker genes, reporter genes or a combination thereof. Another aspect of this invention is a method of preparing a virus-resistant plant, such as a dicot, comprising:

cassettes are employed to transfer two or more virus coat protein transformation-susceptible genes into plants, such as members of the *Cucurbitaceae* family, along with the associated selectable marker and/or reporter genes.

Thus, this invention provides a genetic engineering methodology by which multiple traits can be manipulated and tracked as a single gene insert, i.e., as a construct which acts as a single gene which segregates as a single Mendelian locus. Although this invention is exemplified via virus resistance genes, in practice, any combination of genes could be linked. Therefore one could track a block of genes that provide traits such as disease resistance, plus enhanced herbicide resistance, plus extended shelf life, and the like, by simply tracking the linked selectable marker or reporter gene which has been incorporated into the transformation vector.

It was also discovered that when multiple tandem genes are inserted, they preferably all exhibit substantially the same degrees of efficacy, and more preferably substantially equal degrees of efficacy, wherein the term "substantial" as it relates to viral resistance is defined with reference to the assays described in the examples herein below. For example if one examines numerous transgenic lines containing an intact ZYMV and WMV-2 coat protein insert, one finds that if a line is immune to infection by ZYMV it is also immune to infection by WMV-2. Similarly, if a line exhibits a delay in symptom development to ZYMV it will also exhibit a delay in symptom development in WMV2. Finally, if a line is susceptible to ZYMV it will be susceptible to WMV-2. This phenomenon is unexpected. If there were not a correlation between the efficacy of each gene in these multiple gene constructs this approach as a tool in plant breeding would probably be prohibitively difficult to use. Even with single gene constructs, one must test numerous transgenic plant lines to find one that displays the appropriate level of efficacy. The probability of finding a line with useful levels of expression can range from 10-15% (depending on the species involved).

If the efficacy of individual genes in a Ti plasmid containing multiple genes were independent, the probability of finding a transgenic line that was resistant to each targeted virus would decrease dramatically. For example, in a species in which there is a 10% probability of identifying a line with resistance using a single gene insert, is transformed with

a triple-gene construct CZW and each gene display an independent levels of efficacy, the probability of finding a line with resistance to CMV, ZYMV and WMV-2 would be 0.1 X 0.1 X 0.1 = 0.001 or 0.1%. However, since the efficacy of multivalent genes is not independent of each other the probability of finding a line with resistance to CMV, ZYMV and WMV-2 is still 10% rather than 0.1%. Obviously this advantage becomes more pronounced as constructs containing four or more genes are used.

Title: Papaya Ringspot Virus Protease Gene
Reference No.: SVS3801P0090

COUNTRY	SERIAL NUMBER	FILING DATE	PATENT NUMBER	ISSUE DATE	EXPIRATION DATE
United States	08/366,490	12/30/94	Issue Fee Paid		
United States	08/860,483	06/26/97	Pending		
Chile	206995	12/29/95	Pending		
Israel	116119	11/23/95	Pending		
India	1550/CAL/95	11/30/95	Pending		
Thailand	029075	12/04/95	Pending		
Europe	969328202	06/07/95	Pending		
Mexico	974792	06/07/95	Pending		
Australia	2818395	06/07/95	Pending		

SUMMARY OF THE INVENTION

This invention provides an isolated and purified DNA molecule that encodes the protease for the FLA83 W-type strain of papaya ringspot virus (PRV) or the protease for the PRV USA P-type (HA attenuated) strain. This invention also provides an isolated and purified DNA molecules that encodes the protease and flanking gene segments for the FLA83 W-type strain of papaya ringspot virus (PRV) or the protease and flanking gene segments for the PRV USA P-type (HA attenuated) strain. The invention also provides a chimeric expression cassette comprising at least one of these DNA molecules, a promoter which functions in plant cells to cause the production of an RNA molecule, and at least one polyadenylation signal comprising 3' nontranslated DNA which functions in plant cells to cause the termination of transcription and the addition of polyadenylated ribonucleotides to the 3' end of the transcribed mRNA sequences, wherein the promoter is operably linked to the DNA molecule, and the DNA molecule is operably linked to the polyadenylation signal. Another embodiment of the invention is exemplified by the insertion of multiple virus gene expression cassettes into one purified DNA molecule, e.g., a plasmid. Preferably, these cassettes include the promoter of the 35S gene of cauliflower mosaic virus and the polyadenylation signal of the cauliflower mosaic virus 35S gene.

Also provided are bacterial cells, and transformed plant cells, containing the chimeric expression cassettes comprising the protease gene derived from the FLA83 W-type strain of papaya ringspot virus (referred to herein as PRV FLA83 W) or from the USA P-type (HA attenuated) strain of PRV, and preferably the 35S promoter of cauliflower mosaic virus and the polyadenylation signal of the cauliflower mosaic virus 35S promoter of cauliflower mosaic virus and the polyadenylation signal of the cauliflower mosaic virus 35S gene. Plants are also provided, wherein the plants comprise a plurality of transformed cells transformed with an expression cassette containing the protease gene derived from the PRV FLA83 W

strain or from the USA P-type (HA attenuated) strain of PRV, and preferably the cauliflower mosaic virus 35S promoter and the polyadenylation signal of the cauliflower mosaic virus gene. Transformed plants of this invention include tobacco, corn, cucumber, peppers, potatoes, soybean, squash, and tomatoes. Especially preferred are members of the *Cucurbitaceae* (e.g., squash and cucumber) family.

Another aspect of this invention is a method of preparing a PRV-resistant plant, such as a dicot, comprising: transforming plant cells with a chimeric expression cassette comprising a promoter functional in plant cells operably linked to a DNA molecule that encodes a protease as described above; regenerating the plant cells to provide a differentiated plant; and identifying a transformed plant that expresses the PRV protease at a level sufficient to render the plant resistant to infection by the specific strains of PRV disclosed herein.

SVS3801P0091US

For: Papaya Ringspot Virus NIa Protease Gene

SUMMARY OF THE INVENTION

This invention provides an isolated and purified DNA molecule that encodes the protease for the FLA83 W-type strain of papaya ringspot virus (PRV) or the protease for the PRV USA P-type (HA attenuated) strain. This invention also provides an isolated and purified DNA molecule that encodes the protease and flanking gene segments for the FLA83 W-type strain of papaya ringspot virus (PRV) or the protease and flanking gene segments for the PRV USA P-type (HA attenuated) strain. The invention also provides a chimeric expression cassette comprising at least one of these DNA molecules, a promoter which functions in plant cells to cause the production of an RNA molecule, and at least one polyadenylation signal comprising 3' nontranslated DNA which functions in plant cells to cause the termination of transcription and the addition of polyadenylated ribonucleotides to the 3' end of the transcribed mRNA sequences, wherein the promoter is operably linked to the DNA molecule, and the DNA molecule is operably linked to the polyadenylation signal. Another embodiment of the invention is exemplified by the insertion of multiple virus gene expression cassettes into one purified DNA molecule, e.g., a plasmid. Preferably, these cassettes include the promoter of the 35S gene of cauliflower mosaic virus and the polyadenylation signal of the cauliflower mosaic virus 35S gene.

Also provided are bacterial cells, and transformed plant cells, containing the chimeric expression cassettes comprising the protease gene derived from the FLA83 W-type strain of papaya ringspot virus (referred to herein as PRV FLA83 W) or from the USA P-type (HA attenuated) strain of PRV, and preferably the 35S promoter of cauliflower mosaic virus and the polyadenylation signal of the cauliflower mosaic virus 35S gene. Plants are also provided, wherein the plants comprise a plurality of transformed cells transformed with an expression cassette containing the protease gene derived from the PRV FLA83 W strain or from the USA P-type (HA attenuated) strain of PRV, and preferably the cauliflower mosaic virus 35S promoter and the polyadenylation signal of the cauliflower mosaic virus gene.

Transformed plants of this invention include tobacco, corn, cucumber, peppers, potatoes, soybean, squash, and tomatoes. Especially preferred are members of the *Cucurbitaceae* (e.g., squash and cucumber) family.

Another aspect of this invention is a method of preparing a PRV-resistant plant, such as a dicot, comprising: transforming plant cells with a chimeric expression cassette comprising a promoter functional in plant cells operably linked to a DNA molecule that encodes a protease as described above; regenerating the plant cells to provide a differentiated plant; and identifying a transformed plant that expresses the PRV protease at a level sufficient to render the plant resistant to infection by the specific strains of PRV disclosed herein.

Title: Potyvirus Coat Protein Genes and Plants Transformed Therewith
Reference No.: SVS3801P0110

COUNTRY	SERIAL NUMBER	FILING DATE	PATENT NUMBER	ISSUE DATE	EXPIRATION DATE
United States	08/358,653	12/19/94	Issue Fee Paid		
Canada	607036	07/31/89	1329561	05/17/94	05/17/1
Australia	3987089	07/20/89	634168	02/18/93	07/20/09
Austria	899087688	07/20/89	0429483	11/12/97	07/20/09
Belgium	899087688	07/20/89	0429483	11/12/97	07/20/09
France	899087688	07/20/89	0429483	11/12/97	07/20/09
Germany	899087688	07/20/89	0429483	11/12/97	07/20/09
Italy	899087688	07/20/89	0429483	11/12/97	07/20/09
Netherlands	899087688	07/20/89	0429483	11/12/97	07/20/09
Sweden	899087688	07/20/89	0429483	11/12/97	07/20/09
Switzerland	899087688	07/20/89	0429483	11/12/97	07/20/09
Great Britain	899087688	07/20/89	0429483	11/12/97	07/20/09
Luxembourg	899087688	07/20/89	0429483	11/12/97	07/20/09
Europe 2	951122282	07/20/89	Pending		
Europe 3	951122290	07/20/89	Pending		

SUMMARY OF THE INVENTION

This invention relates to the following Lettuce Infectious Yellows Virus (LIYV) genes: the coat protein gene [SEQ ID NO: 1], the heat shock protein-70 gene [SEQ ID NO: 6], the RNA polymerase gene [SEQ ID NO:11], the gene encoding open reading frame 3 (ORF) of LIYV RNA1 [SEQ ID NO: 16], and the gene encoding ORF 6 of the LIYV RNA2 [SEQ ID NO: 21].

More specifically, this invention relates an isolated nucleic acid which contains a nucleotide sequence which encodes at least a portion of one of five LIYV proteins: the coat protein, the heat shock protein-70, RNA polymerase, the protein encoded by the gene positioned at ORF 3 of LIYV RNA1, and the protein encoded by the gene positioned at ORF 6 of LIYV RNA2. The nucleotide sequences for these proteins, either in the sense or the antisense orientation, are operably linked to genetic regulatory sequences necessary for gene expression to form plant transformation vectors. Specifically, an LIYV nucleotide sequence, or its antisense complement, is operably linked to and positioned downstream from a promoter and a polyadenylation signal is operably linked and positioned downstream from a nucleotide sequence.

Plant transformation vectors which contain a gene, or a portion of a gene, for a lettuce infectious yellows virus protein, such as the coat protein gene, the heat shock protein-70 gene, the RNA polymerase gene, the LIYV RNA1 ORF 3 gene, and a portion of the LIYV RNA2 ORF 6 gene and, additionally, the necessary genetic regulatory sequences needed for expression of a gene transferred into a plant, are used to transform bacterial or plant cells with the LIYV gene or genes present in the isolated nucleic acid. Furthermore, the invention relates to transgenic plants which are produced from plant cells transformed with an isolated nucleic acid containing a nucleotide sequence or nucleotide sequence fragment from lettuce

infectious yellows virus, the gene or fragment being selected from the group consisting of the coat protein gene, the heat shock protein-70gene, the RNA polymerase gene, the LIYV RNA1 ORF 3 gene, and the LIYV RNA2 ORF 6 gene. In addition, the invention relates to a process of producing transgenic plants which have increased resistance to viral infection.

very limiting

We claim:

1. A method of transforming and regenerating squash plants, which comprises (1) excising shoot tips from germinating squash, (2) transforming embryogenic calli by inoculating the excised squash tissue with Agrobacterium comprising a DNA construct having a beneficial gene and a plant expressible selection marker gene and culturing the resulting explant on an induction media comprising MS media, 2,4,5-T, BAP, and Kn and (3) selectively growing the transformed embryogenic calli on media containing a selection agent for the plant expressible selection marker gene and (4) subjecting the transformed embryogenic calli to an embryogenic regeneration procedure from which whole transformed squash plants can be obtained.

2. A method of transforming and regenerating squash plants, which comprises (1) excising shoot tips from germinating squash seeds, (2) culturing the excised squash tissue on an induction media comprising MS media, 2,4,5-T, BAP, and Kn and introducing foreign DNA having a beneficial gene and a plant expressible selection marker gene into the resulting embryoid tissues by bombardment with microprojectiles and (3) selectively growing the transformed embryogenic calli on media containing a selection agent for the plant expressible selection marker gene and (4) subjecting the transformed embryogenic calli to an embryogenic regeneration procedure from which whole transformed squash plants can be obtained.

3. A method according to claim 1, wherein transformed embryoids are identified by their expression of antibiotic or herbicide resistance.

4. A method of transforming and regenerating squash plants, which comprises (1) excising tissue from mature squash seeds, (2) transforming embryogenic calli by inoculating said excised squash tissue with Agrobacterium comprising a DNA construct having a beneficial gene and a plant expressible selection marker gene and culturing on an induction media comprising MS media, 2,4-D or 2,4,5-T, BAP, and Kn, (3) selectively growing the transformed embryogenic calli on media containing a selection agent for the plant expressible selection marker gene and (4) subjecting the transformed embryogenic calli to an embryogenic regeneration procedure from which whole transformed squash plants can be obtained.

5. A method of transforming and regenerating squash plants, which comprises (1) excising tissue from mature squash seeds, (2) transforming embryogenic calli by culturing the excised squash tissue on an induction media comprising MS media, 2,4-D or 2,4,5-T, BAP, and Kn and introducing foreign DNA having a beneficial gene and a plant expressible selection marker gene into the resulting embryoid by microprojectile bombardment, (3) selectively growing the transformed embryogenic calli on media containing a selection agent for the plant expressible selection marker gene and (4) subjecting the transformed embryogenic calli to an embryogenic regeneration procedure from which whole transformed squash plants can be obtained.

6. A process according to claim 1, wherein the plant expressible selection marker gene is the NPTII gene and the selection agent is kanamycin.

7. A method according to claim 2, wherein transformed embryoids are identified by their expression of antibiotic or herbicide resistance.

8. A method according to claim 4, wherein transformed embryoids are identified by their expression of antibiotic or herbicide resistance.

Title: Somatic Embryogenesis of Squash
Reference No.: SVS3801P0140

COUNTRY	SERIAL NUMBER	FILING DATE	PATENT NUMBER	ISSUE DATE	EXPIRATION DATE
United States	08/349,759	12/05/94	5,677,157	10/14/97	10/14/14
Belgium	908127623	08/22/90	0491733	11/30/94	08/22/10
France	908127623	08/22/90	0491733	11/30/94	08/22/10
Germany	908127623	08/22/90	0491733	11/30/94	08/22/10
Italy	908127623	08/22/90	0491733	11/30/94	08/22/10
Netherlands	908127623	08/22/90	0491733	11/30/94	08/22/10
Spain	908127623	08/22/90	0491733	11/30/94	08/22/10

SVS3801P0143US

For: Somatic Embryogenesis of Squash

SUMMARY OF THE INVENTION

This invention provides a method of regenerating and transforming Cucurbita pepo L. (squash) plants, which belong to the family Cucurbitaceae, which comprises (1) excising squash tissue selected from the group consisting of shoot tips from germinating squash seeds and squash tissue from mature seeds, (3) producing embryogenic calli from said tissues, being either non-transformed or transformed, (4) selectively growing the transformed embryogenic calli on media containing kanamycin, and (5) subjecting the transformed embryogenic calli to an embryogenic regeneration procedure from which whole transformed squash plants can be obtained.

When the excised tissue is shoot tips from germinating squash seed, the transformed embryogenic calli is produced by (a) inoculating said excised squash tissue with virulent or avirulent strains of *Agrobacterium* and (b) culturing the resulting explants on an induction media consisting of MS media, 2, 4, 5-T, BA, Kn.

When the excised tissue is tissue from mature squash seed, the transformed embryogenic calli is produced by (a) inoculating said excised squash tissue with virulent or avirulent strains of *Agrobacterium* and (b) culturing the resulting explants on an induction media consisting of MS media, 2, 4-D or 2, 4, 5-T, BA, Kn.

9. A method according to claim 5, wherein transformed embryoids are identified by their expression of antibiotic or herbicide resistance.

10. A process according to claim 2, wherein the plant expressible selection marker gene is the NPTII gene and the selection agent is kanamycin.

11. A process according to claim 4, wherein the plant expressible selection marker gene is the NPTII gene and the selection agent is kanamycin.

12. A process according to claim 5, wherein the plant expressible selection marker gene is the NPTII gene and the selection agent is kanamycin.

Title: Papaya Ringspot Virus Replicase Gene
Reference No.: SVS3801P0150

COUNTRY	SERIAL NUMBER	FILING DATE	PATENT NUMBER	ISSUE DATE	EXPIRATION DATE
United States	8/860,519	06/30/97	Pending		
Chile	207295	12/29/95	Pending		
Israel	116117	11/23/95	Pending		
India	1549/CAL/95	11/30/95	Pending		
Thailand	029069	12/04/95	Pending		
Europe	959216201	06/07/95	Pending		
Australia	2663795	06/07/95	Pending		

Another aspect of this invention is a method of preparing a PRV-resistant plant, such as a dicot, comprising: transforming plant cells with a chimeric expression cassette comprising a promoter functional in plant cells operably liked to a DNA molecule that encodes a replicase as described above; regenerating the plant cells to provide a differentiated plant; and identifying a transformed plant that expresses the PRV replicase at a level sufficient to render the plant resistant to infection by the specific strain of PRV disclosed herein.

Title: *Brassica Oleracea* ACC Synthase Gene
Reference No.: SVS3801P0160

COUNTRY	SERIAL NUMBER	FILING DATE	PATENT NUMBER	ISSUE DATE	EXPIRATION DATE
United States	08/860,577	06/30/97	Pending		
Chile	207095	12/29/95	Pending		
Israel	116116	11/23/95	Pending		
India	1556/CAL/95	11/30/95	Pending		
Thailand	029070	12/04/95	Pending		
Europe	959230053	06/07/95	Pending		
Australia	2769395	06/07/95	Pending		

SVS3801P0161US

For: Transgenic Plants Expressing ACC Synthase Gene

SUMMARY OF THE INVENTION

This provides a DNA molecule in purified and isolated form comprising DNA encoding the ACC synthase of *Brassica oleracea* plant, such as broccoli, cabbage, cauliflower, brussel sprouts, kale, kohlrabi, etc. The invention also provides chimeric plant expression cassettes, i.e., constructs, comprising a DNA molecule encoding the ACC synthase of *Brassica oleracea*, a promoter and polyadenylation signal functional in plant cells wherein the DNA molecule encoding the ACC synthase of *Brassica oleracea* is operably linked to said promoter and polyadenylation signal, effective to transcribe sense and antisense RNA from the DNA encoding said ACC synthase when employed to transform the genome of a host cell, and to recombinant host cells transformed with this expression cassette. The host cells transformed with this expression cassette. The host cells transformed with this expression cassette can include *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes* cells. This also provides transformed B. oleracea and Cucumis melo plants and plant parts, such as seeds. The invention further provides a method to control ACC synthase production and, thus, the growth and development of *Brassica oleracea* and *Cucumis melo* plants, comprising transforming the plants with a chimeric expression cassette with a DNA molecule encoding *B. oleracea* ACC synthase operably linked to a promoter and polyadenylation signal functional in plants, effective to transcribe sense or antisense RNA from the DNA encoding said ACC synthase. The invention thus provides a method for controlling the maturation and aging of *Brassica oleracea* and *Cucumis melo* plants which allows one to influence, e.g., lengthen, the shelf-life of these plants and fresh vegetable products derived from these plants.

Title: Papaya Ringspot Virus Coat Protein Gene
Reference No.: SVS3801P0180

COUNTRY	SERIAL NUMBER	FILING DATE	PATENT NUMBER	ISSUE DATE	EXPIRATION DATE
United States	08/860,368	06/26/97	Pending		
Chile	207195	12/29/95	Pending		
Israel	116118	11/23/95	Pending		
India	1551/CAL/95	11/30/95	Pending		
Mexico	974791	06/07/95	Pending		
Europe	959245762	06/07/95	Pending		
Australia	2901595	06/07/95	Pending		

SUMMARY OF THE INVENTION

This invention provides an isolated and purified DNA molecule that encodes the coat protein for the FLA83 W-type strain of papaya ringspot virus (PRV). This invention also provides a chimeric expression cassette comprising this DNA molecule, a promoter which functions in plant cells to cause the production of an RNA molecule, and at least one polyadenylation signal comprising 3' nontranslated DNA which functions in plant cells to cause the termination of transcription and the addition of polyadenylated ribonucleotides to the 3' end of the transcribed mRNA sequences, wherein the promoter is operably linked to the DNA molecule, and the DNA molecule is operably linked to the polyadenylation signal. Another embodiment of this invention is exemplified by the insertion of multiple virus gene expression cassettes into one purified DNA molecule, e.g., a plasmid. Preferably, these cassettes include the promoter of the 35S gene of cauliflower mosaic virus and the polyadenylation signal of cauliflower mosaic virus and the polyadenylation signal of the cauliflower mosaic virus 35S gene.

Also provided are bacterial cells, and transformed plant cells, containing the chimeric expression cassettes comprising the coat protein gene derived from the FLA83 W-type strain of papaya ringspot virus (referred to herein as PRV FLA83 W), and preferably the 35S promoter of cauliflower mosaic virus and the polyadenylation signal of the cauliflower mosaic virus 35S gene. Plants are also provided, wherein the plants comprise a plurality of transformed cells transformed with a cassette containing the coat protein gene derived from the PRV FLA83 W strain, and preferably the cauliflower mosaic virus 35S promoter and the polyadenylation signal of the cauliflower mosaic virus gene. Transformed plants of this invention include tobacco, corn, cucumber, peppers, potatoes, soybean, squash, and tomatoes. Especially preferred are members of the Cucurbitaceae (e.g., squash and cucumber) family.

Another aspect of this invention is a method of preparing a PRV-resistant plant, such as a dicot, comprising: transforming plant cells with a chimeric expression cassette comprising a promoter functional in plant cells operably linked to a DNA molecule that encodes a coat protein as described above; regenerating the plant cells to provide a differentiated plant; and identifying a transformed plant that expresses the PRV coat protein at a level sufficient to render the plant resistant to infection by the specific strains of PRV disclosed herein.

PATENT

Title: Cucumber Mosaic Virus Coat Protein Gene
Reference No.: SVS3801P0190

COUNTRY	SERIAL NUMBER	FILING DATE	PATENT NUMBER	ISSUE DATE	EXPIRATION DATE
United States	08/010,425	01/28/93	5,349,128	09/20/94	09/20/11
Australia	4047889	08/02/89	634171	06/11/93	08/02/09
Japan	50854189	08/02/89	Pending		
Austria	899090724	08/02/89	0429497	10/06/93	08/02/09
Belgium	899090724	08/02/89	0429497	10/06/93	08/02/09
France	899090724	08/02/89	0429497	10/06/93	08/02/09
Germany	899090724	08/02/89	0429497	10/06/93	08/02/09
Great Britain	899090724	08/02/89	0429497	10/06/93	08/02/09
Italy	899090724	08/02/89	0429497	10/06/93	08/02/09
Luxembourg	899090724	08/02/89	0429497	10/06/93	08/02/09
Netherlands	899090724	08/02/89	0429497	10/06/93	08/02/09
Switzerland	899090724	08/02/89	0429497	10/06/93	08/02/09
Sweden	899090724	08/02/89	0429497	10/06/93	08/02/09
Canada	608775	08/16/89	1335965	06/20/95	06/20/1

SUMMARY OF THE INVENTION

This invention provides: The coat protein gene from the WL strain of cucumber mosaic virus (CMV-WL).

A plant transformation vector comprising the coat protein gene from CMV-WL, the promoter of the 35S gene of cauliflower mosaic virus and the polyadenulation signal of cauliflower mosaic virus 35S gene.

A bacterial cell containing a plant transformation vector comprising the coat protein gene from CMV-WL, the 35S promoter of cauliflower mosaic virus and the polyadenylation signal of the cauliflower mosaic virus 35S gene.

A transformed plant cell containing the coat protein gene from CMV-WL, the cauliflower mosaic virus 35S promoter and the polyadenulation signal of the cauliflower mosaic virus gene.

A plant comprising transformed cells containing the coat protein gene of CMV-WL, the cauliflower mosaic virus 35S promoter and the polyadenulation signal of the cauliflower mosaic virus gene. Transformed plants of this invention include beets, citrus fruit, corn, cucumber, peppers, potatoes, soybean, squash and tomatoes. Especially preferred are members of the cucurbitaceae (squash, cucumber, i.e.,) and solanaceae (peppers, tomatoes, i.e.) family.

A process for producing virus-resistant plants comprising propagating a plant expressing the coat protein gene from the WL strain of cucumber virus. Especially preferred is the process for producing members of the cucurbitacaea and solanacaea families.

Title: Squash Mosaic Virus Genes and Plants Transformed Therewith
Reference No.: SVS3801P0200

COUNTRY	SERIAL NUMBER	FILING DATE	PATENT NUMBER	ISSUE DATE	EXPIRATION DATE
United States	08/363,560	12/21/94	5,514,570	05/07/96	05/07/13
Chile	199595	12/21/95	Pending		
Israel	116473	12/20/95	Pending		
India	1690/CAL/95	12/20/95	Pending		
Thailand	029338	12/19/95	Pending		

PATENT

SUMMARY OF THE INVENTION

This invention relates to the coat protein genes of Squash Mosaic Virus (SqMV). This invention relates to recombinant DNA molecules that comprise SqMV coat protein genes operably linked to genetic regulatory sequences necessary for gene expression. Furthermore, this invention relates to transgenic plants which comprise recombinant DNA molecules that encode SqMV coat proteins and that are operably linked to genetic regulatory sequences necessary for gene expression. In addition, this invention relates to a process of producing transgenic plants which have increased resistance to SqMV infection.

SVS3801P0215US

For: Cucumber Mosaic Virus Coat Protein Gene

SUMMARY OF THE INVENTION

This invention provides: (1) a DNA fragment which encodes the coat protein from the C strain of cucumber mosaic virus (CMV-C).

(2) A plant transformation vector comprising a DNA fragment which encodes the coat protein from CMV-C, a CaMV 35S promoter of cauliflower mosaic virus and the polyadenylation signal of either the cauliflower mosaic virus 35S gene or the Phaseolin seed storage protein gene.

(3) A bacterial cell containing a plant transformation vector comprising a DNA fragment which encodes the coat protein from CMV-C a CaMV 35S promoter of cauliflower mosaic virus and the polyadenylation signal of either the cauliflower mosaic virus 35S promoter of cauliflower mosaic virus and the polyadenylation signal of either the cauliflower mosaic virus 35S gene or the Phaseolin seed storage protein gene.

(4) A transformed plant cell containing a DNA fragment which encodes the coat protein from CMV-C a CaMV 35S promoter of cauliflower mosaic virus and the polyadenylation signal of either the cauliflower mosaic virus gene or the phaseoline seed storage protein gene.

(5) A plant comprising transformed cells containing a DNA fragment which encodes the coat protein from CMV-C; a CaMV 35S promoter of cauliflower mosaic virus and the polyadenylation signal of either the cauliflower mosaic virus gene or the Phaseolin seed storage protein gene. Transformed plants of this invention include beets, citrus fruit, corn, cucumber, peppers, potatoes, soybean, squash and tomatoes. Especially preferred are members of the Cucurbitaceae (squash, cucumber, i.e.,) and Solanaceae (peppers, tomatoes, i.e.) family.

(6) A process for producing virus-resistant plants comprising propagating a plant expressing the coat protein gene from the C strain of cucumber mosaic virus. Especially

Title: Cucumber Mosaic Virus Coat Protein Gene
Reference No.: SVS3801P0210

COUNTRY	SERIAL NUMBER	FILING DATE	PATENT NUMBER	ISSUE DATE	EXPIRATION DATE
United States	08/365,973	12/28/94	5,623,066	04/22/97	04/22/14
Austria	899014294	12/08/88	0391972	06/15/94	12/08/08
Belgium	899014294	12/08/88	0391972	06/15/94	12/08/08
France	899014294	12/08/88	0391972	06/15/94	12/08/08
Germany	899014294	12/08/88	0391972	06/15/94	12/08/08
Great Britain	899014294	12/08/88	0391972	06/15/94	12/08/08
Italy	899014294	12/08/88	0391972	06/15/94	12/08/08
Luxembourg	899014294	12/08/88	0391972	06/15/94	12/08/08
Netherlands	899014294	12/08/88	0391972	06/15/94	12/08/08
Sweden	899014294	12/08/88	0391972	06/15/94	12/08/08
Switzerland	899014294	12/08/88	0391972	06/15/94	12/08/08
Japan	5013291989	12/08/88	Pending		
Australia	29276/89	12/08/88	621336		12/08/08
China	88109272.X	12/08/88	Pending		

preferred is the process for producing members of the Cucurbitaceae and Solanaceae families.

Title: Expression Cassette for Plants
Reference No.: SVS3801P0220

COUNTRY	SERIAL NUMBER	FILING DATE	PATENT NUMBER	ISSUE DATE	EXPIRATION DATE
Canada	606866	07/27/89	1332718	10/25/94	10/25/1
Australia	3970489	07/20/89	639891	12/06/93	07/20/09
Denmark	899085799	07/20/89	28191	05/11/94	07/20/09
Austria	899085799	07/20/89	0429478	05/11/94	07/20/09
France	899085799	07/20/89	0429478	05/11/94	07/20/09
Belgium	899085799	07/20/89	0429478	05/11/94	07/20/09
Germany	899085799	07/20/89	0429478	05/11/94	07/20/09
Great Britain	899085799	07/20/89	0429478	05/11/94	07/20/09
Italy	899085799	07/20/89	0429478	05/11/94	07/20/09
Luxembourg	899085799	07/20/89	0429478	05/11/94	07/20/09
Netherlands	899085799	07/20/89	0429478	05/11/94	07/20/09
Sweden	899085799	07/20/89	0429478	05/11/94	07/20/09
Switzerland	899085799	07/20/89	0429478	05/11/94	07/20/09

SVS3801P0225US

For: Expression Cassette For Plants

SUMMARY OF THE INVENTION

This invention relates to an expression cassette which can express a desired gene at high levels. This invention relates to an expression vector which comprises an expression cassette. The high level expression vector of this invention comprises: a promoter; a 5' untranslated region which is at least 60% A and T; an initiation codon comprising Kozak's element; a cloning site where a desired gene may be inserted to form a functional expression unit; and a 3' untranslated region which comprises a poly(A) addition signal and flanking sequence which yields high level expression. This invention relates to transformed bacterial and plant cells which contain the expression vector. This invention relates to a process of producing transgenic plants with desirable traits by producing the plants from plant cells which have been transformed with an expression vector which contains gene conferring such traits.

Title: Plants Resistant to C Strains of Cucumber Mosaic Virus
Reference No.: SVS3801P0230

COUNTRY	SERIAL NUMBER	FILING DATE	PATENT NUMBER	ISSUE DATE	EXPIRATION DATE
United States	08/875,233	06/26/97	Pending		
Chile	207395	12/29/95	Pending		
Israel	116115	11/23/95	Pending		
India	1555/CAL/95	11/30/95	Pending		
Europe	959229964	06/07/95	Pending		
Australia	2768795	06/07/95	Pending		

SUMMARY OF THE INVENTION

This invention provides: an isolated and purified DNA molecule that encodes the coat protein for the V27 strain of cucumber mosaic virus (CMV V27), and a chimeric expression cassette comprising this DNA molecule; an isolated and purified DNA molecule that encodes the coat protein for the V33 stain of cucumber mosaic virus (CMV V33), and a chimeric expression cassette comprising this DNA molecule; and an isolated and purified DNA molecule that encodes the coat protein for the V34 stain of cucumber mosaic virus (CMV V34), and a chimeric expression cassette comprising this DNA molecule; and an isolated and purified DNA molecule that encodes the coat protein for the A35 strain of cucumber mosaic virus (CMV A35), and a chimeric expression cassette comprising the DNA molecule. Another embodiment of this invention is expression cassettes into one purified DNA molecule, e.g., a plasmid. Each of these cassettes also includes a promoter which functions in plant cells to cause the production of an RNA molecule, and at least one polyadenylation signal comprising 3' nontranslated DNA which functions in plant cells to cause the termination of transcription and the addition of polyadenylated ribonucleotides to the 3' end of the transcribed mRNA sequences, wherein the promoter is operably linked to the DNA molecule, and the DNA molecule is operably linked to the polyadenylation signal. Preferably, these cassettes include the promoter of the 35S gene of cauliflower mosaic virus and the polyadenylation signal of the cauliflower mosaic virus 35S gene.

Also provided are bacterial cells, and transformed plant cells, containing the chimeric expression cassettes comprising the coat protein genes derived from the CMV V27, CMV V33, CMV V34, or CMV A35 strains, and preferably the 35S gene. Plants are also provided, wherein the plants comprise a plurality of transformed cells containing the chimeric coat protein gene expression cassettes derived from the CMV V27, CMV V33, CMV V34, or CMV A35 strains, and preferably the cauliflower mosaic virus 35S promoter and the

polyadenylation signal of the cauliflower mosaic virus gene. Transformed plants of this invention include tobacco, beets, corn, cucumber, peppers, potatoes, melons, soybean, squash, and tomatoes. Especially preferred are members of the Cucurbitaceae (e.g., squash and cucumber,) and Solanaceae (e.g., peppers and tomatoes) family.

Another aspect of this invention is a method of preparing a CMV-resistant plant, such as a dicot, comprising: transforming plant cells with a chimeric expression cassette comprising a promoter functional in plant cells operably liked to a DNA molecule that encodes a coat protein as described above; regenerating the plant cells to provide a differentiated plant; and identifying a transformed plant that expresses the CMV coat protein at a level sufficient to render the plant resistant to infection by the specific strains of CMV disclosed herein.

Title: Transgenic Plants Exhibiting Heterologous Virus Resistance
Reference No.: SVS3801P0240

COUNTRY	SERIAL NUMBER	FILING DATE	PATENT NUMBER	ISSUE DATE	EXPIRATION DATE
United States	08/860,543	06/30/97	Pending		
Chile	207595	12/29/95	Pending		
Israel	116113	11/23/95	Pending		
Thailand	029072	12/04/95	Pending		
Europe	959220922	06/07/95	Pending		
Australia	2690295	06/07/95	Pending		

SUMMARY OF THE INVENTION

This invention provides a method of providing heterologous virus resistance to a plant susceptible to infection by two or more viruses by expressing a chimeric recombinant DNA molecule in the cells of the plant which encodes a protein of one class of plant virus, such as a potyvirus protein or *cucumovirus* protein, i.e., a coat protein or replicase. Unexpectedly, it was found that plants stablely transformed with such recombinant DNA molecules exhibited heterologous virus resistance, in that they were resistant both to infection by the virus from which the encoded protein was derived or isolated, as well as to infection by at least one unrelated class of virus to which the plant is normally susceptible, such as one or more potyviruses. For example, when the known *cucumovirus* coat protein gene (CMV-C) is expressed in transgenic plants, such as transgenic dicots, it confers protection both against infection by cucumber mosaic virus strains and against infection by zucchini yellow mosaic virus or watermelon mosaic virus-2, i.e., ZYMV and WMV-2. Preferably, the transgenic plant exhibit substantially equal levels of resistance to all of the viruses to which it has become resistant. Although heterologous virus resistance has been demonstrated for closely related viruses, such as potyviruses, it is believed that heterologous virus resistance between unrelated classes of virus has not previously been demonstrated, and the term "heterologous virus resistance" is to be understood in this sense herein below.

Therefore, in a preferred embodiment, this invention provides a method of imparting multi-virus resistance to a plant which is susceptible to viruses, comprising:

(a) transforming cells of said susceptible plant with a chimeric recombinant DNA molecule comprising a promoter functional in cells of said plant and operably linked to a DNA sequence encodoing a protein of a first class of virus which is capable of infecting said plant;

(b) regenerating said plant cells to provide a differentiated plant; and

(c) identifying a transformed plant which expresses the coding DNA sequence so as to render the plant resistant to infection by said first class of virus, wherein the plant is also rendered resistant to infection by at least one other class of virus to which said plant is susceptible.

Another embodiment of this invention provides a method for providing resistance to infection by viruses in a susceptible *Cucurbitaceae* plant which comprises:

(a) transforming Cucurbitaceae plant cells with a DNA molecule encoding a protein from a first class of virus which is capable of infecting said *Cucurbitaceae* plant;

(b) regenerating said plant cells to provide a differentiated plant; and

(c) selecting a transformed *Cucurbitaceae* which is expressed so as to render the plant resistant to infection by said first class of said virus, and to at least one other class of said virus.

This invention is exemplified by the insertion of a virus coat protein (cp) expression cassette into a binary plasmid and subsequent characterization of the resulting plasmid. For example, CMV coat protein expression cassette can be placed in the binary plasmid pPRBN. Subsequently, binary plasmids harboring these expression cassettes are mobilized into *Agrobacterium* and employed to transfer the virus coat protein genes into plants, such as members of the *Cucurbitaceae* family, along with the associated selectable marker and/or reporter genes.

Title: Transgenic Plants Expressing ACC Oxidase Genes
Reference No.: SVS3801P0250

COUNTRY	SERIAL NUMBER	FILING DATE	PATENT NUMBER	ISSUE DATE	EXPIRATION DATE
United States	08/793,666	02/28/97	Pending		
Argentina	333395	09/04/95	Pending		
Chile	1330-95	09/02/95	Pending		
Indonesia	951758	09/04/95	Pending		
Israel	115116	08/31/95	Pending		
India	1052/CAL/95	09/04/95	Pending		
Europe	959222498	06/07/95	Pending		
Australia	2700095	06/07/95	Pending		
Canada	2198708	06/07/95	Pending		
Mexico	971475	06/07/95	Pending		
Saudi Arabia	9616056B	01/30/96	Pending		
Thailand	027816	09/01/95	Pending		

SUMMARY OF THE INVENTION

This invention provides recombinant materials which permit control of the level of ACC oxidase in plants, specifically, *Brassica oleracea* and *Cucumis melo*. This invention is also directed to DNA in purified and isolated form comprising a DNA sequence encoding the enzyme ACC oxidase of *Brassica oleracea* and *Cucumis melo*. This invention is also directed to expression systems effective in expressing the DNA encoding said ACC oxidase and to recombinant hosts transformed with this expression system. The invention is further directed to methods to control ACC oxidase production and, thus, the growth and development of *Brassica oleracea* and *Cucumis melo* plants, using the coding sequences for ACC oxidase in an antisense construct or by replacing the ACC oxidase in an antisense construct or by replacing the ACC oxidase gene by a mutated form thereof. This invention thus provides a method for controlling the maturation and aging of *Brassica oleracea* and *Cucumis melo* plants which allows one to influence, e.g., lengthen, the shelf life of these plants.

Title: Transgenic Plants Expressing Geminivirus Genes
Reference No.: SVS3801P0260

COUNTRY	SERIAL NUMBER	FILING DATE	PATENT NUMBER	ISSUE DATE	EXPIRATION DATE
United States	08/838,151	04/15/97	Pending		
Brazil			Pending		
Australia			Pending		
Europe			Pending		
S. Korea			Pending		
Mexico			Pending		
Israel			Pending		
Japan			Pending		
Turkey			Pending		

SVS3801P0261US

For: Transgenic Plants Expressing Geminivirus Genes

SUMMARY OF THE INVENTION

The invention involves production of transgenic plants containing DNA encoding AC1/C1 wildtype and mutant sequences that negatively interfere with trans with geminiviral replication during infection. The resulting transgenic plants are resistant to viral infection.

Title: Seedless Tomatoes and Method for Making the Same
Reference No.: SVS3801P0270

COUNTRY	SERIAL NUMBER	FILING DATE	PATENT NUMBER	ISSUE DATE
United States	08/957,867	10/27/97	Pending	
PCT			Pending	

SUMMARY OF THE INVENTION

This invention involves tomatoes (*Lycopersicon esculentum*) that are substantially seedless. The tomatoes of this invention are about 100% seedless. The seedless tomatoes of this invention are made by crossing a tomato plant (*Lycopersicon esculentum*) containing at least one parthenocarpic gene as the male parent with a male sterile tomato plant (*Lycopersicon esculentum*) containing at least one parthenocarpic gene as the female parent. The male and female parental lines may contain any parthenocarpic gene such as pat, pat-2, pat-3, pat-4, and pat-5, sha, and sds. The parthenocarpic gene(s) in the male and female parental lines should be identical in order to insure the production of the seedless tomatoes of this invention.

The seedless tomatoes of this invention retain the size of fruit of the parent lines, and therefore a means is provided for obtaining seedless tomatoes of commercially acceptable size. The seedless tomatoes of this invention also have good flavor (sugar and acid balance) and do not exhibit any malformations such as puffiness.

Title: Plant Potyvirus Expression Vector With a Gene for Protease
Reference No.: SVS3801P0280

COUNTRY	SERIAL NUMBER	FILING DATE	PATENT NUMBER	ISSUE DATE	EXPIRATION DATE
United States	07/752,972	08/30/91	5,162,601	11/10/92	11/22/09

For: Plant Potyvirus Expression Vector with a Gene For Protease

SUMMARY OF THE INVENTION

This invention relates to a recombinant multigene comprising a plurality of structural genes and a plurality of DNA sequences which encode peptide linkers. In this invention, one of the structural genes encodes a protease, the DNA sequences encoding the peptide linkers are adjacent to the DNA sequences which encode the structural genes and the peptide linkers contain an amino acid sequence which the protease recognizes as a proteolytic cleavage site.

This invention additionally relates to transgenic plants which comprise such a recombinant multigene transgene. Furthermore, this invention relates to host cells transformed with a recombinant multigene. Additionally, this invention relates to transgenic animals which comprise a recombinant multigene transgene.

This invention relates to a method of producing a plurality of polypeptides in a host by incorporating and expressing in the host a recombinant, multigene comprising a plurality of structural genes which encode such polypeptides and a plurality of DNA sequences which encode peptide linkers between the structural genes. One structural gene encodes a protease which recognizes and cleaves the peptide linkers.

Title: *Lactuca Sativa* Cultivar Exhibiting Resistance to Downy Mildew and Corky Root Rot
Reference No.: SVS3801P0290

COUNTRY	SERIAL NUMBER	FILING DATE	PATENT NUMBER	ISSUE DATE
United States	08/986,624	12/08/97	Pending	

SVS3801P0290US

For: *Lactuca Sativa* Cultivar Exhibiting Resistance to Downy Mildew and Corky Root Rot

SUMMARY OF THE INVENTION

This invention relates to a new crisphead *Lactuca sativa* cultivar referred to as Sharp Shooter. Sharp Shooter exhibits vigorous growth and resistance to downy mildew pathotypes I, IIA, III, and IV and corky root rot pathotype CA1. In addition, Sharp Shooter has a color of 146A according to the R.H.S. Colour Chart published by the Royal Horticultural Society of London, England. Furthermore, Sharp Shooter weighs from about 10% to about 40% greater than a comparable crisphead *Lactuca sativa* cultivar. Specifically, mature heads of Sharp Shooter weigh from about 820.0 grams to about 960.0 grams, preferably about 890 grams. Seeds of Sharp Shooter have been deposited with the American Type Culture Collection (ATCC) in Rockville, Maryland and have been assigned ATCC Accession No. 209461.

This invention also relates to a *Lactuca sativa* plant produced by growing the seed of Sharp Shooter that have ATCC Accession number 209461. This invention also relates to a *Lactuca sativa* plant that has all the physiological and morphological characteristics of a *Lactuca sativa* plant grown from seed of ATCC Accession No. 209461.

Finally, this invention relates to a F_1 hybrid *Lactuca sativa* plant having Sharp Shooter as a parent.

Title: A Starchless Variety of *Pisum sativum*
Reference No.: SVS3801P0300

COUNTRY	SERIAL NUMBER	FILING DATE	PATENT NUMBER	ISSUE DATE
United States	08/986,616	12/08/97	Pending	

Foreign Filings due 12/08/98

SVS3801P0300US

For: A Starchless Variety Of Pisum Sativum

SUMMARY OF THE INVENTION

This invention relates to a new variety of pea, *Pisum sativum*, that is resistant to Fusarium Wilt Fungus and Powdery Mildew Fungus and which contains a recessive gene, referred to as the bsg gene. A *Pisum sativum* variety that contains the bsg gene produces peas which exhibit an elevated level of sucrose. More specifically, the peas containing the bsg gene contain from about 12% to about 25% higher levels of sucrose than wrinkled pea varieties that contain the r gene. Additionally, the peas of this invention exhibit a 20% decrease level of alcohol insoluble solids, when compared to wrinkled peas that contain the r gene.

Title: A Starchless Variety of *Pisum Sativum* having Elevated Levels of Sucrose
Reference No.: SVS3801P0301

COUNTRY	SERIAL NUMBER	FILING DATE	PATENT NUMBER	ISSUE DATE
United States	09/015,711	01/29/98	Pending	

Foreign Filings due by 12/08/98

SVS3801P0301US (A Continuation-in-Part Application of SVS 3801P0300 US)

For: A Starchless Variety of *Pisum Sativum* Having Elevated Levels of Sucrose

SUMMARY OF THE INVENTION

This invention relates to a new variety of *Pisum sativum*, which is resistant to Fusarium Wilt Fungus and Powdery Mildew Fungus and which contains within its genome, a homozygous recessive gene, referred to as the bsg gene. A *Pisum sativum* variety that contains the bsg gene within its genome produces peas (known in the art as immature seeds) which exhibit an elevated level of sucrose and a decreased level of alcohol insoluble solids when compared with peas produced from a *Pisum sativum* variety that does not contain the bsg gene homozygous within its genome.

The peas of this invention contain from about 6.0 to about 7.5 percent fresh weight of sucrose when measured at a tenderometer value of from about 90 to about 110 and from about 6.5 to about 8.0 percent by weight of alcohol insoluble solids when measured at a tenderometer value of about 105. Moreover, the peas of this invention contain from about 5 to about 30 percent fresh weight more sucrose than peas produced from a *Pisum sativum* variety that does not contain the bsg gene homozygous within its genome. Additionally, the peas of this invention exhibit twenty (20) percent less alcohol insoluble solids when compared with peas from a *Pisum sativum* that does not contain the bsg gene homozygous within its genome.

Additionally, this invention relates to a process for producing peas of a *Pisum sativum* variety that contain higher levels of sucrose and lower levels of alcohol insoluble solids than peas from a *Pisum sativum* variety that does not contain the bsg gene homozygous within its genome. The process involves crossing a *Pisum sativum* variety or line that contains the bsg gene homozygous within its genome with a second *Pisum sativum* variety or line that contains the bsg

gene homozygous within its genome, collecting the resulting mature seeds, planting the mature seeds, growing the mature seeds into *Pisum sativum* plants, selecting *Pisum sativum* plants with desirable phenotypic traits; allowing the plants to self-pollinate until a uniform line is produced, allowing the *Pisum sativum* line to self-pollinate, and collecting the resulting peas.

In another embodiment, the process involves crossing a *Pisum* sativum variety or line that contains the bsg gene homozygous within its genome with a second Pisum *sativum* variety or line which does not contain the bsg gene within its genome, collecting dry, mature seeds, planting the collected dry, mature seeds, growing the mature seeds into *Pisum sativum* plants, allowing the plants to self-pollinate, collecting the resulting dry, mature seeds, selecting highly wrinkled mature seeds that do not contain organized starch grains and which do not stain purple when treated with a solution of iodine and potassium iodide, planting said highly wrinkled mature seeds, growing the mature seeds into *Pisum sativum* plants, selecting plants with desirable phenotypic traits, allowing the plants to self-pollinate until a uniform *Pisum sativum* line is produced, allowing the *Pisum sativum* line selected to self-pollinate, and collecting the resulting peas. The *Pisum sativum* variety or line that does not contain the bsg gene within its genome can contain any combination of the genes such as the r, rb, R or Rb homozygous within its genome. The peas produced by the process of this invention contain from about 6.0 to about 7.5 percent fresh weight of sucrose when measured at a tenderometer value of from about 90 to about 110 and from about 6.5 to about 8.0 percent by weight of alcohol insoluble solids when measured at a tenderometer value of about 105.

This invention also contemplates a process of producing highly wrinkled mature seed of a *Pisum sativum* variety that contains the bsg gene within its genome. In one embodiment the process involves crossing a *Pisum sativum* variety or line that contains the bsg gene within its genome with a second *Pisum sativum* variety or line that contains the bsg gene within its genome and collecting the resulting mature seeds.

In another embodiment, the process involves crossing a *Pisum sativum* variety or line that contains the bsg gene within its genome with a *Pisum sativum* variety or line that does not

contain the bsg gene within its genome, collecting mature seeds, planting the collected mature seeds, growing the mature seeds into *Pisum sativum* plants, allowing the plants to self-pollinate, collecting mature seeds, selecting highly wrinkled seeds that do not contain organized starch grains, planting said mature seeds and growing the seeds into *Pisum sativum* plants, selecting plants with desirable phenotypic traits, allowing the plants to self-pollinate until a uniform *Pisum sativum* line is produced, allowing the *Pisum sativum* line to self -pollinate and collecting the mature seeds.

This invention also contemplates *Pisum sativum* varieties grown from the mature seed described above and peas harvested from said varieties.

SCHEDULE A
TO RELEASE AND REASSIGNMENT OF PATENTS

REGISTERED PATENTS
AND PATENTS APPLICATIONS

Title: Cytoplasmic Male Sterile *Brassica Oleracea* Plants which Contain the Polima CMS Cytoplasm and are Male Sterile at High and Low Temperatures
Reference No.: SVS3801P0050

COUNTRY	SERIAL NUMBER	FILING DATE	PATENT NUMBER	ISSUE DATE	EXPIRATION DATE
United States	09/029,709	09/11/95	Pending		
Europe	959428285	09/11/95	Pending		
Australia	4404496	09/11/95	Pending		
Brazil	PCT/US95/11497	09/11/95	Pending		
Canada	2231423	09/11/95	Pending		
China	95197954	09/11/95	Pending		
Czech Republic	PCT/US95/11497	09/11/95	Pending		
Hungary	PCT/US95/11497	09/11/95	Pending		
Japan	PCT/US95/11497	09/11/95	Pending		
Norway	981050	09/11/95	Pending		
New Zealand	298533	09/11/95	Pending		
Poland	PCT/US95/11497	09/11/95	Pending		
Romania	PCT/US95/11497	09/11/95	Pending		
Russian Federation	98106843	09/11/95	Pending		
Ukraine	PCT/US95/11497	09/11/95	Pending		
South Korea	98701783	09/11/95	Pending		
Macedonia	PCT/US95/11497	09/11/95	Pending		

Title: *Lycopersicon Pimpinellifolium* as a Source of Resistance to the Plant Pathogen *Phytophthora Infestans*
Reference No.: SVS3801P060

COUNTRY	SERIAL NUMBER	FILING DATE	PATENT NUMBER	ISSUE DATE
United States	08/621,352	5/22/98	Issue Fee Paid	
Europe			Pending	
Australia			Pending	
Brazil			Pending	
Canada			Pending	
China			Pending	
Israel			Pending	
Japan			Pending	
South Korea			Pending	

Title: Transgenic Plants Expressing DNA Construct Containing A Plurality of Genes to Impart Virus Resistance
Reference No.: SVS3801P0080

COUNTRY	SERIAL NUMBER	FILING DATE	PATENT NUMBER	ISSUE DATE	EXPIRATION DATE
United States	08/860,379	06/25/97	Pending		
Chile	207495	12/29/95	Pending		
Spain	109495	12/30/95	Pending		
Israel	116114	11/23/95	Pending		
India	1554/CAL/95	11/30/95	Pending		
Thailand	029071	12/04/95	Pending		
Europe	959228750	06/07/95	Pending		
Australia	2761395	06/07/95	Pending		
China	951972073	06/07/95	Pending		
Mexico	974794	06/07/95	Pending		

Title: Papaya Ringspot Virus Protease Gene
Reference No.: SVS3801P0090

COUNTRY	SERIAL NUMBER	FILING DATE	PATENT NUMBER	ISSUE DATE	EXPIRATION DATE
United States	08/366,490	12/30/94	Issue Fee Paid		
United States	08/860,483	06/26/97	Pending		
Chile	206995	12/29/95	Pending		
Israel	116119	11/23/95	Pending		
India	1550/CAL/95	11/30/95	Pending		
Thailand	029075	12/04/95	Pending		
Europe	969328202	06/07/95	Pending		
Mexico	974792	06/07/95	Pending		
Australia	2818395	06/07/95	Pending		

Title: Papaya Ringspot Virus Replicase Gene
Reference No.: SVS3801PQ150

COUNTRY	SERIAL NUMBER	FILING DATE	PATENT NUMBER	ISSUE DATE	EXPIRATION DATE
United States	8/860,519	06/30/97	Pending		
Chile	207295	12/29/95	Pending		
Israel	116117	11/23/95	Pending		
India	1549/CAL/95	11/30/95	Pending		
Thailand	029069	12/04/95	Pending		
Europe	959216201	06/07/95	Pending		
Australia	2663795	06/07/95	Pending		

Title: *Brassica Oleracea* ACC Synthase Gene
Reference No.: SVS3801P0160

COUNTRY	SERIAL NUMBER	FILING DATE	PATENT NUMBER	ISSUE DATE	EXPIRATION DATE
United States	08/860,577	06/30/97	Pending		
Chile	2[illegible]7095	12/29/95	Pending		
Israel	116116	11/23/95	Pending		
India	1556/CAL/95	11/30/95	Pending		
Thailand	029070	12/04/95	Pending		
Europe	959230053	06/07/95	Pending		
Australia	2769395	06/07/95	Pending		

Title: Papaya Ringspot Virus Coat Protein Gene
Reference No.: SVS3801P0180

COUNTRY	SERIAL NUMBER	FILING DATE	PATENT NUMBER	ISSUE DATE	EXPIRATION DATE
United States	08/860,368	06/26/97	Pending		
Chile	207195	12/29/95	Pending		
Israel	116118	11/23/95	Pending		
India	1551/CAL/95	11/30/95	Pending		
Mexico	974791	06/07/95	Pending		
Europe	959245762	06/07/95	Pending		
Australia	2901595	06/07/95	Pending		

Title: Cucumber Mosaic Virus Coat Protein Gene
Reference No.: SVS3801P0190

COUNTRY	SERIAL NUMBER	FILING DATE	PATENT NUMBER	ISSUE DATE	EXPIRATION DATE
United States	08/010,425	01/28/93	5,349,128	09/20/94	09/20/11
Australia	4047889	08/02/89	634171	06/11/93	08/02/09
Japan	50854189	08/02/89	Pending		
Austria	8990907z4	08/02/89	0429497	10/06/93	08/02/09
Belgium	899090724	08/02/89	0429497	10/06/93	08/02/09
France	899090724	08/02/89	0429497	10/06/93	08/02/09
Germany	899090724	08/02/89	0429497	10/06/93	08/02/09
Great Britain	899090724	08/02/89	0429497	10/06/93	08/02/09
Italy	899090724	08/02/89	0429497	10/06/93	08/02/09
Luxembourg	899090724	08/02/89	0429497	10/06/93	08/02/09
Netherlands	899090724	08/02/89	0429497	10/06/93	08/02/09
Switzerland	899090724	08/02/89	0429497	10/06/93	08/02/09
Sweden	899090724	08/02/89	0429497	10/06/93	08/02/09
Canada	608775	08/16/89	1335965	06/20/95	06/20/1

Title: Plants Resistant to C Strains of Cucumber Mosaic Virus
Reference No.: SVS3801P0230

COUNTRY	SERIAL NUMBER	FILING DATE	PATENT NUMBER	ISSUE DATE	EXPIRATION DATE
United States	08/875,233	06/26/97	Pending		
Chile	207395	12/29/95	Pending		
Israel	116115	11/23/95	Pending		
India	1555/CAL/95	11/30/95	Pending		
Europe	959229964	06/07/95	Pending		
Australia	2768795	06/07/95	Pending		

Title: Transgenic Plants Exhibiting Heterologous Virus Resistance
Reference No.: SVS3801P0240

COUNTRY	SERIAL NUMBER	FILING DATE	PATENT NUMBER	ISSUE DATE	EXPIRATION DATE
United States	08/860,543	06/30/97	Pending		
Chile	207595	12/29/95	Pending		
Israel	116113	11/23/95	Pending		
Thailand	029072	12/04/95	Pending		
Europe	959220922	06/07/95	Pending		
Australia	2690295	06/07/95	Pending		

Title: Transgenic Plants Expressing ACC Oxidase Genes
Reference No.: SVS3801P0250

COUNTRY	SERIAL NUMBER	FILING DATE	PATENT NUMBER	ISSUE DATE	EXPIRATION DATE
United States	08/793,666	02/28/97	Pending		
Argentina	333395	09/04/95	Pending		
Chile	1330-95	09/02/95	Pending		
Indonesia	951758	09/04/95	Pending		
Israel	115116	08/31/95	Pending		
India	1052/CAL/95	09/04/95	Pending		
Europe	959222498	06/07/95	Pending		
Australia	2700095	06/07/95	Pending		
Canada	2198708	06/07/95	Pending		
Mexico	971475	06/07/95	Pending		
Saudi Arabia	9616056B	01/30/96	Pending		
Thailand	027816	09/01/95	Pending		

Title: Plant Potyvirus Expression Vector With a Gene for Protease
Reference No.: SVS3801P0280

COUNTRY	SERIAL NUMBER	FILING DATE	PATENT NUMBER	ISSUE DATE	EXPIRATION DATE
United States	07/752,972	08/30/91	5,162,601	11/10/92	11/22/09

SEMINIS VEGETABLE SEEDS DOCKET

Title: Genetic Factor Responsible For A Defective Endosperm Phenotype In Seeds, Plants Comprising Said Factor and Their Use In Hybridization Processes
Reference No.: SVS3801P0010

COUNTRY	SERIAL NUMBER	FILING DATE	PATENT NUMBER	ISSUE DATE
United States	08/687,502	07/19/96	Pending	
Europe	959075755	01/20/95	Pending	