PLNT2530
2024
Unit 10d
Applications of Plant Biotechnology in Agriculture

Novel Traits

Novel Traits - Arctic Non-browning Apples

Petition for Determination of nonregulated status: Arctic Apple (Malus x domestica) Events GD743 and GS784). Submitted by Okanagan Specialty Fruits Inc., Summerland BC, Canada. Feb. 2012. Approved in US by APHIS 2014.
http://www.aphis.usda.gov/brs/aphisdocs/10_16101p.pdf

The Arctic Non-browning apple is a good case study, illustrating the process of developing a transgenic crop, from concept to market.

Problem: Apples turn brown when cut or damaged due to polyphenol oxidases (PPO. PPOs reduce phenolic compounds to O-quinones, which in turn can react with amino acids or proteins to produce brown compounds. Many O-quinones can also form polymers with brown color. In apples, the primary substrate resulting in browning is chlorogenic acid.

Solution: Downregulation of polyphenoloxidase genes in apples would provide several benefits:
"i) minimize shrinkage due to harvest and postharvest damage
ii) reduce need for antibrowning chemicals on cut fruit products
iii) promote the inclusion of apples in the cut fruit market"

Arctic Non-browning Apples - PPO genes

Successful transgene-induced gene silencing usually requires a minimum of 30 - 35 bp of perfect match between siRNA and target mRNA.

Problem: There are four distinct families of PPO genes in apple: PPO2, GPO3, APO5 and pSR7. Sequence similarity between these families is only about 61 to 75%. No one sequence can be used to suppress all genes in all four families.

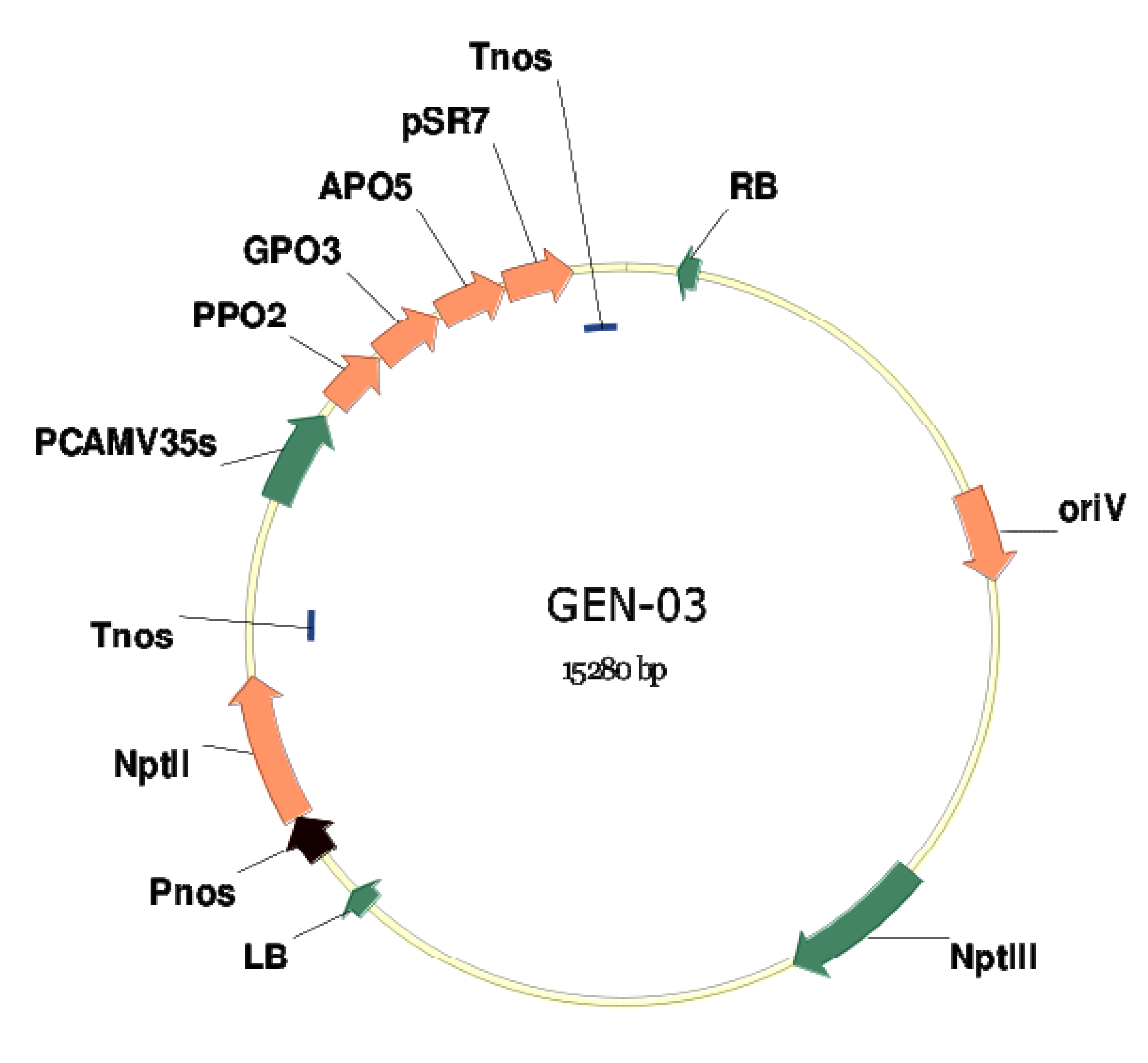
Solution: Create a single chimeric RNA containing sequences conserved among each of the four families.

Arctic Non-browning Apples - Gene silencing construct GEN3

The chimeric gene contains sequences conserved among each of the four PPO families.

Thus, a single over-expressed RNA can silence all genes in these families.

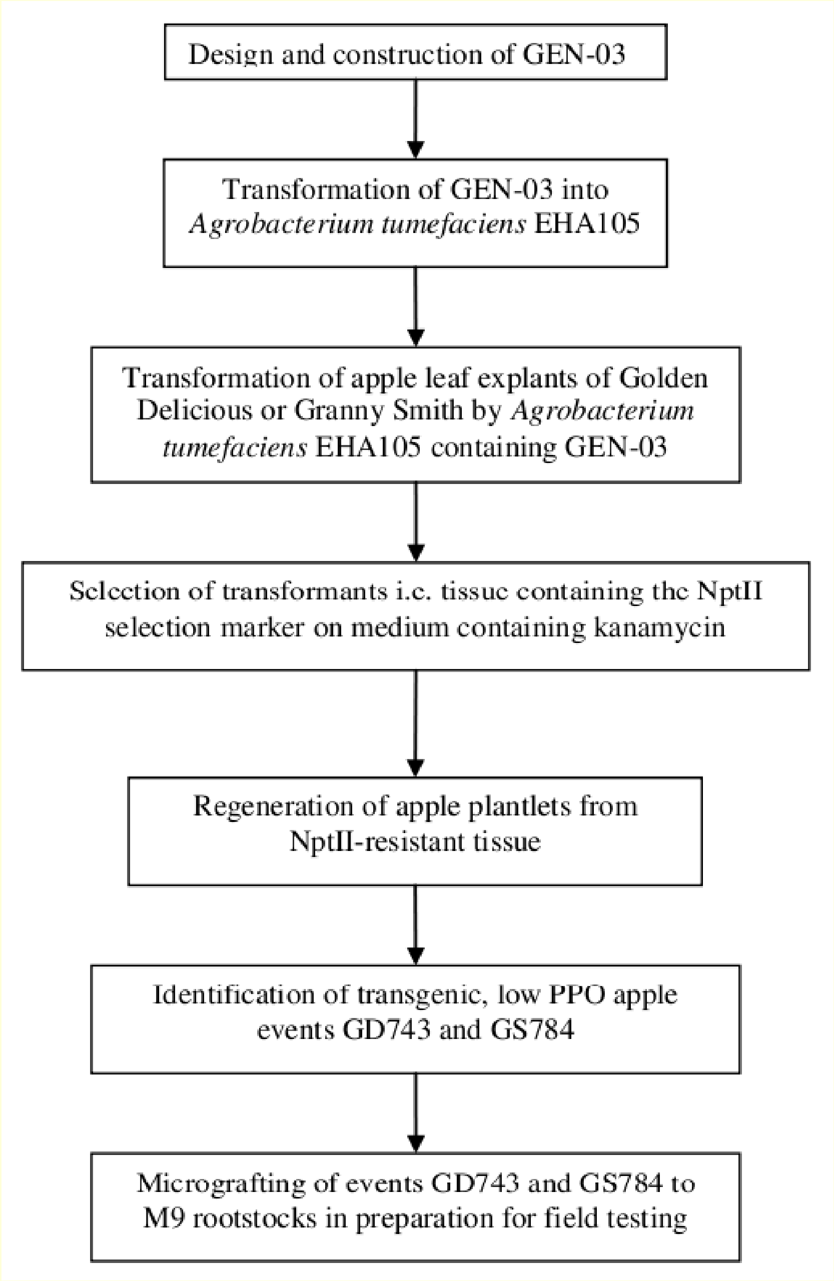
The 35S promoter was found to express the chimeric gene at a sufficient level to suppress PPO activity in transgenic apple.



Arctic Non-browning Apples - How do you do genetic engineering on trees?

Transgenic lines:
Event: GD743 (parent Golden Delicious); 2 unlinked insertions at two independent loci
Event: GS784 (parent Granny Smith); 4 unlinked insertions of GEN-03 at four independent loci

Apple trees take 5 years to reach maturity. However, cuttings from plantlets can be grafted onto existing rootstocks to allow more rapid production of apples for testing.



Arctic Non-browning Apples - How do you do genetic engineering on trees?

2002 - 2004

Seedling regenerates from tissue explant.

The seedling can be grafted onto conventional root stock, as shown in the bottom panel.

Within 3 months, the seedling has grown into a "whip tree" that is sturdy enough to transfer to a screen house to condition it for the field.

(*The screen house gives the sapling some shelter, while exposing it to ambient temperature, humidity and light conditions.*)

Figure 11: Micrografting ArcticTM Apple Shoot to M9 Rootstocks

Arctic Non-browning Apples - How do you do genetic engineering on trees?

Field Trials:

The row shown contains events GD743 and GS784, as well as controls.

2004 - 4th leaf stage

2007 - saplings appear healthy with lush foliage

Note automated irrigation system below.

2004

2007

Figure 12: Trial Block - Washington

Arctic Non-browning Apples - Evaluation

In 2008 the first crop of fruit was available for evaluation.

Figure 13: Whole Apple Images of GD743 and GS784

Arctic Non-browning Apples - Evaluation

Table 14: PPO Activity in GD743 and GS784 - Tissue Culture Leaves

Event	Mean SpActivity[1]	n[2]	PPO Suppression[3]
GD743	593	2	77 %
GD	2561	6	
GS784	289	2	87 %
GS	2166	4	

[1] SpActivity = Specific Activity of PPO.
[2] n = number of pooled tissue culture leaf samples per event.
[3] PPO Suppression = ((Mean SpActivity of Control – Mean SpActivity of Event) / Mean SpActivity of Control)*100

Table 15: PPO Activity in GD743 and GS784 - Greenhouse Leaves

Event	Mean SpActivity[1]	n[2]	PPO Suppression[3]
GD743	150	6	93 %
GD	2248	10	
GS784	338	3	93 %
GS	4978	2	

[1] SpActivity = Specific Activity of PPO.
[2] n = number of pooled greenhouse leaf samples per event. In the greenhouse, generally, only one pooled leaf sample was taken from each tree. Therefore, the number of samples approximately equals the number of trees sampled.
[3] PPO Suppression = ((Mean SpActivity of Control – Mean SpActivity of Event) / Mean SpActivity of Control)*100

Arctic Non-browning Apples - Evaluation

Table 16: PPO Activity in GD743 and GS784 - Field Leaves

Event	Mean SpActivity[1]	*S*	n[2]	PPO Suppression[3]
GD743	207	104	14	82 %
GD	1165	390	6	
GS784	315	95	10	76 %
GS	1297	245	8	

[1] SpActivity = Specific Activity of PPO.

[2] n = number of pooled field leaf samples per event. In the field, generally, two pooled leaf samples were taken from each tree. Therefore, the number of samples approximately equals twice the number of trees sampled.

[3] PPO Suppression = ((Mean SpActivity of Control – Mean SpActivity of Event) / Mean SpActivity of Control)*100

Up to this point we've only been able to test leaves, but what we're really interested in is PPO levels in fruit.

Arctic Non-browning Apples - Evaluation

Whew! After years of product development, only now do we know that it actually works in fruit!

2005

Table 17: PPO Activity in GD743 and GS784 - Immature Fruit

Event	Mean SpActivity[1]	*S*	n	PPO Suppression[2]
GD743	4519	3703	4	94 %
GD	75160	43329	8	
GS784	-	-	-	Not determined
GS	68171	26351	11	

[1] SpActivity = Specific Activity of PPO.
[2] PPO Suppression = ((Mean SpActivity of Control – Mean SpActivity of Event) / Mean SpActivity of Control)*100

2007

Table 18: PPO Activity in GD743 and GS784 - Mature Fruit

Event	Mean SpActivity[1]	*S*	n	PPO Suppression[2]
GD743	294	173	10	91 %
GD	3176	1235	10	
GS784	520	259	10	90 %
GS	5390	2341	10	

[1] SpActivity = Specific Activity of PPO.
[2] PPO Suppression = ((Mean SpActivity of Control – Mean SpActivity of Event) / Mean SpActivity of Control)*100

Arctic Non-browning Apples - Evaluation

Field Trials - 2006 - 2014

Table 22: Washington State Field Trial Notifications and Permits

Notification or Permit No.	Effective Date	Date Expires	Reporting Status
03-073-07n	April 13, 2003	Dec. 31, 2006	Annual report submitted
04-097-02n	May 6, 2004	Dec. 31, 2007	Annual report submitted
06-087-10n	May 1, 2006	May 1, 2007	Annual report submitted
06-087-09n	May 1, 2006	May 1, 2007	Annual report submitted
07-086-111n	April 27, 2007	April 27, 2008	Annual report submitted, extended by ePermit
07-086-110n	April 27, 2007	April 27, 2008	Annual report submitted, extended by ePermit
07-086-106n	May 30, 2007	May 30, 2008	Annual report submitted, extended by ePermit
07-086-107n	May 31, 2007	May 31, 2008	Annual report submitted, extended by ePermit
07-348-101r	April 1, 2008	March 31, 2011	Annual report submitted
11-056-102r	April 1, 2011	April 1, 2014	New in 2011

Table 23: New York State Field Trial Notifications and Permits

Notification or Permit No.	Date Issued	Date Expires	Reporting Status
05-046-14n	March 24, 2005	March 24, 2009	Annual report submitted, extended by ePermit
07-355-101r	April 1, 2008	April 1,2011	Annual report submitted
11-067-105r	March 31, 2011	March 31, 2014	New in 2011

Arctic Non-browning Apples - Evaluation

Bruising test

Transgenics and parental controls were subjected to impact bruising to simulate damage during handling.

Bruises were allowed to develop for 2 hr. and skin was peeled to reveal underlying flesh color.

Transgenics(left) show no apparent browning, whereas controls (right) show obvious browning.

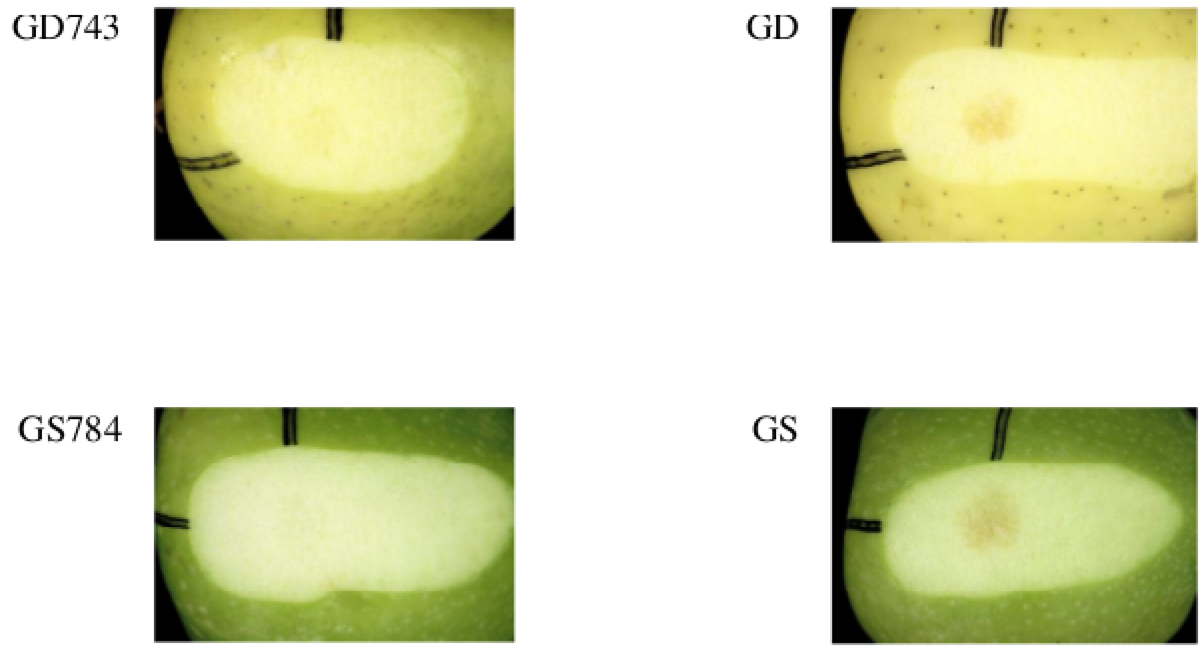


Figure 14: Bruise Response in GD743 and GS784

Arctic Non-browning Apples - Evaluation

Slicing test

Transgenics and parental controls were sliced, washed and stored at 5 °C for 3 weeks in Ziploc bags.

Transgenics(left) show no apparent browning, whereas controls (right) show obvious browning.

GD743

Figure 15: Apple Slices of GD743 and GD Control

Arctic Non-browning Apples - Evaluation

Juicing test

Transgenics and parental controls were sliced, cored and juiced with skins.

"The resulting apple juice was left overnight at room temperature for color development. Notably full color development occurred in the untransformed parents within minutes of juicing."

<u>Observation</u>: Consumers might expect brown color in juice and cider products, so Non-browning apples might not be marketable for those applications.

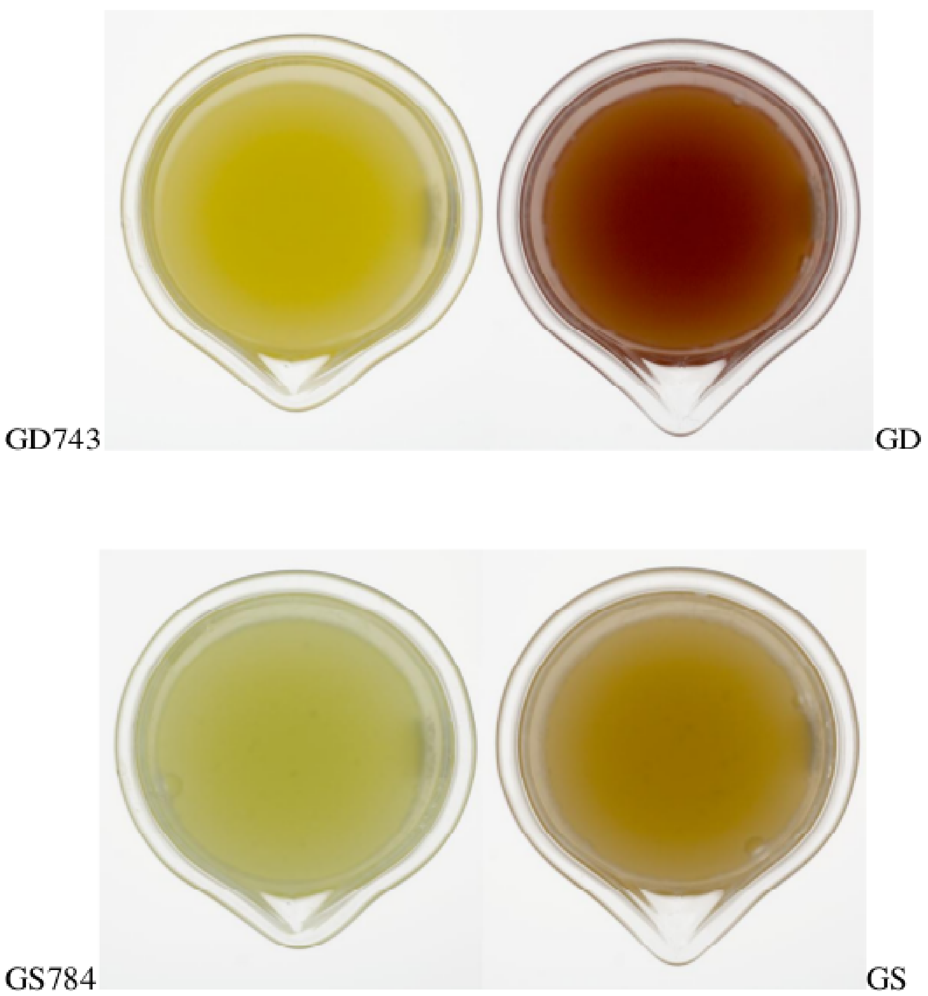


Figure 16: Apple Juice Made From GD743 and GS784

Arctic Non-browning Apples - Commercial availability

2016 - First commercial harvest

2017 - Sales in selected stores

2021 - Available as a snack food sold in packets

Arctic Golden Press Release

http://www.arcticapples.com/arctic-apples-r/arctic-golden/

Arctic Non-browning Apples - Significance

- Demonstrates practical genetic engineering on a tree species
- Engineering of a post-harvest trait
  - Authors may be underestimating the value of protecting apples from bruising and subsequent browning in storage
- Creates a value-added product with obvious benefits to the consumer
  - Most previous GM crops have been targeted to producers, not consumers
- Benefits to consumer may reverse some of the negative image of GM crops.
- Non-browning apples appear to fill a product niche. Marketed as sliced fruit product, but may not be suitable for juice or cider. **The next generation of GM crops may target specific niches in product lines.**

Novel Traits - Simplot Innate Potatoes

Waltz E (2015) Nature Biotech. 33:12-13. doi:10.1038/nbt0115-12

Uses RNAi to knock out two genes:

- **PPO5 - polyphenoloxidase**
    - inhibits browning
    - less bruising
    - longer shelf life
    - reduces waste in food service and manufacturing by allowing longer time between cutting and processing or cooking
- **asparagine synthase 1 (Asn1)**
    - In frying, Asn reacts with sugars to form acrylamide
    - acrylamide is a known carcinogen
    - when fried, Innate potatoes have 50 - 70% less acrylamide

Only gene fragments were used to induce RNAi pathways.

"We've done the math. Before potatoes ever reach the consumer, there is 400 million pounds of potato waste that we could save if Innate potatoes were adopted in the fresh market," says Haven Baker, general manager and vice president of plant sciences at Simplot.