An Overview of Arctic Apples: Basic Facts and Characteristics

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Apple fruits turn brown quickly after being sliced or bitten. Although this is a natural phenomenon, browning has been considered as an undesirable trait that often discourages consumption and causes unnecessary waste. To address the long standing issue of fruit browning in apple, Okanagan Specialty Fruits (OSF), a Canadian firm located in British Columbia that is funded by its current president Mr. Neal Carter who is also an apple grower, has developed a series of new apple varieties from widely grown existing apple varieties, such as Golden Delicious (GD), Granny Smith (GS), Gala and Fuji, which do not turn brown for over two weeks under appropriate conditions. These new strains of nonbrowning apples are not natural sports from their mother plants, but a carefully developed product using genetic engineering (GE) or modification (GM) by scientists in OSF. The firm has collectively named such genetically engineered nonbrowning apples Arctic Apples. OSF has been seeking market access in Canada and the US for Arctic Golden Delicious and Arctic Granny Smith for the last few years. In this article, I will briefly describe relevant scientific background and facts about Arctic Golden Delicious and Arctic Granny Smith in a Q&A format so that apple growers can make an informed decision on whether or not to grow Arctic Apples once they become available.

> **"Arctic Golden Delicious and Artic Granny Smith Apples, which are genetically modified varieties that do not brown when cut, will be available for commercial planting once deregulated. This article describes the relevant scientific background and facts about these apples so that growers can make an informed decision on whether or not to grow Arctic Apples once they become available."**

Why Do Apples Turn Brown After Being Cut?

Apple browning is caused by a polymer compound of pigment that primarily consists of quinones. Quinones are produced from phenols, which are common in apple fruit cells, through the action of a class of enzymes called polyphenol oxidases (PPOs). In intact fruit cells, phenols and PPOs are separated in different compartments. When cells are damaged by slicing or biting, phenols and PPOs come into contact and react to produce quinones, which will eventually become the pigmented polymer compound (together with amino acids and proteins). Therefore, PPOs are the key enzymes responsible for apple browning. To stop or minimize fruit browning, reducing the activities of PPOs has long been considered as an effective approach. What OSF accomplished in Arctic Apples can be largely attributed to their success in reducing the activities of PPOs in fruit cells.

How Are the Activities of PPOs Reduced in Arctic Apples?

One of the commonly used methods in reducing the activity of a specific enzyme in living cells is to suppress the expression of mRNA from the gene targeted. There are several means scientists can use to achieve specific gene expression suppression. OSF took an approach called co-suppression, a technique that allows specifically suppressing mRNA from one or more target genes depending upon the DNA sequences. One required element for this technique, obviously, is DNA sequence information for gene(s) targeted. Another element is that a second copy (partial or complete) of the target gene(s) is transformed into cells and expressed at high levels. Such high level expression of the transgene will trigger a process called RNA interference (RNAi) that will chop mRNA from both the target gene and the transgene into small pieces to kill the mRNA, leading to co-suppression of their mRNA. The procedure of co-suppression used to develop Arctic Apples is briefed described below:

1) Through literature searches and its own studies, OSF identified ten PPO encoding genes in the apple genome. Based on DNA sequence similarities, OSF categorized the ten genes into four groups, namely PPO2, GPO3, APO5 and pSR7 (PGAS), respectively, which are names of a representative gene from each group. Since the sequences are of high identity within a group but low identity between groups, OSF smartly decided to target the four representative genes to suppress the ten PPO genes.
2) OSF produced a DNA fragment of 450 bp (base pairs) from each of the four genes and then combined them into a single hybrid transgene *PGAS*.
3) The hybrid transgene *PGAS* was assembled into a vector equipped with other genetic elements necessary for plant transformation. The assembled vector, called GEN-03 (Figure 1, Table 1), allows the transgene *PGAS* to be integrated into the apple genome and expressed at high levels in apple cells.
4) OSF transformed apple cells with bacterium *Agrobacterium tumefaciens* carrying vector GEN-03, and then screened and selected for transgenic plants with the expected transgene *PGAS*.
5) OSF evaluated for reduced- or non-browning characteristic in fruit.

What External DNA Molecule is Present in Arctic Apples?

The extra DNA molecule integrated into Arctic Apples is a fragment of 4,560 bp (Figure 1, Table 1). Such DNA is usually called transfer DNA or T-DNA. The T-DNA of 4,560 bp accounts for about 0.0006% of the apple genome of 750 Mb (million base pairs). There are eight genetic elements contained in the T-DNA, including the hybrid transgene *PGAS* which suppresses the PPO encoding genes, the *nptII* (neomycin phosphotransferase type II) marker gene for kanamycin resistance selection in plant cells, and other non-protein encoding sequences primarily for regulating the expression of the two genes. The hybrid transgene *PGAS* is of 1,810 bp and accounts

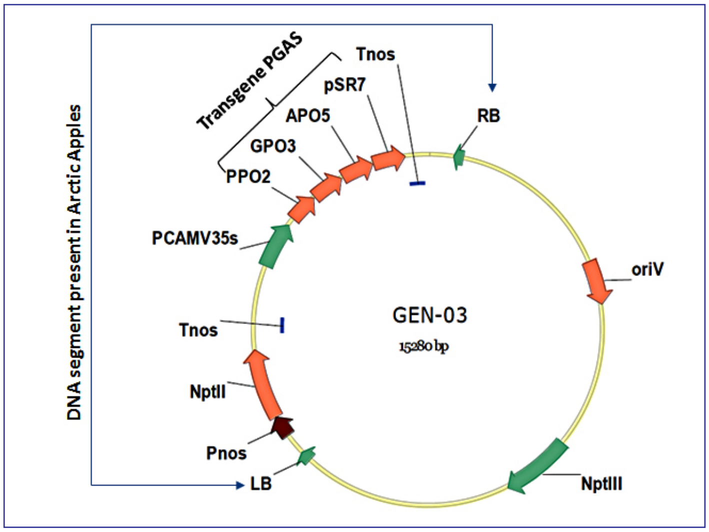

Figure 1. Diagram of vector GEN-03 (adapted from Carter (2012)). The DNA segment (T-DNA) from LB to RB containing the hybrid transgene PGAS is indicated. Size and function of each genetic element in the T- DNA are noted in Table 1. NptIII is a kanamycin and neomycin resistant gene and used as a selection marker in bacterial cells. OriV is a genetic element necessary for vector replication in bacterial cells.

for 39.7% of the T-DNA integrated in Arctic Apples. As mentioned previously, transgene *PGAS* was directly derived from the apple genome. Gene *nptII* is of 980 bp (making up 21.5% of the T-DNA) and exists in a range of soil micro-organisms naturally. The gene confers resistance to antibiotics kanamycin and neomycin that have limited use in medicine nowadays. The sources of other elements are either plant bacterium (*A. tumefaciens*) or plant virus (cauliflower mosaic virus).

What Percentage of the Activities of PPOs Are Suppressed in Arctic Apples?

The activities of PPO are reduced significantly in Arctic Apples (Table 2). Compared with their non-transgenic controls, Arctic Apples showed 76% - 82% of reduction in PPO activities in leaves and 90% - 91% of reduction in mature fruit. Such high percentage reductions in PPO activities in mature fruit directly explain why Arctic Apples show little browning.

Where is the NPTII Protein (Resistant to Kanamycin) Detected in Arctic Apples?

The NPTII protein concentrations were analyzed in leaves and in mature fruit (Table 3). In leaves, the protein was detected with much higher concentration in Arctic Apples than in non-transgenic controls (GD and GS). In mature fruit, however, the protein was detected at a low concentration identical across all samples regardless of transgenic or non-transgenic, suggesting that the NPTII protein is not detectable in mature fruit of Arctic Apples. (For growers and consumers, the non-detectable NPTII protein in mature fruit alleviates the concern on the NPTII protein intake if Arctic Apples are consumed.)

How do Arctic Apples Differ from Their Non-transgenic Mother Varieties?

The most visible difference of Arctic Apples from their non-transgenic mother varieties is nonbrowning when being cut. Fruit slices of Arctic Apples can be stored for over two weeks without turning brown in a Ziploc bag in the fridge. Most apple varieties, including Golden Delicious and Granny Smith, become brown in minutes or hours after being sliced. 'Cortland,' an apple variety known most for its slow browning rate, would show moderate browning in 24 hours after being sliced. The nonbrowning characteristic of Arctic Apples retains in juice as well, making their juice color close to that of the fruit flesh. Since Arctic Apples are genetically engineered with an insertion of the T-DNA of 4,560 bp, the genome of Arctic Apples is slightly larger (by 0.0006%) than that of non-transgenics. As there are two genes in the transformed T-DNA segment, Arctic Apples have two genes more than their non-transgenic mother varieties.

How Similar are Arctic Apples to Their Non-transgenic Mother Varieties in Performance and Nutrition?

OSF evaluated a number of important traits of Arctic Apples in comparison with their non-transgenic mother varieties in field trials conducted in Washington and New York States. They did not find any significant differences in these traits when compared correspondingly in pairs. The traits evaluated include 1) agronomic performance,

Table 1. Size and function of genetic elements in the T-DNA present in Arctic Apples[1]

Genetic Element	Size (Kb)	Function and Source
LB	0.15	A left border from plant bacterium *Agrobacterium tumefaciens*
PNOS	0.31	A transcription promoter from *A. tumefaciens*
nptII	0.98	Neomycin phosphotransferase type II gene resistance to kanamycin and neomycin and used as selection marker in plant tissue culture.
TNOS (1)	0.26	A transcription terminator from *A. tumefaciens*
PCAMV35s	0.65	A transcription promoter from cauliflower mosaic virus
PGAS	1.81	The co-suppression transgene designed to suppress apple PPO genes
TNOS (2)	0.26	A transcription terminator from *A. tumefaciens*
RB	0.14	A right border from *A. tumefaciens*
Total	**4.56**	

[1] According to Carter (2012);

Table 2. Relative suppression of PPO activities in Arctic Apples[1]

Arctic Apples	Organ	PPO Suppressed (%)
Arctic Golden Delicious	Leaves	82
	Immature fruit	94
	Mature fruit	91
Arctic Granny Smith	Leaves	76
	Immature fruit	ND
	Mature fruit	90

[1] According to Carter (2012); ND: Not determined

Table 3. Presence of protein NptII in leaves and mature fruit of Arctic Apples[1]

	Protein NptII[2]	
Group	Leaves	Mature fruit
Arctic Golden Delicious	5.0	0.1
GD Control	0.0	0.1
Arctic Granny Smith	3.8	0.1
GS Control	0.1	0.1

[1] According to Carter (2012); [2] expressed as ng (Protein NptII)/ g (fresh weight)

such as tree growth rate, flower cluster number and fruit number at harvest; 2) pest and disease characteristics, such as scab, mildew, fire blight, aphids, mites, Japanese beetle, codling moth, tentiform leafminer, burr knot, leaf spot, russet, Campylomma, and fruit rot after storage; and 3) fruit nutrition and composition, such as fat, protein, moisture, ash, carbohydrates, calories, sugar content, dietary fiber, potassium, vitamin C, ORAC (oxygen radical absorbance capacity), and phenolics.

How Much Distance is Needed Between Plantings of Transgenic and Non-transgenic Apples to Prevent Cross-pollination?

Based on a recent published study (Tyson et al. 2011) specifically designed to investigate and predict transgenic seed contamination resulting from cross-pollination in apple, a physical distance of 600 ft (183 M) separating transgenic and non-transgenic would be sufficient to prevent from cross- pollination (Table 4). This is primarily attributed to the fact that apple is an exclusively insect-pollinated crop. Although the study did not use Arctic Apples directly, the data and conclusions apply for any transgene in apple. Another important fact to know is that when cross-pollination occurs, the seeds will be the only part in fruit that will carry the transgene as the rest of the fruit, including the flesh, are all developed from the pollen recipient tree and therefore are non-transgenic.

What are the Other Potential Risks Associated with Gene-flows From Arctic Apples to the Wild?

Apples are largely consumed fresh. As a result, the seeds are discarded along with the core and then carried away. Animals and birds eating apples can distribute apple seeds unpredictably. Although most apple seeds on the ground do not usually lead to a tree, a fraction of these dispersed seeds will germinate and become trees in wild or unintended places. If deregulated, the seeds from Arctic Apples will be dispersed similarly as any other apples, allowing a possible channel through which the transgene might flow to the wild. To address this potential risk, more comprehensive studies are needed.

Table 4. Transgenic seeds found from nearby non-transgenic fruit[1]

Distance from transgenic source (m (ft))	Seeds tested	Transgenic seed (%)
18 (30)	623	0.16
18 (60)	383	0
27 (90)	641	0
37 (120)	809	0
46 (150)	1606	0.25
55 (180)	2064	0.05
64 (210)	2959	0.17
73 (240)	1940	0.05
82 (270)	2068	0.48
101 (330)	2394	0.08
110 (360)	1602	0.06
119 (390)	1015	0.1
128 (420)	769	0
137 (450)	1243	0.24
146 (480)	30	0
155 (510)	30	0
165 (540)	666	0
174 (570)	125	0
183 (600)	115	0

[1] Adapted from Tyson et al (2011)

What is the Current Status of Arctic Apples in Regulatory Procedures and When Will They be Deregulated?

OSF has initiated its petition to the US and Canada governments for deregulation of Arctic Apples. In US, the petition was filed in May 2010 to the U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS) and the Food and Drug Administration (FDA). APHIS watches whether or not the performance of transgenic crops against pest insects and diseases is significantly weaker than that of their non-transgenic controls. FDA examines whether or not GM crops could cause health issues when consumed by people. APHIS posted OSF's petition of 163 pages (Carter 2012) in July 2012 for the first of two public comment sessions. This comment session concluded in September 2012. The second public comment period will last for 30 days, and is expected in next few months in 2013.

In Canada, the petition was submitted in December 2011 to Canadian Food Inspection Agency (CFIA) and Health Canada, which have similar charges as APHIS and FDA, respectively. CFIA posted OSF's Notice of Submission in May 2012 to allow public to comment on Arctic Apples for about two months. Currently, the petition is still being reviewed by CFIA and Health Canada. OSF anticipates full deregulation of Arctic Apples in both Canada and US later in 2013. The trees of Arctic Apples will be available to commercial nurseries and growers once the deregulation status is granted. OSF expects selling of the fruit of Arctic Apples will begin in North American grocery stores in 2014 or 2015.

Are Arctic Apples Safe for Human Consumption?

Instead of directly answering this question, I would like to provide some of the facts regarding GM crops and leave for you to form your own answer. According to data from USDA, corn, soybean and cotton grown in the US are mostly genetically modified. In 2012, for example, GM varieties in these three crops accounted for 88%, 93% and 94%, respectively. GM crops, in fact, have been present in the US food supply system since 1996. Globally, GM crop hectares had been increased by 100-fold, from 1.7 million hectares in 1996, to 170 million hectares in 2012. The Hawaiian papaya (Rainbow and SunUp) has been the first GM fruit in the US markets for years. If approved, Arctic Apples will be the second GM fruits available to US consumers.

Literature Cited

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