

05-22-2000



101363914

MRO  
5-3-00

### RECORDATION FORM COVER SHEET TRADEMARKS ONLY

TO: The Commissioner of Patents and Trademarks: Please record the attached original document(s) or copy(ies).

#### Submission Type

New

Resubmission (Non-Recordation)  
Document ID # \_\_\_\_\_

Correction of PTO Error  
Reel # \_\_\_\_\_ Frame # \_\_\_\_\_

Corrective Document  
Reel # \_\_\_\_\_ Frame # \_\_\_\_\_

#### Conveyance Type

Assignment  License

Security Agreement  Nunc Pro Tunc Assignment

Merger

Change of Name

Other \_\_\_\_\_

Effective Date  
Month Day Year  
6 28 99

#### Conveying Party

Mark if additional names of conveying parties attached

Name Sentiris, Inc. Execution Date  
Month Day Year  
7 6 99

Formerly \_\_\_\_\_

Individual  General Partnership  Limited Partnership  Corporation  Association

Other \_\_\_\_\_

Citizenship/State of Incorporation/Organization Delaware

#### Receiving Party

Mark if additional names of receiving parties attached

Name Harris Trust and Savings Bank, as Agent

DBA/AKA/TA \_\_\_\_\_

Composed of \_\_\_\_\_

Address (line 1) 111 West Monroe Street

Address (line 2) P.O. Box 755

Address (line 3) Chicago IL 60690  
City State/Country Zip Code

Individual  General Partnership  Limited Partnership  If document to be recorded is an assignment and the receiving party is not domiciled in the United States, an appointment of a domestic representative should be attached. (Designation must be a separate document from Assignment.)

Corporation  Association

Other \_\_\_\_\_

Citizenship/State of Incorporation/Organization Delaware

05/22/2000 JBNBZZ 0000048 75333586

FOR OFFICE USE ONLY

01 FC:481  
02 FC:482

40.00 DP  
1125.00 DP

Public burden reporting for this collection of information is estimated to average approximately 30 minutes per Cover Sheet to be recorded, including time for reviewing the document and gathering the data needed to complete the Cover Sheet. Send comments regarding this burden estimate to the U.S. Patent and Trademark Office, Chief Information Officer, Washington, D.C. 20231 and to the Office of Information and Regulatory Affairs, Office of Management and Budget, Paperwork Reduction Project (0651-0027), Washington, D.C. 20503. See OMB Information Collection Budget Package 0651-0027, Patent and Trademark Assignment Practice. DO NOT SEND REQUESTS TO RECORD ASSIGNMENT DOCUMENTS TO THIS ADDRESS.

Mail documents to be recorded with required cover sheet(s) information to:

TRADEMARK  
REEL: 002073 FRAME: 0412

**Domestic Representative Name and Address**

Enter for the first Receiving Party only.

Name

Address (line 1)

Address (line 2)

Address (line 3)

Address (line 4)

**Correspondent Name and Address**

Area Code and Telephone Number

Name

Address (line 1)

Address (line 2)

Address (line 3)

Address (line 4)

**RETURN TO:**  
Federal Research Corporation  
400 Seventh St., N.W., Suite 101  
Washington, DC 20004

**Pages**

Enter the total number of pages of the attached conveyance document including any attachments.

#

**Trademark Application Number(s) or Registration Number(s)**

Mark if additional numbers attached

Enter either the Trademark Application Number or the Registration Number (DO NOT ENTER BOTH numbers for the same property).

**Trademark Application Number(s)**

**Registration Number(s)**

<input type="text" value="75/333586"/>	<input type="text" value="75/172061"/>	<input type="text" value="74/391403"/>	<input type="text" value="1829947"/>	<input type="text" value="1226105"/>	<input type="text" value="2015005"/>
<input type="text" value="74/391044"/>	<input type="text" value="75/334715"/>	<input type="text" value="75/334716"/>	<input type="text" value="1476131"/>	<input type="text" value="1224451"/>	<input type="text" value="2104778"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text" value="1848683"/>	<input type="text" value="2011585"/>	<input type="text" value="1595730"/>

**Number of Properties**

Enter the total number of properties involved.

#

**Fee Amount**

Fee Amount for Properties Listed (37 CFR 3.41):

\$

Method of Payment:

Enclosed

Deposit Account

Deposit Account

(Enter for payment by deposit account or if additional fees can be charged to the account.)

Deposit Account Number:

#

Authorization to charge additional fees:

Yes

No

**Statement and Signature**

To the best of my knowledge and belief, the foregoing information is true and correct and any attached copy is a true copy of the original document. Charges to deposit account are authorized, as indicated herein.

Jane P Miles

Name of Person Signing

Jane P Miles

Signature

8/13/99

Date Signed

**RECORDATION FORM COVER SHEET  
CONTINUATION  
TRADEMARKS ONLY**

FORM PTO-1618C  
Expires 06/30/99  
OMB 0651-0027

U.S. Department of Commerce  
Patent and Trademark Office  
**TRADEMARK**

**Conveying Party**

Enter Additional Conveying Party

Mark if additional names of conveying parties attached

Execution Date  
Month Day Year

Name

Formerly

Individual  General Partnership  Limited Partnership  Corporation  Association

Other

Citizenship State of Incorporation/Organization

**Receiving Party**

Enter Additional Receiving Party

Mark if additional names of receiving parties attached

Name

DBA/AKA/TA

Composed of

Address (line 1)

Address (line 2)

Address (line 3)

City

State/Country

Zip Code

Individual  General Partnership  Limited Partnership

If document to be recorded is an assignment and the receiving party is not domiciled in the United States, an appointment of a domestic representative should be attached (Designation must be a separate document from the Assignment.)

Corporation  Association

Other

Citizenship/State of Incorporation/Organization

**Trademark Application Number(s) or Registration Number(s)**

Mark if additional numbers attached

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**Trademark Application Number(s)**

<input type="text"/>	<input type="text"/>	<input type="text"/>
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**Registration Number(s)**

<input type="text" value="2104796"/>	<input type="text" value="1698477"/>	<input type="text" value="1240536"/>
<input type="text" value="1605842"/>	<input type="text" value="1312900"/>	<input type="text" value="1605843"/>
<input type="text" value="1844621"/>	<input type="text" value="916946"/>	<input type="text" value="970462"/>
<input type="text" value="2001018"/>	<input type="text" value="1226827"/>	<input type="text" value="1151174"/>
<input type="text" value="2028321"/>	<input type="text" value="2130422"/>	<input type="text" value="2032614"/>
<input type="text" value="2104797"/>	<input type="text" value="965840"/>	<input type="text" value="1995037"/>
<input type="text" value="1968604"/>	<input type="text" value="1992938"/>	<input type="text" value="1965805"/>

**RECORDATION FORM COVER SHEET  
CONTINUATION  
TRADEMARKS ONLY**

FORM PTO-1618C  
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U.S. Department of Commerce  
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**Trademark Application Number(s)**

**Registration Number(s)**

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<input type="text" value="1872713"/>	<input type="text" value="1363648"/>	<input type="text" value="572327"/>
<input type="text" value="1968198"/>	<input type="text" value="1226104"/>	<input type="text" value="1773800"/>
<input type="text" value="1254701"/>	<input type="text" value="1988450"/>	<input type="text" value="1965754"/>
<input type="text" value="1256326"/>	<input type="text"/>	<input type="text"/>
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**SEMINIS, INC.**  
**SEMINIS VEGETABLE SEEDS, INC.**

**SECURITY AGREEMENT RE: INTELLECTUAL PROPERTY**

This Security Agreement Re: Intellectual Property (the "*Agreement*") is dated as of June 28, 1999, by and among Seminis, Inc., a Delaware corporation (the "*Company*"), and the other parties executing this Agreement under the heading "Debtors" (the Company and such other parties being hereinafter referred to collectively as the "*Debtors*" and individually as a "*Debtor*"), each with its mailing address as set forth on its signature page hereto, and Harris Trust and Savings Bank, an Illinois banking corporation ("*Harris*"), with its mailing address at 111 West Monroe Street, P.O. Box 755, Chicago, Illinois 60690, acting as agent hereunder for the Lenders hereinafter identified and defined (Harris acting as such agent and any successor or successors to Harris acting in such capacity being hereinafter referred to as the "*Agent*");

**P R E L I M I N A R Y   S T A T E M E N T S**

A. The Company, Seminis Vegetable Seeds, Inc., a California corporation ("*SVS*"), SVS Holland B.V., a private company with limited liability incorporated under the laws of The Netherlands ("*SVS Holland*" and, together with the Company and SVS, individually a "*Borrower*" and collectively the "*Borrowers*"), and Harris, individually and as agent, have entered into a Credit Agreement dated as of June 28, 1999 (such Credit Agreement as the same may be amended, modified or restated from time to time being hereinafter referred to as the "*Credit Agreement*"), pursuant to which Harris and other lenders which are and from time to time become party to the Credit Agreement (Harris and such other lenders which are and from time to time hereafter become party to the Credit Agreement being hereinafter referred to collectively as the "*Lenders*" and individually as a "*Lender*") have agreed, subject to certain terms and conditions, to extend credit and make certain other financial accommodations available to the Borrowers

B. The Borrowers may from time to time enter into one or more Interest Rate Protection Agreements with one or more of the Lenders party to the Credit Agreement for the purpose of hedging or otherwise protecting the Borrowers against changes in interest rates applicable to Term Loans under the Credit Agreement (the liability of the Borrowers in respect of such agreements with such Lenders being hereinafter referred to as the "*Hedging Liability*").

C. As a condition to extending credit to the Borrowers under the Credit Agreement, the Lenders have required, among other things, that each Debtor grant to the Agent a lien on and security interest in the personal property of such Debtor described herein subject to the terms and conditions hereof.

D. The Company owns, directly or indirectly, all or substantially all of the equity interests in each other Debtor and the Company provides each other Debtor with financial, management, administrative, and technical support which enables such Debtor to conduct its business in an orderly and efficient manner in the ordinary course.

US SEMINIS TRADEMARKS

15-Sep-98

Trademark	CountryName	CaseNumber	Application Number	Filing Date	Registration Number	Registration Date	Action Type	PTO/Base Date
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ARISTOGENES, INC. THE SIGNATURE OF ....

Cancelled 31 VEGETABLE SEEDS	United States of America	2241.T5			1640709	09-Apr-91		
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..... SUPERIOR SEEDS & DESIGN; NO SECTION 8 FILED; CANCELLED UNDER SECTION 8 AS OF 010797

BRUINSMA SEEDS & DESIGN

Registered 31 SEEDS OF OPEN POLLINATED AND HYBRID VARIETIES OF TOMATOES, CUCUMBERS, PEPPERS, EGGPLANTS, MELONS AND SQUASH, SEEDS OF OPEN POLLINATED VARIETIES OF LETTUCE AND ENDIVE, ALL FOR AGRICULTURAL PURPOSES	United States of America	2241.T10	74/250892	02-Mar-92	1829947	05-Apr-94		
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①

Aff 1 Yr	05-Apr-99
Aff 6 Mon	05-Oct-99
Aff of Use	05-Apr-00
Renew Wn	05-Oct-03
Renewal	05-Apr-04

CALIFORNIA

Registered 31 SEEDS FOR HORTICULTURAL PURPOSES, NAMELY, BEET, CAULIFLOWER, CANTALOUPE, CABBAGE, CARROT, CUCUMBER, EGGPLANT, ENDIVE, LETTUCE, ONION, PARSLEY, HOT PEPPER, SWEET PEPPER, PUMPKIN, RADISH, SQUASH, TOMATO AND WATERMELON	United States of America	2241.T15	73/322087	03-Aug-81	1226105	01-Feb-83		
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②

Renew Wn	01-Aug-02
Renewal	01-Feb-03

**US SEMINIS TRADEMARKS**

15-Sep-98

Trademark	CountryName	CaseNumber	Application Number	Filing Date	Registration Number	Registration Date	Action Type	PTO/Base Date
<b>DUSTX</b>								
Registered 40	United States of America	2241.T40	74/681042	30-May-95	2015005	12-Nov-96	(3)	
SEED PROCESSING SERVICES, NAMELY THE COATING OF AGRICULTURAL SEEDS FOR OTHERS								
							Aff 1 Yr	12-Nov-01
							Aff 6 Mon	12-May-02
							Aff of Use	12-Nov-02
							Renew Wn	12-May-06
							Renewal	12-Nov-06

**GENECORP & DESIGN**

Registered 31	United States of America	2241.T45	73/666318	30-Apr-87	1476131	09-Feb-88	(4)	
AGRICULTURAL SEEDS								

Renew Wn 09-Aug-07  
Renewal 09-Feb-08

**GOLD SHIELD**

Registered 31	United States of America	2241.T50	73/299751	05-Mar-81	1224451	18-Jan-83	(5)	
HORTICULTURAL SEED								

Renew Wn 18-Jul-02  
Renewal 18-Jan-03

**US SEMINIS TRADEMARKS**

15-Sep-98

Trademark	CountryName	CaseNumber	Application Number	Filing Date	Registration Number	Registration Date	Action Type	P/O/Base Date
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**HYPEEL**

Registered 31 United States of America 2241.T55 75/080794 29-Mar-96 2104778 14-Oct-97 **6**

SEEDS FOR AGRICULTURAL PURPOSES, NAMELY TOMATO AND PRODUCE, NAMELY FRESH FRUITS AND VEGETABLES

Aff 1 Yr	14-Oct-02
Aff 6 Mon	14-Apr-03
Aff of Use	14-Oct-03
Renew Wn	14-Apr-07
Renewal	14-Oct-07

**IMPACT**

Registered 31 United States of America 2241.T60 74/382561 26-Apr-93 1848683 09-Aug-94 **7**

SEEDS FOR AGRICULTURAL PURPOSES, NAMELY, LETTUCE, AND PRODUCE, NAMELY, FRESH FRUITS AND FRESH VEGETABLES

Aff 1 Yr	09-Aug-99
Aff 6 Mon	09-Feb-00
Aff of Use	09-Aug-00
Renew Wn	09-Feb-04
Renewal	09-Aug-04



15-Sep-98

US SEMINIS TRADEMARKS

Trademark	CountryName	CaseNumber	Application Number	Filing Date	Registration Number	Registration Date	Action Type	PTO/Base Date
INCOTEC	United States of America	2241.T65	74/681044	30-May-95	2011585	29-Oct-96	8	
Registered 1, 7							Aff 1 Yr	29-Oct-01
							Aff 6 Mon	29-Apr-02
							Aff of Use	29-Oct-02
							Renew Wn	29-Apr-06
							Renewal	29-Oct-06

CHEMICALS FOR USE IN COATING AGRICULTURAL SEEDS; APPARATUS FOR USE IN APPLYING CHEMICAL COATINGS AND PESTICIDES TO AGRICULTURAL SEEDS

INCOTEC	United States of America	2241.T65A	73/803484	30-May-89	1595730	08-May-90	9	
Registered 40							Renew Wn	08-Nov-99
							Renewal	08-May-00

SEED PROCESSING SERVICES, NAMELY, THE COATING OF AGRICULTURAL AND HORTICULTURAL SEEDS FOR OTHERS  
SEC 8 ACPT 051396

INTELLIGENT SEEDS	United States of America	2241.T68	75/333586	31-Jul-97			10	
Pending 31							AMEND ST	29-Jan-99

VEGETABLE SEEDS, FLOWER SEEDS, FRUIT SEEDS, AND HORTICULTURAL SEEDS

**US SEMINIS TRADEMARKS**

15-Sep-98

Trademark	CountryName	CaseNumber	Application Number	Filing Date	Registration Number	Registration Date	Action Type	PTO/Base Date
<b>NEMA</b>								
Registered 31	United States of America	2241.T75	75/084751	08-Apr-96	2104796	14-Oct-97	11	
	SEEDS FOR AGRICULTURAL PURPOSES, NAMELY TOMATO AND PRODUCE, NAMELY FRESH FRUITS AND VEGETABLES							
							Aff 1 Yr	14-Oct-02
							Aff 6 Mon	14-Apr-03
							Aff of Use	14-Oct-03
							Renew Wn	14-Apr-07
							Renewal	14-Oct-07
<b>ONGARD</b>								
Registered 31	United States of America	2241.T80	74/097100	14-Sep-90	1698477	30-Jun-92	12	
	FILM COATING FOR SEEDS FORMERLY 1830.T29							
							Renew Wn	30-Dec-01
							Renewal	30-Jun-02
<b>PACESETTER</b>								
Registered 31	United States of America	2241.T85	73/347683	29-Jan-82	1240536	31-May-83	13	
	TOMATO SEEDS FORMERLY 1830.T32							
							Renew Wn	01-Dec-02
							Renewal	31-May-03

Trademark

CountryName	CaseNumber	Application Number	Filing Date	Registration Number	Registration Date	Action Type	PTO/Base Date
United States of America	2241.T90	73/811496	10-Jul-89	1605842	10-Jul-90		
<p><b>PETOCOAT AND DESIGN</b></p> <p>Registered 31 HORTICULTURAL SEEDS; NAMELY, VEGETABLE SEED AND FLOWER SEED 8&amp;15 ACPT 032196</p>							
United States of America	2241.T95	73/460759	13-Jan-84	1312900	08-Jan-85		
<p><b>PETOPPLUS</b></p> <p>Registered 1 HORTICULTURAL SEED TREATMENT NAMELY, CHEMICAL TREATMENT FOR PROMOTING GROWTH OF VEGETABLE SEEDS</p>							
United States of America	2241.T96	73/811709	10-Jul-89	1605843	10-Jul-90		
<p><b>PETOPPLUS &amp; DESIGN</b></p> <p>Registered 31 HORTICULTURAL SEEDS; NAMELY, VEGETABLE SEED AND FLOWER SEED 8&amp;15 ACPT 032196</p>							

14

15

16

**US SEMINIS TRADEMARKS**

15-Sep-98

Trademark	CountryName	CaseNumber	Application Number	Filing Date	Registration Number	Registration Date	Action Type	P/O/Base Date
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PETOSEED

Registered 31	United States of America	2241.T99	74/424890	12-Jul-94	1844621	12-Jul-94		
VEGETABLE AND FRUIT SEEDS FOR PLANTING								
							RCRD ASGN	29-Dec-98
							Aff 1 Yr	12-Jul-99
							Aff 6 Mon	12-Jan-00
							Aff of Use	12-Jul-00
							Renew Wn	12-Jan-04
							Renewal	12-Jul-04

PETOSEED PS & DESIGN

Registered 31	United States of America	2241.T100	72/340822	15-Oct-69	916946	27-Jul-71		
SEED FOR HORTICULTURAL PURPOSES, SPECIFICALLY PEPPER, TOMATO, CUCUMBER, SQUASH, CANTALOUPE, EGGPLANT AND WATERMELON SEED								
							Renew Wn	27-Jan-01
							Renewal	27-Jul-01

PS

Registered 31	United States of America	2241.T105	72/439630	30-Oct-72	970462	16-Oct-73		
SEED FOR HORTICULTURAL PURPOSES-NAMELY, PEPPER, TOMATO, CUCUMBER, SQUASH, CANTALOUPE, EGGPLANT AND WATERMELON SEED								
							Renew Wn	16-Apr-03
							Renewal	16-Oct-03

**US SEMINIS TRADEMARKS**

15-Sep-98

Trademark	CountryName	CaseNumber	Application Number	Filing Date	Registration Number	Registration Date	Action Type	PTO/Base Date
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**QUALITY SEED FOR QUALITY ...**

Abandoned 16	United States of America	2241.110A	74/695124	29-Jun-95				
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ELECTRONIC TRANSMISSIONS CONCERNING SEEDS AND VEGETABLES  
... PRODUCE; ABANDON PER CLIENT 091098

**QUALITY SEED FOR QUALITY ...**

Registered 16	United States of America	2241.T110	74/695117	29-Jun-95	2001018	17-Sep-96		
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PERIODICALS, NAMELY NEWSLETTERS CONCERNING FRESH VEGETABLES  
... PRODUCE; FORMERLY 1830.T45B

Aff 1 Yr	17-Sep-01
Aff 6 Mon	17-Mar-02
Aff of Use	17-Sep-02
Renew Wn	17-Mar-06
Renewal	17-Sep-06

**QUICK PILL**

Registered 31	United States of America	2241.T115	73/321577	31-Jul-81	1226827	08-Feb-83		
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SEEDS FOR GROWING ASPARAGUS BEANS, BEETS, BROCCOLIS, BRUSSELS SPROUTS, CABBAGES, CARROTS, CAULIFLOWERS, CELERIACS, CELERIES, CHARDS, CHIERVILS, CHICORIES, CORNS, CRESSSES, CUCUMBERS, EGGPLANTS, ENDIVES, HERBS, KOHLRABIES, LEEKS, LETTUCES, MELONS, ONIONS, PARSLEYS, PEAS, PEPPERS, RADISHES, SCORZONERAS, SPINACHES, SQUASHES, TOMATOES, TURNIPS, WATERCRESSES, WATERMELONS

Renew Wn	08-Aug-02
Renewal	08-Feb-03

US SEMINIS TRADEMARKS

15-Sep-98

Trademark	CountryName	CaseNumber	Application Number	Filing Date	Registration Number	Registration Date	Action Type	PTO/Base Date
ROYAL SLUIS & DESIGN								
Registered 31	United States of America	2241.T125	73/189081	12-Oct-78	1151174	14-Apr-81	Renew Wn Renewal	14-Oct-00 14-Apr-01
	VEGETABLE AND FLOWER SEEDS, PELLETTED VEGETABLE AND FLOWER SEEDS, FRESH FRUITS, VEGETABLES AND FLOWERS							

SEED DEVELOPMENTS

Abandoned 42	United States of America	2241.T130	74/695118	29-Jun-95				
	INFORMATION SERVICES CONCERNING THE PRODUCE TRADE RENDERED BY COMPUTER BY MEANS OF A GLOBAL COMPUTER NETWORK							
	ABANDON PER CLIENT PER DNB 070797; FORMERLY 1830.T44A							

SEED DEVELOPMENTS

Registered 16	United States of America	2241.T130A	74/695104	29-Jun-95	2028321	07-Jan-97	Renew Wn Renewal	07-Jan-02 07-Jul-02 07-Jan-03 07-Jul-06 07-Jan-07
	PERIODICALS, NAMELY, NEWSLETTERS CONCERNING THE PRODUCE TRADE							
	FORMERLY 1830.T44B							

US SEMINIS TRADEMARKS

15-Sep-98

Trademark	CountryName	CaseNumber	Application Number	Filing Date	Registration Number	Registration Date	Action Type	PTO/Base Date
SEEDS FOR THE WORLD								
Registered 31	United States of America	2241.T135	75/224899	13-Jan-97	2130422	20-Jan-98	Aff 1 Yr Aff 6 Mon Aff of Use Renew Wn Renewal	20-Jan-03 20-Jul-03 20-Jan-04 20-Jul-07 20-Jan-08
	VEGETABLE SEEDS AND FLOWER SEEDS							

SEMINIS

Allowed  
31  
United States of America 2241.T140 75/172061 26-Sep-96  
VEGETABLE SEEDS, AND FLOWER SEEDS

Use/Ext2 17-Feb-99  
Use/Ext3 17-Aug-99  
Use/Ext4 17-Feb-00  
Stmnt+Ext5 17-Aug-00  
Ext5 Expr 17-Feb-01

SENECA

Pending  
31  
United States of America 2241.T136 74/391403 17-May-93

SEEDS FOR AGRICULTURAL PURPOSES; NAMELY, FIELD CORN, ORNAMENTAL CORN, SWEET CORN, POPCORN, SQUASH, CUCUMBER, PUMPKIN, MELON, PEA, BEAN, PEPPER, CARROT, BROCCOLI, SUNFLOWER, ALFALFA, GRASS, TURF, CLOVER AND FORAGE MIXTURE SEEDS

RESP STAT 27-Jan-99

**US SEMINIS TRADEMARKS**

15-Sep-98

Trademark	CountryName	CaseNumber	Application Number	Filing Date	Registration Number	Registration Date	Action Type	PTO/Base Date
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**SENECA HYBRIDS**

Pending 31	United States of America	2241.T137	74/391044	17-May-93		27		
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SEEDS FOR AGRICULTURAL PURPOSES; NAMELY, FIELD CORN, ORNAMENTAL CORN, SWEET CORN, POPCORN, SQUASH, CUCUMBER, PUMPKIN, MELON, PEA, BEAN, PEPPER, CARROT, BROCCOLI, SUNFLOWER, ALFALFA, GRASS, TURF, CLOVER, AND FORAGE MIXTURE SEEDS

**SENECA HYBRIDS**

Abandoned 42	United States of America	2241.T137A	74/391045	17-May-93			RESP STAI	27-Jan-99
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RESEARCH AND DEVELOPMENT OF AGRICULTURAL SEED PRODUCTS FOR OTHERS

**SENECOAT**

Registered 1	United States of America	2241.T138	74/558313	08-Aug-94	2032614	28		
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COATINGS FOR SEEDS FOR AGRICULTURAL PLANTING PURPOSES

Aff 1 Yr	21-Jan-02
Aff 6 Mon	21-Jul-02
Aff of Use	21-Jan-03
Renew Wn	21-Jul-06
Renewal	21-Jan-07

**SMART SEEDS**

Pending 31	United States of America	2241.T143	75/334715	31-Jul-97		29		
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VEGETABLE SEEDS, FLOWER SEEDS, FRUIT SEEDS, AND HORTICULTURAL SEEDS



**US SEMINIS TRADEMARKS**

15-Sep-98

Trademark	CountryName	CaseNumber	Application Number	Filing Date	Registration Number	Registration Date	Action Type	PTO/Base Date
<b>SPECTRUM</b>								
Registered 31	United States of America	2241.T145	75/084752	08-Apr-96	2104797	14-Oct-97	30	
	SEEDS FOR AGRICULTURAL PURPOSES, NAMELY TOMATO AND PRODUCE, NAMELY FRESH FRUITS AND VEGETABLES							
							Aff 1 Yr	14-Oct-02
							Aff 6 Mon	14-Apr-03
							Aff of Use	14 Oct 03
							Renew Wn	14-Apr-07
							Renewal	14-Oct-07
<b>SPLIT PILL &amp; DESIGN</b>								
Registered 31	United States of America	2241.T155	72/441231	15-Nov-72	965840	14-Aug-73	31	
	PELLETED SEEDS FOR CULTIVATION PLANTS							
							Renew Wn	14-Feb-03
							Renewal	14-Aug-03
<b>SPLITKOTE</b>								
Registered 1	United States of America	2241.T150A	74/681045	30-May-95	1995037	20-Aug-96	32	
	NON-BIODECIDAL, GROWTH-PROMOTING CHEMICALS FOR USE IN COATING AGRICULTURAL SEEDS							
							Aff 1 Yr	20-Aug-01
							Aff 6 Mon	20-Feb-02
							Aff of Use	20-Aug-02
							Renew Wn	20-Feb-06
							Renewal	20-Aug-06

US SEMINIS TRADEMARKS

15-Sep-98

Trademark	CountryName	CaseNumber	Application Number	Filing Date	Registration Number	Registration Date	Action Type	P/O/Base Date	
SPLITKOTE	United States of America	2241.T150B	74/686520	06-Jun-95	1968604	16-Apr-96	33	Aff 1 Yr	16-Apr-01
								Aff 6 Mon	16-Oct-01
								Aff of Use	16-Apr-02
								Renew Wn	16-Oct-05
								Renewal	16-Apr-06

SEED PROCESSING SERVICES, NAMELY THE COATING OF AGRICULTURAL SEEDS FOR OTHERS

SPRINGKOTE

Registered 40	United States of America	2241.T160	74/681040	30-May-95	1992938	13-Aug-96	34	Aff 1 Yr	13-Aug-01
								Aff 6 Mon	13-Feb-02
								Aff of Use	13-Aug-02
								Renew Wn	13-Feb-06
								Renewal	13-Aug-06

NON-BIODEGRADABLE, GROWTH-PROMOTING CHEMICALS FOR USE IN COATING AGRICULTURAL SEEDS

SPRINGKOTE

Registered 40	United States of America	2241.T160A	74/685285	06-Jun-95	1965805	02-Apr-96	35	Aff 1 Yr	02-Apr-01
								Aff 6 Mon	02-Oct-01
								Aff of Use	02-Apr-02
								Renew Wn	02-Oct-05
								Renewal	02-Apr-06

SEED PROCESSING SERVICES, NAMELY THE COATING OF AGRICULTURAL SEEDS FOR OTHERS

US SEMINIS TRADEMARKS

15-Sep-98

Trademark	CountryName	CaseNumber	Application Number	Filing Date	Registration Number	Registration Date	Action Type	P/O/Base Date
TARGET	United States of America	2241.T1165	74/382559	26-Apr-93	1872713	10-Jan-95		
Registered 31								
HYBRID LETTUCE SEEDS SOLD ONLY TO COMMERCIAL LETTUCE GROWERS THROUGH A DEALER NETWORK, AND NOT SOLD AT RETAIL								
							Aff 1 Yr	10-Jan-00
							Aff 6 Mon	10-Jul-00
							Aff of Use	10-Jan-01
							Renew Wn	10-Jul-04
							Renewal	10-Jan-05

ULTRASEED

Registered

31

VEGETABLE SEEDS  
FORMERLY 1830.T39

97

19-Feb-85

73/522953

2241.T1170

United States of America

1363648

01-Oct-85

Renew Wn

Renewal

01-Apr-05

01-Oct-05

VIGORPAK

Registered

31

VEGETABLE SEED  
FORMERLY 1830.T41

98

24-Mar-53

572327

71/623943

23-Jan-52

United States of America

24-Mar-53

Renew Wn

Renewal

24-Sep-02

24-Mar-03

US SEMINIS TRADEMARKS

15-Sep-98

Trademark	CountryName	Number	Application Number	Filing Date	Registration Date	Action Type	P/O/Base Date
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X3R

Registered 31	United States of America	2241.T180	74/648541	20-Mar-95	1968198		
VEGETABLE SEEDS							
						Aff 1 Yr	16-Apr-01
						Aff 6 Mon	16-Oct-01
						Aff of Use	16-Apr-02
						Renew Wn	16-Oct-05
						Renewal	16 Apr-06

X5R

Allowed 29, 31	United States of America	2241.T185	75/334716	31-Jul-97			
PROCESSED PEPPERS; FRESH PEPPERS							
						Use/Ext1	18-Feb-99
						Use/Ext2	18-Aug-99
						Use/Ext3	18-Feb-00
						Use/Ext4	18-Aug-00
						Stmnt+Ext5	18-Feb-01
						Ext5 Expr	18-Aug-01

**SCHEDULE B-2**

**TO SECURITY AGREEMENT  
RE: INTELLECTUAL PROPERTY**

**TRADEMARK LICENSES**

None

US SEMINIS TRADEMARKS

15-Sep-98

Trademark	CountryName	CaseNumber	Application Number	Filing Date	Registration Number	Registration Date	Action Type	PTO/Base Date
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CALIFORNIA & DESIGN

Registered 31	United States of America	2241.T16	73/322086	03-Aug-81	1226104	01-Feb-83		
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SEEDS FOR HORTICULTURAL PURPOSES, NAMELY, BEET, CAULIFLOWER, CANTALOUPE, CABBAGE, CARROT, CUCUMBER, EGGPLANT, ENDIVE, LETTUCE, ONION, PARSLEY, HOT PEPPER, SWEET PEPPER, PUMPKIN, RADISH, SQUASH, TOMATO AND WATERMELON

Renew Wn 01-Aug-02  
Renewal 01-Feb-03

CUKETTE

Registered 31	United States of America	2241.T20	74/185602	16-Jul-91	1773800	25-May-93		
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42

CUCUMBER SEEDS FOR AGRICULTURAL PURPOSES  
FORMERLY 1830.T15

Aff 6 Mon 25-Nov-98  
AFF OF USE 25-May-99  
Renew Wn 25-Nov-02  
Renewal 25-May-03

DENHOLM SEEDS

Registered 31	United States of America	2241.T25	73/362392	30-Aug-82	1254701	18-Oct-83		
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FLOWER SEEDS FOR HORTICULTURAL PURPOSES

Renew Wn 18-Apr-03  
Renewal 18-Oct-03

**US SEMINIS TRADEMARKS**

15-Sep-98

Trademark	CountryName	CaseNumber	Application Number	Filing Date	Registration Number	Registration Date	Action Type	PTO/Base Date
<b>DESIGN ONLY</b>								
Registered 1, 7	United States of America	2241.T30	74/701306	14-Jul-95	1988450	23-Jul-96		
CHEMICALS FOR USE IN COATING AGRICULTURAL SEEDS; MACHINE APPARATUS FOR USE IN APPLYING CHEMICAL COATINGS AND PESTICIDES TO AGRICULTURAL SEEDS (TWO CIRCLES)								
							Aff 1 Yr	23-Jul-01
							Aff 6 Mon	23-Jan-02
							Aff of Use	23-Jul-02
							Renew Wn	23-Jan-06
							Renewal	23-Jul-06
<b>DESIGN ONLY</b>								
Registered 40	United States of America	2241.T30A	74/965754	30-May-95	1965754	02-Apr-96		
SEED PROCESSING SERVICES, NAMELY THE COATING OF AGRICULTURAL SEEDS FOR OTHERS (TWO CIRCLES)								
							Aff 1 Yr	02-Apr-01
							Aff 6 Mon	02-Oct-01
							Aff of Use	02-Apr-02
							Renew Wn	02-Oct-05
							Renewal	02-Apr-06
<b>DS &amp; DESIGN</b>								
Registered 31	United States of America	2241.T35	73/362393	30-Sep-82	1256326	01-Nov-83		
FLOWER SEEDS FOR HORTICULTURAL PURPOSES								
							Renew Wn	01-May-03
							Renewal	01-Nov-03

**SCHEDULE C-1**

**TO SECURITY AGREEMENT  
RE: INTELLECTUAL PROPERTY**

**COPYRIGHTS**

U.S. COPYRIGHT  
REG. NO. (AUTHOR)

TITLE

DATE OF REG.

None

PENDING U.S. COPYRIGHT  
APPLICATION NO. (AUTHOR)

TITLE

FILING DATE

None



**SCHEDULE D**

**PERMITTED ENCUMBRANCES**

None

E. Each Debtor will benefit, directly or indirectly, from credit and other financial accommodations extended by the Lenders to the Borrowers.

NOW, THEREFORE, for and in consideration of the execution and delivery by the Lenders of the Credit Agreement, and other good and valuable consideration, receipt whereof is hereby acknowledged, the parties hereto hereby agree as follows:

SECTION 1. GRANT OF SECURITY INTEREST IN THE COLLATERAL; OBLIGATIONS SECURED.

(a) Each Debtor hereby grants, bargains, sells, transfers, conveys, assigns, mortgages and pledges to the Agent for the ratable benefit of the Lenders, and grants to the Agent for the ratable benefit of the Lenders a security interest in, and acknowledges and agrees that the Agent has and shall continue to have for the ratable benefit of the Lenders a continuing security interest in, any and all right, title and interest of each Debtor, whether now existing or hereafter acquired or arising, in and to the following:

(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-1 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-2 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) *Trademarks.* Trademarks and Trademark registrations, whether now owned or hereafter adopted or acquired, or in which such Debtor now has or hereafter acquires any rights (the term "*Trademarks*" means and includes (i) all trademarks, trade names, trade styles, service marks and logos, all prints and labels on which said trademarks, trade names, trade styles, service marks and logos have appeared or appear and all designs and general intangibles of like nature, all registrations and recordings thereof, and all applications in connection therewith, 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, extensions or renewals thereof), including, without limitation, each Trademark registration listed on Schedule B-1 hereto, and all of the goodwill of the business connected with the use of, and symbolized by, each Trademark and Trademark registration and all customer lists and other records of such Debtor relating to the distribution of products bearing, or rendition of services otherwise relating to, a Trademark;

(iv) *Trademark Licenses.* Trademark Licenses, whether now owned or hereafter acquired, or in which such Debtor now has or hereafter acquires any rights (the term "*Trademark Licenses*" means and includes any written agreement granting to any person any right to use or exploit any Trademark or Trademark registration of another person), including, without limitation, the agreements described in Schedule B-2 hereto, and all of the goodwill of the business connected with the use of, and symbolized by, each Trademark licensed and all royalties and other sums due or to become due under or in respect of such Debtor's Trademark Licenses, together with the right to sue for and collect all such royalties and other sums.

(v) *Copyrights.* Copyrights and Copyright registrations, whether now owned or hereafter adopted or acquired, or in which such Debtor now has or hereafter acquires any rights (the term "*Copyrights*" means and includes (i) all copyrights, whether or not published or registered, and all works of authorship and other intellectual property and the rights therein, including, without limitation, copyrights for computer programs and data bases, copyrightable materials, and all tangible property embodying such copyrights or copyrightable materials, all registrations and recordings thereof, and all applications in connection therewith, including, without limitation, registrations, recordings and applications in the United States Copyright 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 renewals, derivative works, enhancements, modifications, new releases and other revisions thereof, and (iii) all accounts receivable, income, royalties, damages and payments now or hereafter due and/or payable with respect thereto, including, without limitation, payments under all licenses entered into in connection therewith, and (iv) all rights corresponding thereto throughout the world), including, without limitation, each Copyright registration listed on Schedule C-1 hereto;

(vi) *Copyright Licenses.* Copyright Licenses, whether now owned or hereafter acquired, or in which such Debtor now has or hereafter acquires any rights (the term "*Copyright Licenses*" means and includes any written agreement granting to any person the right to use or exploit any Copyright or Copyright registration of another person, including, without limitation, the right to use the foregoing to prepare for sale or distribution and sell or distribute any and all inventory now or hereafter owned by such Debtor and now or hereafter covered by such licenses), including, without limitation, the license and subscription agreements listed on Schedule C-2 hereto, and all royalties and

other sums due or to become due under or in respect of such Debtor's Copyright Licenses, together with the right to sue for and collect all such royalties and other sums;

(vii) *Know-How and Trade Secret Collateral.* All know-how, inventions, processes, methods, information, data, plans, blueprints, specifications, designs, drawings, engineering reports, test reports, material standards, processing standards and performance standards, to the extent that the foregoing pertain to manufacturing, production or processing operations of such Debtor and constitute trade secrets of such Debtor, and all licenses or other similar agreements granted to or by such Debtor with respect to any of the foregoing;

(viii) *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;

(ix) *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

(x) *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, (ii) any claim by such Debtor against third parties for damages by reason of past, present or future infringement or dilution of any Trademark or Trademark registration or of any Trademark licensed under any Trademark License, or for injury to the goodwill of the business connected with the use of, or symbolized by, any Trademark or Trademark registration or of any Trademark licensed under any Trademark License, (iii) any claim of such Debtor against third parties for damages by reason of past, present or future infringements of any Copyright or Copyright registration or of any Copyright licensed under any Copyright License, and (iv) 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), (ii), (iii) and (iv);

all of the foregoing being herein sometimes referred to as the "*Collateral*"; provided that the Collateral shall not include any license agreement under which any Debtor is licensee which, by its terms, prohibits the security interest contemplated by this Agreement. All terms which are used herein which are defined in the Uniform Commercial Code of the State of Illinois ("*UCC*") shall have the same meanings herein as such terms are defined in the UCC, unless this Agreement shall otherwise specifically provide

(b) This Agreement is made and given to secure, and shall secure, the prompt payment and performance of (i) any and all indebtedness, obligations and liabilities of the Debtors, and of any of them individually, to the Agent and the Lenders, and to any of them individually, under or in connection with or evidenced by the Credit Agreement, the Notes of the Borrowers heretofore or hereafter issued under the Credit Agreement and the obligations of the Borrowers to reimburse the Agent and the Lenders for the amount of all drawings on all L/Cs issued for the account of the Borrowers pursuant to the Credit Agreement, and all other obligations of the Borrowers under any and all applications for L/Cs, and any and all liability of the Debtors, and of any of them individually, arising under or in connection with or otherwise evidenced by agreements with any one or more of the Lenders with respect to any Hedging Liability, and any and all liability of the Debtors, and of any of them individually, arising under any guaranty issued by it relating to the foregoing or any part thereof, in each case whether now existing or hereafter arising (and whether arising before or after the filing of a petition in bankruptcy), due or to become due, direct or indirect, absolute or contingent, and howsoever evidenced, held or acquired and (ii) subject to the provisions of Section 12.8 of the Credit Agreement, any and all expenses and charges, legal or otherwise, suffered or incurred by the Agent and the Lenders, and any of them, in collecting or enforcing any of such indebtedness, obligations and liabilities or in realizing on or protecting or preserving any security therefor, including, without limitation, the lien and security interest granted hereby (all of the indebtedness, obligations, liabilities, expenses and charges described above being hereinafter referred to as the "Secured Obligations"). Notwithstanding anything in this Agreement to the contrary, the right of recovery against any Debtor (other than the Company to which this limitation shall not apply) under this Agreement shall not exceed \$1.00 less than the amount which would render such Debtor's obligations under this Agreement void or voidable under applicable law, including fraudulent conveyance law.

## SECTION 2. TERMS DEFINED IN CREDIT AGREEMENT.

All capitalized terms used herein without definition shall have the same meanings herein as such terms have in the Credit Agreement. The term "Debtor" and "Debtors" as used herein shall mean and include the Debtors collectively and also each individually, with all grants, representations, warranties and covenants of and by the Debtors, or any of them, herein contained to constitute joint and several grants, representations, warranties and covenants of and by the Debtors; *provided, however*, that unless the context in which the same is used shall otherwise require, any grant, representation, warranty or covenant contained herein related to the Collateral shall be made by each Debtor only with respect to the Collateral owned by it or represented by such Debtor as owned by it.

## SECTION 3. NO RELEASE

Nothing set forth in this Agreement shall relieve any Debtor from the performance of any term, covenant, condition or agreement on such Debtor's part to be performed or observed under or in respect of any of the Collateral or from any liability to any party under or in respect of any of the Collateral or impose any obligation on the Agent or any Lender to perform or observe any such term, covenant, condition or agreement on such Debtor's part to be so performed or observed or impose any liability on the Agent or any Lender for any act or omission on the part of such Debtor relative thereto or for any breach of any representation or warranty on the part of

any Debtor contained in this Agreement or under or in respect of the Collateral or made in connection herewith or therewith.

#### SECTION 4. USE OF COLLATERAL.

Notwithstanding anything to the contrary contained in this Agreement, until an Event of Default hereunder has occurred and is continuing and thereafter until otherwise notified by the Agent, each Debtor may continue to exploit, license, use, enjoy and protect the Collateral throughout the world and the Agent shall from time to time execute and deliver, upon written request of each Debtor, any and all instruments, certificates or other documents, in the form so requested, necessary or appropriate in the reasonable judgment of such Debtor to enable such Debtor to continue to exploit, license, use, enjoy and protect the Collateral throughout the world.

#### SECTION 5. REPRESENTATIONS AND WARRANTIES OF THE DEBTORS.

Each Debtor hereby represents and warrants to the Agent and the Lenders as follows:

(a) Each Debtor is, and, as to the Collateral acquired by it from time to time after the date hereof, each Debtor will be, the owner or, as applicable, licensee of all the Collateral. Each Debtor's rights in the Collateral are and shall remain free and clear of any lien, pledge, security interest, encumbrance, license, assignment, collateral assignment or charge of any kind, including, without limitation, any filing of or agreement to file a financing statement as debtor under the Uniform Commercial Code or any similar statute, except for the lien and security interest created by this Agreement and except as permitted by Schedule D attached hereto and Sections 7.10(a), (e), (f) and (k) of the Credit Agreement (collectively, the "*Permitted Encumbrances*"). Each Debtor has made no previous assignment, conveyance, transfer or agreement in conflict herewith. Each Debtor further represents and warrants to the Agent and each Lender that Schedules A-1, A-2, B-1, B-2, C-1 and C-2 hereto, respectively, are true and correct lists of all Patents, Patent Licenses, Trademarks, Trademark Licenses, Copyrights and Copyright Licenses registered in the United States owned or used by such Debtor as of the date of this Agreement and that Schedules A-1, A-2, B-1, B-2, C-1 and C-2 are true and correct with respect to the matters set forth therein as of the date of this Agreement.

(b) Each Debtor has full corporate power to pledge and grant a security interest in all the Collateral pursuant to this Agreement.

(c) No authorization, consent, approval, license, qualification or exemption from, nor any filing, declaration or registration with, any court, governmental agency or regulatory authority, or with any securities exchange or any other party, is required in connection with (i) each Debtor's execution, delivery or performance of this Agreement, (ii) each Debtor's grant of a security interest (including the priority thereof when the appropriate filings have been made and accepted) in the Collateral in the manner and for the purpose contemplated by this Agreement or (iii) the rights of the Agent and Lenders created hereby, except those that have already been obtained or made and those referred to in paragraph (f) of this *Section 5*.

(d) Each Debtor has made all filings and recordations consistent with such Debtor's current practice to protect its interests in the Collateral.

(e) Each Debtor owns directly or has rights to use all the Collateral necessary for or of importance to the business of such Debtor in the ordinary course as presently conducted. The use of the Collateral by each Debtor does not, to the best of such Debtor's knowledge after due inquiry, infringe on the rights of any party, nor has any claim of such infringement been made.

(f) Upon filings and the acceptance thereof in the appropriate offices under the Uniform Commercial Code and in the United States Patent and Trademark Office and the United States Copyright Office, this Agreement will create a valid and duly perfected first priority lien and security interest in the Collateral located in the United States of America subject to no prior liens or encumbrances other than Permitted Encumbrances.

(g) To the best of each Debtor's knowledge after due inquiry, no claim has been made and remains outstanding that any Debtor's use of any of the Collateral does or may violate the rights of any third person which violation could reasonably be expected to have a material adverse effect on the financial condition or results of operations of the Company and its Subsidiaries taken as a whole.

#### SECTION 6. COVENANTS AND AGREEMENTS OF THE DEBTORS.

Each Debtor hereby covenants and agrees with the Agent and the Lenders as follows:

(a) On a continuing basis, each Debtor will, at the expense of such Debtor, subject to any prior licenses, encumbrances and restrictions and prospective licenses, encumbrances and restrictions permitted hereunder, make, execute, acknowledge and deliver, and file and record in the proper filing and recording places within the United States of America, all such instruments, including, without limitation, appropriate financing and continuation statements and collateral agreements, and take all such action, as may reasonably be deemed necessary or advisable by the Agent (i) to carry out the intent and purposes of this Agreement, (ii) to assure and confirm to the Agent the grant and perfection of a first priority security interest in the Collateral for the benefit of the Lenders or (iii) to enable the Agent to exercise and enforce its rights and remedies hereunder with respect to any Collateral.

(b) Without limiting the generality of the foregoing paragraph (a) of this Section 6, each Debtor (i) will not enter into any agreement that would impair or conflict with such Debtor's obligations hereunder, (ii) will, promptly following its becoming aware thereof, notify the Agent of (x) any material final adverse determination in any proceeding in the United States Patent and Trademark Office or United States Copyright Office with respect to any of the Collateral or (y) any material final adverse determination in any federal, state, local or foreign court or administrative bodies regarding such Debtor's claim of ownership in or right to use any of the Collateral, its right to register any such Collateral or its right to keep and maintain such registration;

(iii) will properly maintain and care for the Collateral to the extent necessary for the conduct of the business of such Debtor in the ordinary course as presently conducted and consistent with such Debtor's current practice; (iv) will not grant or permit to exist any lien or encumbrance upon or with respect to the Collateral or any portion thereof except the Permitted Encumbrances and will not execute any security agreement or financing statement covering any of the Collateral except in the name of the Agent; (v) will not permit to lapse or become abandoned, settle or compromise any pending or future material litigation or material administrative proceeding with respect to any Collateral without the prior written consent of the Agent or, subject to Section 7.12 of the Credit Agreement, contract for sale or otherwise sell, convey, assign or dispose of, or grant any option with respect to, the Collateral or any material portion thereof; (vi) upon any responsible officer of such Debtor obtaining knowledge thereof, will promptly notify the Agent in writing of any event which may reasonably be expected to materially and adversely affect the value of the Collateral, the ability of such Debtor or the Agent to dispose of the Collateral or the rights and remedies of the Agent in relation thereto, including, without limitation, a levy or threat of levy or any legal process against the Collateral; (vii) will diligently keep reasonable records respecting the Collateral; (viii) hereby authorizes the Agent, in its sole discretion, to file one or more financing or continuation statements relative to all or any part of the Collateral without the signature of such Debtor where permitted by law (and the Agent shall provide copies of such financing or continuation statements to the applicable Debtor after filing the same, but the Agent's failure to give such copies shall not affect the solidity or enforceability of the relevant filing); (ix) during the existence of an Event of Default will furnish to the Agent from time to time statements and schedules further identifying and describing the Collateral and such other materials evidencing or reports pertaining to the Collateral as the Agent may reasonably request, all in reasonable detail; (x) will pay when due any and all material taxes, levies, maintenance fees, charges, assessments, licenses fees and similar taxes or impositions payable in respect of the Collateral except to the extent being contested in good faith by appropriate proceedings which preclude interference with the operation of the business of such Debtor in the ordinary course; and (xi) comply in all material respects with all laws, rules and regulations applicable to the Collateral.

(c) If any Debtor shall (i) obtain any rights to any new invention (whether or not patentable), know-how, trade secret, design, process, procedure, formula, diagnostic test, service mark, trademark, trademark registration, trade name, copyright, copyright registration, or license or (ii) become entitled to the benefit of any patent, patent application, service mark, trademark, trademark application, trademark registration, copyright, copyright application, copyright registration, license renewal or copyright renewal or extension, or patent for any reissue, division, continuation, renewal, extension, or continuation-in-part of any Patent or any improvement on any Patent, the provisions of this Agreement shall automatically apply thereto and the same shall automatically constitute Collateral and be and become subject to the assignment, lien and security interest created hereby without further action by any party, all to the same extent and with the same force and effect as if the same had originally been Collateral hereunder. If any Debtor so obtains or becomes entitled to any of the foregoing rights described in clauses (i) and (ii) above, such Debtor shall give written notice thereof to the Agent on a



quarterly basis. Each Debtor agrees, promptly following written request therefor by the Agent, to confirm the attachment of the lien and security interest created hereby to any such rights described in clauses (i) and (ii) above by execution of an instrument in form and substance reasonably acceptable to the Agent.

(d) Each Debtor shall, consistent with such Debtor's current practice, prosecute diligently applications for the Patents, Trademarks and Copyrights now or hereafter pending that in such Debtor's reasonable judgment would be materially beneficial to the business of such Debtor in the ordinary course, make application on unpatented but patentable inventions and registrable but unregistered Trademarks and Copyrights that in such Debtor's reasonable judgment would be materially beneficial to the business of such Debtor in the ordinary course, file and prosecute opposition and cancellation proceedings and do all acts necessary to preserve and maintain all its material rights in the Collateral, unless as to any Patent, Trademark or Copyright, in the reasonable judgment of such Debtor, such Patent, Trademark or Copyright is no longer necessary or useful to the business of such Debtor. Any expenses incurred in connection with such actions shall be borne by such Debtor.

#### SECTION 7. GRANT OF LICENSE TO PATENTS, TRADEMARKS, COPYRIGHTS, ETC.

Without in any way limiting the scope of the lien and security interest created hereby, each Debtor hereby grants to the Agent for the ratable benefit of the Lenders an irrevocable, nonexclusive license and right to use all of such Debtor's Patents, Patent applications, Patent Licenses, Trademarks, Trademark registrations, Trademark Licenses, trade names, trade styles, Copyrights, Copyright registrations, Copyright Licenses and similar intangibles in the processing, production, marketing, distribution or sale by the Agent of all or any part of its collateral for the Secured Obligations in connection with and solely in connection with any foreclosure or other realization on such collateral to the extent permitted by law and any applicable license or other intellectual property agreement. The license and rights granted the Agent hereby shall be exercisable without the payment of any royalty, fee, charge or any other compensation to such Debtor or any other party. Such license and rights shall include reasonable access to all records in which any of the licensed items may be recorded or stored. Such license and rights shall be absolute and unconditional to the extent used for the purpose stated above.

#### SECTION 8. SUPPLEMENTS. FURTHER ASSURANCES.

Each Debtor (i) agrees that it will join with the Agent in executing and, at such Debtor's own expense, file and refile, or permit the Agent to file and refile, such financing statements, continuation statements and other instruments and documents (including without limitation this Agreement) in such offices (including, without limitation, the United States Patent and Trademark Office and the United States Copyright Office) as the Agent may reasonably deem necessary or appropriate in order to perfect and preserve the rights and interests granted to the Agent hereunder, and the Agent shall provide copies of such filings to the applicable Debtor after such filing is made, but the Agent's failure to provide such copies shall not affect the validity or enforceability of the relevant filing, and (ii) hereby authorizes the Agent to file and refile such instruments and documents and any other instruments or documents related thereto without the

signature of such Debtor where permitted by law and (iii) agrees to do such further acts and things, and to execute and deliver to the Agent such additional instruments and documents, as the Agent may reasonably require to carry into effect the purposes of this Agreement or to better assure and confirm unto the Agent its respective rights, powers and remedies hereunder. All of the foregoing are to be at the sole cost of each Debtor. Any costs of the foregoing incurred by the Agent shall be payable in accordance with Section 12.8 of the Credit Agreement and shall constitute additional Secured Obligations hereunder.

#### SECTION 9. THE AGENT MAY PERFORM.

During any time that any Debtor fails to perform any agreement contained herein and the expiration of any applicable grace period under Section 8.1 of the Credit Agreement after receipt of a written request to do so from the Agent, the Agent may itself perform, or cause performance of, such agreement, and the expenses of the Agent, including the fees and expenses of its counsel, so incurred in connection therewith shall be payable by the Debtors under Section 12.8 of the Credit Agreement.

#### SECTION 10. REMEDIES UPON DEFAULT.

(a) The occurrence of any event or the existence of any condition which is specified as an "Event of Default" under the Credit Agreement shall constitute an "*Event of Default*" hereunder.

(b) Upon the occurrence and during the continuation of any Event of Default hereunder, (i) the Agent shall have, in addition to all other rights provided herein or by law, the rights and remedies of a secured party under the Uniform Commercial Code as enacted in the State of Illinois and any successor statute(s) thereto (regardless of whether such Uniform Commercial Code is the law of the jurisdiction where the rights or remedies are asserted and regardless of whether such Uniform Commercial Code applies to the affected Collateral), and (ii) further the Agent may, without demand and without advertisement, notice, hearing or process of law, all of which each Debtor hereby waives, at any time or times, sell and deliver any or all of the Collateral at public or private sale, for cash, upon credit or otherwise, at such prices and upon such terms as the Agent deems advisable, in its sole discretion, subject to any restrictions contained in any applicable license or other intellectual property agreement. Any requirement of reasonable notice shall be met if such notice is personally served on or mailed, postage prepaid, to each Debtor in accordance with Section 16(b) hereof at least ten (10) days before the time of sale or other event giving rise to the requirement of such notice; *however*, no notification need be given to any Debtor if such Debtor has signed, after an Event of Default hereunder has occurred, a statement renouncing any right to notification of sale or other intended disposition. The Agent shall not be obligated to make any sale or other disposition of the Collateral regardless of notice having been given. The Agent or any Lender may be the purchaser at any such sale. Each Debtor hereby waives all of its rights of redemption from any such sale. Subject to the provisions of applicable law, the Agent may postpone or cause the postponement of the sale of all or any portion of the Collateral by announcement at the time and place of such sale, and such sale may, without further notice, be made at the time and place to which the sale was postponed or the Agent may further postpone such sale by announcement made at such time and place.

(c) Without in any way limiting the foregoing, upon the occurrence and during the continuation of any Event of Default hereunder, the Agent may, to the full extent permitted by applicable law, with ten (10) days' prior notice to each Debtor, and without advertisement, notice, hearing or process of law of any other kind, all of which each Debtor hereby waives, (i) exercise any and all rights as beneficial and legal owner of the Collateral, including, without limitation, any and all consensual rights and powers with respect to the Collateral and (ii) sell or assign or grant a license to use, or cause to be sold or assigned or granted a license to use, any or all of the Collateral or any part hereof, in each case free of all rights and claims of any Debtor therein and thereto. In that connection, the Agent shall have the right to cause any or all of the Collateral to be transferred of record into the name of the Agent or its nominee as well as the right to impose (i) such limitations and restrictions on the sale or assignment of the Collateral as the Agent may deem to be necessary or appropriate to comply with any law, rule or regulation, whether federal, state or local, having applicability to the sale or assignment and (ii) requirements for any necessary governmental approvals.

(d) In the event the Agent shall have instituted any proceeding to enforce any right, power or remedy under this Agreement by foreclosure, sale, entry or otherwise, and such proceeding shall have been discontinued or abandoned for any reason or shall have been determined adversely to the Agent, then and in every such case each Debtor, the Agent and each Lender shall be restored to their respective former positions and rights hereunder with respect to the Collateral, and all rights, remedies and powers of the Agent and the Lenders shall continue as if no such proceeding had been instituted.

(e) Failure by the Agent to exercise any right, remedy or option under this Agreement or any other agreement between any Debtor and the Agent or provided by law, or delay by the Agent in exercising the same, shall not operate as a waiver; no waiver shall be effective unless it is in writing, signed by the party against whom such waiver is sought to be enforced and then only to the extent specifically stated. For purposes of this Agreement, an Event of Default shall be construed as continuing after its occurrence until the same is cured or waived in writing by the Lenders or the Required Banks, as the case may be, in accordance with the terms of the Credit Agreement. Neither the Agent, nor any Lender, nor any party acting as attorney for the Agent or any Lender, shall be liable hereunder for any acts or omissions or for any error of judgment or mistake of fact or law other than their gross negligence or willful misconduct. The rights and remedies of the Agent under this Agreement shall be cumulative and not exclusive of any other right or remedy which the Agent or the Lenders may have.

#### SECTION 11. THE AGENT APPOINTED ATTORNEY-IN-FACT.

Each Debtor hereby irrevocably appoints the Agent, its nominee, or any other person whom the Agent may designate as such Debtor's attorney-in-fact, with full authority in the place and stead of such Debtor and in the name of such Debtor, the Agent or otherwise, upon the occurrence and during the continuation of any Event of Default hereunder, from time to time in the Agent's discretion, to take any action and to execute any instrument which the Agent may deem necessary or advisable to accomplish the purposes of this Agreement, including, without limitation, to prosecute diligently any patent, trademark or copyright or any application for Patents, Trademarks or Copyrights pending as of the date of this Agreement or thereafter until

this Agreement has terminated in accordance with Section 14 hereof, to make application on unpatented but patentable inventions and registrable but unregistered Trademarks and Copyrights, to file and prosecute opposition and cancellation proceedings, to do all other acts necessary or desirable to preserve all rights in Collateral and otherwise to file any claims or take any action or institute any proceedings which the Agent may deem necessary or desirable to enforce the rights of the Agent and the Lenders with respect to any of the Collateral. Each Debtor hereby ratifies and approves all acts of any such attorney and agrees that neither the Agent nor any such attorney will be liable for any acts or omissions nor for any error of judgment or mistake of fact or law other than their gross negligence or willful misconduct. The foregoing power of attorney, being coupled with an interest, is irrevocable until this Agreement has terminated in accordance with Section 14 hereof.

**SECTION 12. APPLICATION OF PROCEEDS.**

The proceeds and avails of the Collateral at any time received by the Agent upon the occurrence and during the continuation of any Event of Default shall, when received by the Agent in cash or its equivalent, be applied by the Agent in accordance with Section 3.5 of the Credit Agreement. The Debtors shall remain liable to the Agent and the Lenders for any deficiency. Any surplus remaining after the termination of this Agreement in accordance with Section 14 hereof shall be returned to the Company, as agent for the Debtors, or to whomsoever the Agent reasonably determines is lawfully entitled thereto.

**SECTION 13. INDEMNIFICATION: LITIGATION.**

(a) Each Debtor shall have the right to commence and prosecute in its own name, as real party in interest, for its own benefit and at its own expense, such applications for protection of the Collateral, suits, proceedings or other actions for infringement, unfair competition, dilution or other damage as are in its reasonable business judgment necessary to protect the Collateral. The Agent and the Lenders shall provide all reasonable and necessary cooperation in connection with any such suit, proceeding or action, including, without limitation, joining as a necessary party, at the Debtor's expense

(b) Upon the occurrence and during the continuation of any Event of Default hereunder, the Agent shall have the right, but shall in no way be obligated, to file applications for protection of the Collateral or bring suit in the name of the Debtors, the Agent or the Lenders to enforce the Collateral. In the event of such suit, each Debtor shall, at the request of the Agent, do any and all lawful acts and execute any and all documents required by the Agent in aid of such enforcement.

**SECTION 14. TERMINATION AND RELEASE.**

This Agreement is made for collateral purposes only. This Agreement shall be a continuing agreement in every respect and shall remain in full force and effect until (a) the commitments of the Lenders to extend credit to or for the account of the Borrowers under the Credit Agreement shall have terminated and all of the Secured Obligations, both for principal and interest, then due and payable have been fully paid and satisfied, or (b) the liens and security

interest have been terminated pursuant to Section 1.8(b) of the Credit Agreement. Upon such termination of this Agreement, the Agent shall, upon the request and at the expense of the Debtors, forthwith assign, transfer and deliver, against receipt and without recourse to the Agent, such of the Collateral as may then be in the possession of the Agent and as shall not have been sold or otherwise applied pursuant to the terms hereof to or on the order of each Debtor. Said assignment, transfer and delivery shall include an instrument in form recordable in the United States Patent and Trademark Office or the United States Copyright Office, as the case may be, by which the Agent shall terminate, release and, without representation, recourse or warranty, reassign to each Debtor all rights in each Patent, Patent License, Trademark, Trademark License, Copyright and Copyright License, including each registration thereof and application therefor, conveyed and transferred to the Agent pursuant to this Agreement.

#### SECTION 15. THE AGENT

In acting under or by virtue of this Agreement, the Agent shall be entitled to all the rights, authority, privileges and immunities provided in Section 10 of the Credit Agreement, all of which provisions of said Section 10 are incorporated by reference herein with the same force and effect as if set forth herein in their entirety. The Agent hereby disclaims any representation or warranty to the Lenders or any other holders of the Obligations concerning the perfection of the liens and security interests granted hereunder or in the value of any of the Collateral.

#### SECTION 16. MISCELLANEOUS.

(a) This Agreement cannot be changed or terminated orally. This Agreement shall create a continuing lien on and security interest in the Collateral and shall be binding upon each Debtor, its successors and assigns and shall inure, together with the rights and remedies of the Agent and the Lenders hereunder, to the benefit of the Agent, the Lenders and their successors and permitted assigns; *provided, however*, that each party hereto may assign its rights or delegate its duties hereunder only in accordance with Sections 10.13, 12.10, 12.16 and 12.17 of the Credit Agreement. Without limiting the generality of the foregoing, and subject to the provisions of the Credit Agreement, any Lender may assign or otherwise transfer any indebtedness held by it secured by this Agreement to any other person, and such other person shall thereupon become vested with all the benefits in respect thereof granted to such Lender herein or otherwise.

(b) All communications provided for herein shall be in writing, except as otherwise specifically provided for hereinabove, and shall be deemed to have been given or made, if to any Debtor when given to such Debtor in accordance with Section 12.7 of the Credit Agreement, or if to the Agent or any Lender, when given to such party in accordance with Section 12.7 of the Credit Agreement.

(c) No Lender shall have the right to institute any suit, action or proceeding in equity or at law for the foreclosure or other realization upon any Collateral subject to this Agreement or for the execution of any trust or power hereof or for the appointment of a receiver, or for the enforcement of any other remedy under or upon this Agreement; it being understood and intended that no one or more of the Lenders shall have any right in any manner whatsoever to affect, disturb or prejudice the lien and security interest of this Agreement by its or their action or

to enforce any right hereunder, and that all proceedings at law or in equity shall be instituted, had and maintained by the Agent in the manner herein provided for the benefit of the Lenders.

(d) In the event that any provision hereof shall be deemed to be invalid or unenforceable by reason of the operation of any law or by reason of the interpretation placed thereon by any court, this Agreement shall be construed as not containing such provision, but only as to such jurisdictions where such law or interpretation is operative, and the invalidity or unenforceability of such provision shall not affect the validity of any remaining provisions hereof, and any and all other provisions hereof which are otherwise lawful and valid shall remain in full force and effect. Without limiting the generality of the foregoing, in the event that this Agreement shall be deemed to be invalid or otherwise unenforceable with respect to any Debtor, such invalidity or unenforceability shall not affect the validity of this Agreement with respect to the other Debtors.

(e) The lien and security interest herein created and provided for stand as direct and primary security for the Secured Obligations of the Borrowers arising under or otherwise relating to the Credit Agreement as well as for any of the other Secured Obligations. No application of any sums received by the Lenders in respect of the Collateral or any disposition thereof to the reduction of the Secured Obligations or any part thereof shall in any manner entitle any Debtor to any right, title or interest in or to the Secured Obligations or any collateral or security therefor, whether by subrogation or otherwise, unless and until this Agreement shall have terminated in accordance with Section 14 hereof. Each Debtor acknowledges that the lien and security interest hereby created and provided are absolute and unconditional and shall not in any manner be affected or impaired by any acts of omissions whatsoever of the Agent, any Lender or any other holder of any Secured Obligations, and without limiting the generality of the foregoing, the lien and security interest hereof shall not be impaired by any acceptance by the Lenders or any other holder of any Secured Obligations of any other security for or guarantors upon any of the Secured Obligations or by any failure, neglect or omission on the part of the Agent, any Lender or any other holder of any Secured Obligations to realize upon or protect any of the Secured Obligations or any collateral or security therefor. The lien and security interest hereof shall not in any manner be impaired or affected by any sale, pledge, surrender, compromise, settlement, release, renewal, extension, indulgence, alteration, substitution, exchange, change in, modification or disposition of any of the Secured Obligations or of any collateral or security therefor, or of any guaranty thereof, or of any instrument or agreement setting forth the terms and conditions pertaining to any of the foregoing. The Lenders may at their discretion at any time grant credit to the Borrowers without notice to the other Debtors in such amounts and on such terms as the Lenders may elect (all of such to constitute additional Secured Obligations) without in any manner impairing the lien and security interest created and provided for herein. In order to realize hereon and to exercise the rights granted the Agent and the Lenders hereunder and under applicable law, there shall be no obligation on the part of the Agent, any Lender or any other holder of any Secured Obligations at any time to first resort for payment to the Borrowers or to any other Debtor or to any guaranty of the Secured Obligations or any portion thereof or to resort to any other collateral, security, property, liens or any other rights or remedies whatsoever, and the Agent and the Lenders shall have the right to enforce this Agreement against any Debtor or any of its Collateral irrespective of whether or not other proceedings or steps seeking resort to or realization upon or from any of the foregoing are pending.

(f) This Agreement shall be deemed to have been made in the State of Illinois and shall be governed by, and construed in accordance with, the laws of the State of Illinois. The headings in this Agreement are for convenience of reference only and shall not limit or otherwise affect the meaning of any provision hereof.

(g) Each Debtor hereby submits to the non-exclusive jurisdiction of the United States District Court for the Northern District of Illinois and of any Illinois state court sitting in the City of Chicago for purposes of all legal proceedings arising out of or relating to this Agreement, the other Loan Documents or the transactions contemplated hereby or thereby. Each Debtor irrevocably waives, to the fullest extent permitted by law, any objection which it may now or hereafter have to the laying of the venue of any such proceeding brought in such a court and any claim that any such proceeding brought in such a court has been brought in an inconvenient form. EACH DEBTOR, THE AGENT, AND EACH LENDER HEREBY IRREVOCABLY WAIVES ANY AND ALL RIGHT TO TRIAL BY JURY IN ANY LEGAL PROCEEDING ARISING OUT OF OR RELATING TO THIS AGREEMENT, ANY OTHER LOAN DOCUMENT OR THE TRANSACTIONS CONTEMPLATED HEREBY OR THEREBY.

(h) This Agreement may be executed in any number of counterparts and by different parties hereto on separate counterpart signature pages, each constituting an original, but all together one and the same agreement.

[SIGNATURE PAGES TO FOLLOW]

IN WITNESS WHEREOF, each Debtor has caused this Agreement to be duly executed and delivered in Chicago, Illinois as of the date first above written.

"DEBTORS"

SEMINIS, INC.

By \_\_\_\_\_  
Its \_\_\_\_\_

Address:

2901 N. Ventura Road  
Suite 250  
Oxnard, California 94142-0007  
Attention: \_\_\_\_\_

SEMINIS VEGETABLE SEEDS, INC

By \_\_\_\_\_  
Its \_\_\_\_\_

Address:


1905 Lirio Avenue  
Saticoy, California 93007  
Attention: \_\_\_\_\_

*Intellectual Property*



Accepted and agreed to in Chicago, Illinois as of the date first above written.

HARRIS TRUST AND SAVINGS BANK,  
as Agent as aforesaid for the Lenders

By   
Its Vice President

Address:

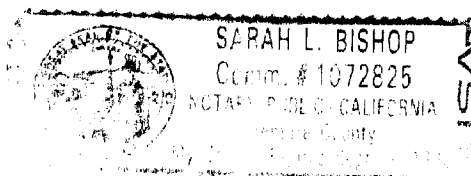
111 West Monroe Street  
P.O. Box 755  
Chicago, Illinois 60690  
Attention: Agribusiness Division

STATE OF CALIFORNIA            )  
  ) SS.  
COUNTY OF VENTURA         )

On July 6, 1999 before me, Sarah L. Bishop, Notary Public, personally appeared Alejandro Rodriguez Graue, personally known to me to be the person whose name is subscribed to the within instrument and acknowledged to me that he executed the same in his authorized capacity, and that by his signature on the instrument the person, or the entity upon behalf of which the person acted, executed the instrument.

WITNESS my hand and official seal.

  
NOTARY'S SIGNATURE

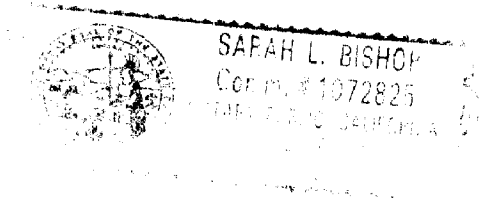


STATE OF CALIFORNIA            )  
  ) SS.  
COUNTY OF VENTURA         )

On July 6, 1999 before me, Sarah L. Bishop, Notary Public, personally appeared Alejandro Rodriguez Graue, personally known to me to be the person whose name is subscribed to the within instrument and acknowledged to me that he executed the same in his authorized capacity, and that by his signature on the instrument the person, or the entity upon behalf of which the person acted, executed the instrument.

WITNESS my hand and official seal.

  
NOTARY'S SIGNATURE



STATE OF ILLINOIS

SS

COUNTY OF COOK

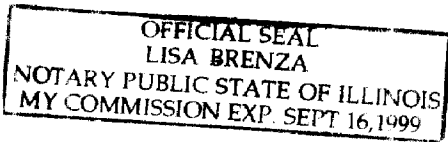
I, LISA BRENZA, a Notary Public in and for said County, in the State aforesaid, do hereby certify that C. Scott Place, Vice President of Harris Trust and Savings Bank, an Illinois banking corporation, who is personally known to me to be the same person whose name is subscribed to the foregoing instrument as such Vice President, appeared before me this day in person and acknowledged that she signed and delivered the said instrument as her own free and voluntary act and as the free and voluntary act and deed of said corporation for the uses and purposes therein set forth.

Given under my hand and notarial seal, this 17th day of August, 1999.

Lisa Brenza  
Notary Public

(NOTARIAL SEAL)

LISA BRENZA  
(Type or Print Name)



My Commission Expires:

\_\_\_\_\_

**SCHEDULE A-1**

**TO SECURITY AGREEMENT  
RE: INTELLECTUAL PROPERTY**

**U.S. PATENT NUMBERS  
AND PENDING U.S. PATENT APPLICATION NUMBERS**

Schedule A - 1

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	

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 incompatibility.

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  $\alpha$ -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 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 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 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 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  $\alpha$ -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 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 phosphorus.

(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 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.

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, curcubits, 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, cucurbits, 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 accession 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		



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<sub>1</sub> 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 from 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<sub>1</sub> 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.

**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	

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.: SVS3801F0080

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		

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 or 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:

- (a) transforming plant cells with a chimeric recombinant DNA molecule comprising a plurality of DNA sequences, each comprising a promoter functional in said plant cells, operably linked to a DNA sequence, which encodes a protein associated with a virus which is capable of infection said plant;
- (b) regenerating said plant cells to provide a differentiated plant; and
- (c) identifying a transformed plant which expresses the DNA sequences so as to render the plant resistant to infection by said viruses, preferably at substantially equal levels of resistance to infection by each virus.

Yet another object of this invention is to provide 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 plurality of proteins from viruses which are capable of infecting said *Cucurbitaceae* plant;
- (b) regenerating said plant cells to provide a differentiated plant; and
- (c) selecting a transformed *Cucurbitaceae* which expresses the virus proteins at levels sufficient to render the plant resistant to infection by said viruses.

It is a further object of this invention to provide multivirus resistant transformed plant which contains stably-integrated DNA sequences encoding virus proteins.

It is still a further object of this invention to provide virus resistant transformed plant cells which contain a plurality of viral genes, i.e., 2-7 or more genes, which are expressed as virus proteins from the same virus strain, from different virus strains as from different members of the virus group, such as the potyvirus group.

This invention is exemplified primarily by the insertion of multiple virus cp expression cassettes into a binary plasmid and subsequent characterization of resulting plasmids. Combinations of CMV, ZYMV, WMV-2, SQMV, and PRV coat protein expression cassettes were placed in the binary plasmid pPRBN. Subsequently, binary plasmids harboring multiple cp expression cassettes were mobilized into *Agrobacterium* for use in plant transformation procedures. Binary plasmids harboring multiple expression

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  $0.1 \times 0.1 \times 0.1 = 0.001$  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.



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.

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-70 gene, 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

**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		

for: Papaya Ringspot Virus Replicase Gene

### SUMMARY OF THE INVENTION

This invention provides an isolated and purified DNA molecule that encodes the replicase for the FLA83 W-type strain of papaya ringspot virus (PRV). The 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 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 replicase 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 an expression cassettes comprising the replicase 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 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		

**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.

## 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 cucurbitaceae and solanaceae 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	116475	12/20/95	Pending		
India	1690/CAL/95	12/20/95	Pending		
Thailand	029338	12/19/95	Pending		

**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.

### 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	2927689	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

TRADEMARK  
REEL: 002073 FRAME: 0524

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 strain 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 strain 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 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 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 stably 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 encoding 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	1151-6	08/31/95	Pending		
India	052/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	027876	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		

**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

**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	

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<sub>1</sub> 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				

**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	

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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-2**

**TO SECURITY AGREEMENT  
RE: INTELLECTUAL PROPERTY**

PATENT LICENSES

U.S. PATENT NUMBER

DATE ISSUED

LICENSE AGREEMENT

None

**SCHEDULE B-1**

**TO SECURITY AGREEMENT  
RE: INTELLECTUAL PROPERTY**

REGISTERED U.S. TRADEMARKS  
AND TRADEMARK APPLICATIONS