Norfolk Plant Sciences

Purple Tomato Case Study:
Completed USDA and FDA review processes

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SCRA Workshop

September 19-21, 2023

Outline

- Company Background
- Product Background
- Market Overview
- Regulatory Strategy
- FDA BNF Submission
- USDA RSR Submission
- Regulatory Timeline
- Lessons Learned

Norfolk Plant Sciences Background

Norfolk Plant Sciences was founded in 2008 by Profs Cathie Martin and Jonathan Jones of the John Innes Centre and the Sainsbury Laboratory. The company is built on technologies that aim to use the best plant science to improve health, nutrition and well-being.

Prof. Cathie Martin
John Innes Center, UK
Fellow of the Royal Society
Rank Prize for Nutrition in 2022

Acknowledgements

Jeff Stein

Jim Ligon

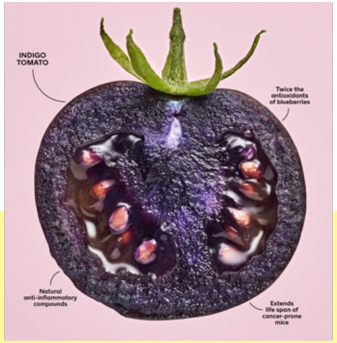
Lisa Zannoni

Prof. Jonathan Jones
The Sainsbury Laboratory
Fellow of the Royal Society
US National Academy of Sciences

Norfolk's Innovative New Product: The Purple Tomato

DIFFERENTIATED

The New York Times



NUTRITION

INNOVATION

- Norfolk Plant Sciences reported developing a Purple Tomato through genetic engineering, 2008
- Submitted FDA BNF in March, 2020. Completed June 2023
- Submitted USDA RSR in August, 2021. Completed September, 2022

  (first RSR decision under SECURE Rule)

NORFOLK
PLANT SCIENCES

Anthocyanins: Pigments present in several familiar fruit and vegetables

Interest in the project started from published studies on health benefits

Dietary anthocyanins have protective effects against myocardial infarction and coronary heart disease (**Cassidy et al., 2013**) as well as ameliorative effects in those already at risk of atherosclerosis (**Zhu et al., 2012**).

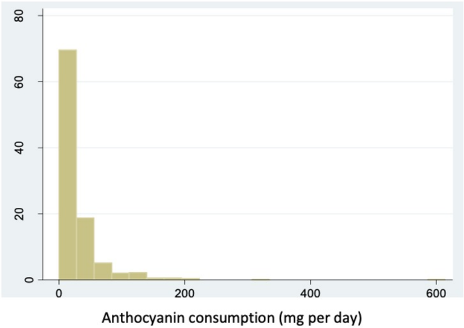


Figure 2: Estimation of the daily consumption of anthocyanins by Italians n=450 **(Rizzi *et al.*, 2016)**

Most people eating western diets consume very little anthocyanins

~12 mg/day in the US

Intervention studies target 300 mg/day

Anthocyanins are produced by a few introgressed tomato varieties currently on sale in Europe and the USA

OSU: Oregon state University

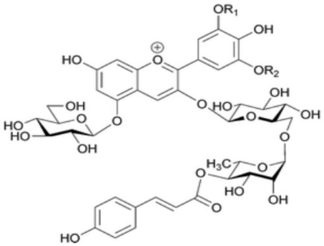
Indigo Rose, a truly purple tomato, from OSU's program to breed for high levels of antioxidants

Purple tomato debuts as 'Indigo Rose'

January 27, 2012

CORVALLIS, Ore. – The "Indigo Rose" tomato steps out this year as the first "really" purple variety to come from a program at Oregon State University that is seeking to breed tomatoes with high levels of antioxidants

Interior of the Indigo Rose tomato



R1 and R2 = H = delphinidin 3-*O*-(coumaroyl)rutinoside-5-*O*-glucoside also known as Nasunin
R1 = CH3 and R2 = H = petunidin 3-*O*-(coumaroyl)rutinoside-5-*O*-glucoside

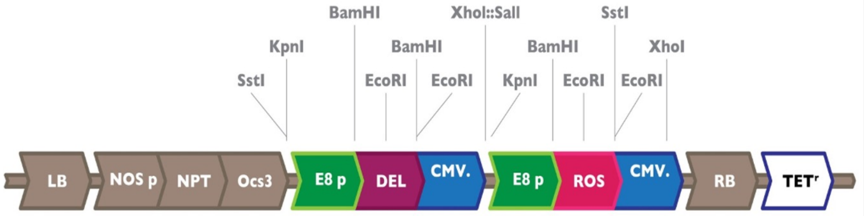
Figure 3. Structure of major anthocyanins produced in tomato leaves of WT control plants and in purple tomato fruit of *Del/Ros1*-N plants

Same Anthocyanin as Norfolk's tomato

Skin only & Light-activated

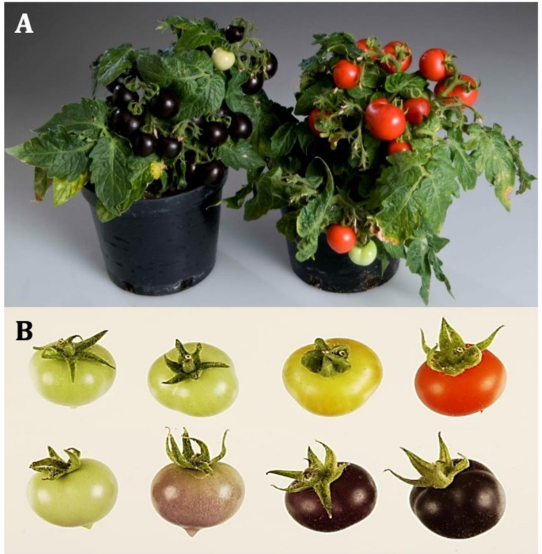
Generation of transgenic tomato plants (after Snapdragon discovery)

Schematic representation of binary vector pDEL.ROS:



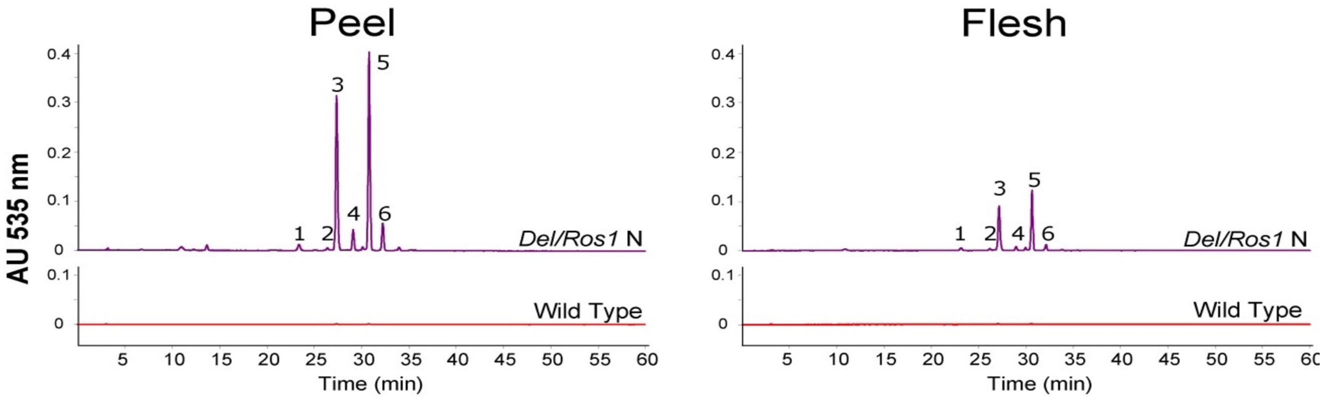
LB: T-DNA left border sequence
NOSp: Nopaline synthase promoter region from *A.tumefaciens*
NPT: Neomycin phosphotransferase gene conferring resistance to Kanamycin from Tn5
Ocs3: Octopine synthase transcriptional termination region from *A.tumefaciens*
E8p: E8 promoter region from tomato
DEL: *Delila* cDNA from snapdragon
CMV: Cauliflower mosaic virus transcriptional termination region
ROS: *Rosea1* cDNA from snapdragon
RB: T-DNA right border sequence
TET^r: Tetracycline resistance gene from pRK290

Figure 5. (A) Comparison of the growth and development of a *Del/Ros1* tomato (left) in the MicroTom variety with a nontransgenic wildtype MicroTom tomato (right). (B) Fruit derived from the wildtype MicroTom (top) compared to fruit from the Purple Tomato (bottom) harvested at the mature green (left), breaker (second from left), breaker plus 2 days (third from left), and red ripe (right) stages.



Butelli et al. (2008) *Nature Biotechnology*

Anthocyanin characterization

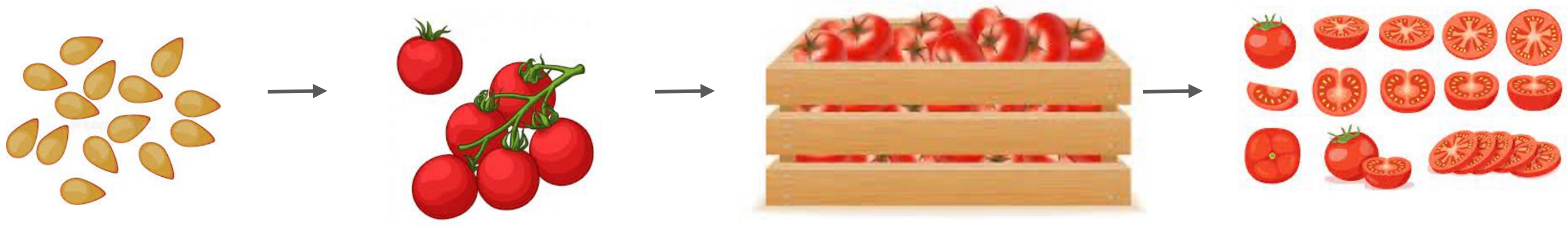


Peak	Identification	λ_{max} (nm)	$[M+H]^+$ *(m/z)*	Detected fragments
1	Delphinidin 3-(caffeoyl)-rutinoside-5-glucoside	529	935.24	773.23 (M^+-Glc), 465.11 (dpd+Glc), 303.05 (dpd)
2	Delphinidin 3(cis-coumaroyl) rutinoside-5-glucoside	533	919.27	757.22 (M^+-Glc), 465.12 (dpd+Glc), 303.06 (dpd)
3	Delphinidin 3-(trans-coumaroyl)-rutinoside-5-glucoside	529	919.28	757.19 (M^+-Glc), 465.11 (dpd+Glc), 303.05 (dpd)
4	Delphinidin 3-(feruloyl)-rutinoside-5-glucoside	529	949.24	787.21 (M^+-Glc), 465.10 (dpd+Glc), 303.06 (dpd)
5	Petunidin 3-(trans-coumaroyl)-rutinoside-5-glucoside	531	933.26	771.21 (M^+-Glc), 479.10 (ptd+Glc), 317.07 (ptd)
6	Petunidin 3-(feruloyl)-rutinoside-5-glucoside	530	963.27	801.23 (M^+-Glc), 479.13 (ptd+Glc), 317.07 (ptd)

Butelli et al. (2008) *Nature Biotechnology*

Tomato Value Chain: Multi-step process to reach the consumer

US Tomato Sales: $13 Billion (Retail and Food Service)



Seed Companies
- Global Tomato Seed Sales ~$1B
- 5 market leaders make up 50% of sales
- Tomato seed sales in the US are less than 1% of growers total input costs

Grower, Packer, Shipper
- Mostly **open-field** producers located in Mexico, Florida and California
- Rapidly expanding protected culture in Mexico

Wholesalers, Retailers, Food Service
- Wholesalers repack for retail or food service distributors
- Retailers sell to consumers
- Food service providers distribute to restaurants

Consumers
- Uses - salads, condiments, snacks, salsas, cooked products
- Per capita consumption: >20 pounds per year
- Snacker, **greenhouse** tomatoes command the highest pricing, deliver highest, consistent quality

Norfolk's Place in the Market

Marketing

Initial Channels (2023): →

- Farmers Markets
- Independent Restaurants

Future Channels: →

- Retail
- Food Service
- Home Gardeners

Enabled by

Initial Production (2023):

- Mid- and Low-tech greenhouses

Future Production:

- High-tech greenhouses
- Open field

Regulatory Strategy

Norfolk initial regulatory strategy (2013)

- USDA deregulation = too expensive; FDA = possible
- Product strategy: Produce a purple tomato juice, with seeds filtered out and incinerated.
  Grow in contained greenhouses
- Began generating nutrient composition data for FDA BNF (Studies in 2016 and 2018)
- Pre-submission meeting with FDA in 2017 & 2019; Submitted in March, 2020 (BNF 178)

Subsequent strategy (2021, Post-USDA Secure Rule Announcement):

- USDA = may be possible
- Pre-submission meeting with USDA; Submitted August 2021
- Product strategy: Began considering additional uses (fresh tomatoes)
- EPA = Consulted to confirm not in scope for FIFRA

Data Submitted: FDA

Take home message:

Composition of purple tomato is substantially equivalent, in the range of conventional tomatoes

Exception is anthocyanins, which are at similar ranges to high-accumulating berries (like blueberries)

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Data Submitted: FDA

Stability of novel proteins during gastric digestion

Figure 19: Delila - digestion time course (in min)

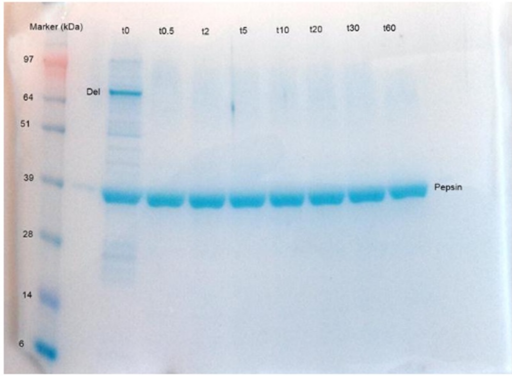

Figure 19: shows a gel of Delila (Del) protein digested with pepsin in SGF indicating that Del is rapidly digested with no full-length protein detectable after 0.5 min.

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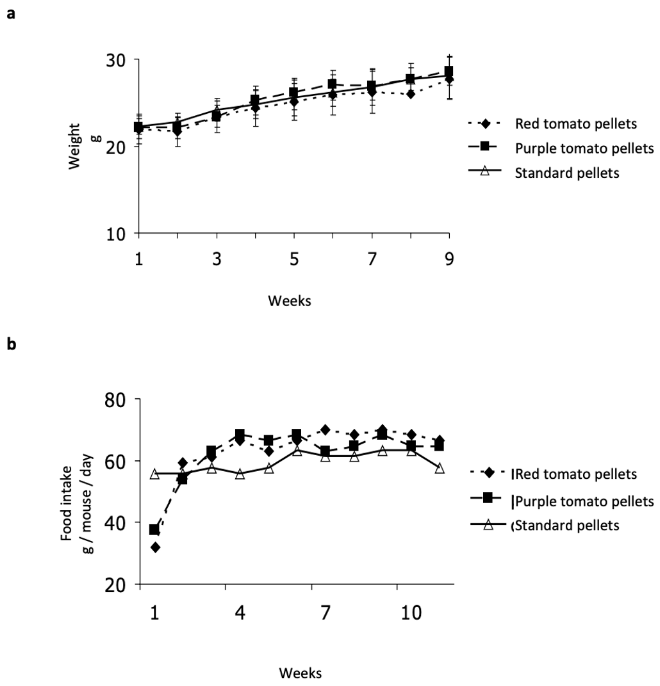

Figure 21: Body weight (**a**) and energy intake (**b**) of WT C57B16 mice fed with pellets of the standard diet, pellets supplemented with 10% red tomato powder and pellets supplemented with 10% purple tomato powder.

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Table 13: Compositional (nutrient) analysis of Purple Tomato (*Del/Ros1*-N) and red (wild-type) tomato juices.

	RED tomato juice	PURPLE tomato juice
Moisture (g/100g FW)	95.7 ± 0.10	95.68 ± 0.21
Protein (g/100g FW)**	0.7 ± 0.04	0.66 ± 0.04
Ash (g/100g FW)	0.48 ± 0.02	0.48 ± 0.04
Carbohydrate (g/100g FW)	3.12 ± 0.16	2.84 ± 0.37
Fructose (g/100g FW)	1.2 ± 0.10	1.06 ± 0.04
Galactose (g/100g FW)	<0.1	<0.1
Glucose (g/100g FW)	1 ± 0.08	0.84 ± 0.04
Lactose (g/100g FW)	<0.5	<0.1
Maltose (g/100g FW)	<0.1	<0.1
Sucrose (g/100g FW)	<0.1	<0.1
Total Sugar (g/100g FW)	2.26 ± 0.16	1.9 ± 0.08
Total Fat (g/100g FW)	<0.5	<0.9
Total Fibre (g/100g FW)	<0.5	<0.8
Energy kCal (kCal)	15.24 ± 0.47	15.94 ± 0.90
Energy kJ (kJ)	64.94 ± 1.97	67.44 ± 3.65
Salt NaCl (g/100g FW)	<0.05	<0.025
Monounsat FAs (g/100g FW)	<0.5	<0.1
Polyunsat FAs (g/100g FW)	<0.5	<0.4
Sat FAs (g/100g FW)	<0.5	<0.5
Trans FAs (g/100g FW)	<0.5	<0.1
Mg (mg/100g FW)	7.63±15.7	8.86 ± 17.1
K (mg/100g FW)	253 ± 16	267 ± 17
Na (mg/100g FW)	<20	<10
beta carotene (ug/100g FW)	267 ± 4.00	334.2 ± 36.62
folate (ug/100g FW)	85.16 ± 6.83	62.64 ± 3.38
Vitamin C (mg/100g FW)	6.33 ± 1.62	2.295 ± 0.94
Vitamin K1 (ug/100g FW)	<0.8	<0.8
Lycopene (mg/l)	51.34 ± 2.74	36.5 ± 2.54

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Data Submitted: FDA

Table 10 **Nutrient composition of Tomatoes – standard, raw**

Analyte	units	RED	PURPLE	USDA (avg)	USDA (min)	USDA (max)	McCance and Widdowson 2019
moisture	g/100g	94.72 ± 0.12	95.1 ± 0.12	94.52	92.7	95.73	94.6
Crude protein	g/100g	0.64 ± 0.05	0.70 ± 0.09	0.88	0.59	1.06	0.5
Ash	g/100g	0.32 ± 0.02	0.48 ± 0.02	0.50	0.37	0.60	n/a
CHO	g/100g	3.06 ± 0.29	3.26 ± 0.27	3.89	n/a	n/a	3
Fructose	g/100g	1.46 ± 0.05	1.22 ± 0.09	1.37	1.1	2.32	1.6
Galactose	g/100g	<0.1 ± 0	0.1 ± 0	0.00	0.00	0.00	0.00
Glucose	g/100g	1.26 ± 0.02	1.04 ± 0.08	1.25	0.49	2.67	1.4
Lactose	g/100g	<0.1 ± 0	0.1 ± 0	0.00	0.00	0.00	0.00
Maltose	g/100g	<0.1 ± 0	0.1 ± 0	0.00	0.00	0.00	0.00
Sucrose	g/100g	<0.1 ± 0	0.1 ± 0	0.00	0.00	0.02	0.00
Total sugar	g/100g	2.72 ± 0.06	2.24 ± 0.17	2.63	1.59	5.01	3.00
Total fibre AOAC	g/100g	1.1 ± 0.2	0.7 ± 0.1	1.2	0.7	2	1
Energy kcal	kcal/100g	18.4 ± 0.83	16.8 ± 0.77	18	n/a	n/a	4
Energy kJ	kJ/100g	81.8 ± 1.78	71 ± 2.95	74	n/a	n/a	61
Total fat	g/100g	<0.3 ± 0.04	<0.3 ± 0	0.2	0.07	0.80	0.10
Salt	g/100g	<0.025 ± 0	<0.025 ± 0	n/a	n/a	n/a	n/a
Mono unsat FAs	g/100g	<0.1 ± 0	<0.1 ± 0	0.031	n/a	n/a	0.03
Poly unsat FAs	g/100g	<0.1 ± 0	<0.1 ± 0	0.083	n/a	n/a	0.05
Sat Fas	g/100g	<0.1 ± 0	<0.1 ± 0	0.028	n/a	n/a	0.03
Trans FAs	g/100g	<0.1 ± 0	<0.1 ± 0	n/a	n/a	n/a	0
Mg	g/100g	0.01 ± 0.00	0.01 ± 0.00	0.011	0.007	0.015	0.008
K	g/100g	0.16 ± 0.00	0.23 ± 0.01	0.237	0.144	0.385	0.0223
Na	g/100g	<0.01 ± 0	0.01 ± 0.00	0.005	0.001	0.024	0.002
beta carotene	ug/100g	451.6 ± 42.4	661.8 ± 64.8	449	184	572	349
Folate B9	ug/100g	8.92 ± 0.28	14.22 ± 0.31	13.7	7.8	19.8	23
Ascorbate C	mg/100g	6.86 ± 0.18	8.1 ± 0.86	15	1	36	22
Phylloquinone K1	ug/100g	2.69 ± 0.14	1.99 ± 0.1	7.9	2.2	60	6
Lycopene	mg / kg	n/a	20.9	25.73	11.36	34.19	n/a

Average and Min/Max values available online:
USDA Food Composition Databases Show Foods—Tomatoes, Red, Ripe, Raw, Year Round
https://ndb.nal.usda.gov/ndb/ accessed 09-18-2018

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Data Submitted: USDA

Take home message:

Purple tomatoes do not pose an increased plant pest risk, compared with standard tomatoes

Norfolk Plant Sciences

Information Supporting a Regulatory Status Review of Tomato Genetically Engineered to Produce Increased Levels of Anthocyanins

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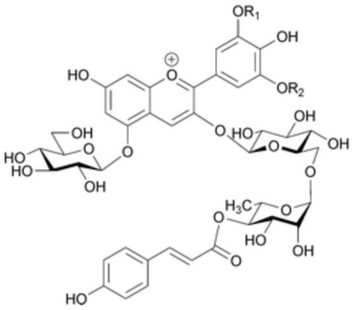
Data Submitted: USDA

Table 1: Anthocyanin content of different foods (Manach, 2004).

Foodstuff	Anthocyanin (mg /100 g FW)	Foodstuff	Anthocyanin (mg /100 g FW)
Eggplant	750	Radish	11-60
Blackberry	83-326	Raspberry	10-60
Blackcurrant	130-400	Red cabbage	125-210
Blueberry	25-497	Red currant	80-420
Cherry	100-400	Red grape	15-375
Chokeberry	200-1000	Red onions	7-21
Cranberry	60-200	Red wine	24- 150
Elderberry	450-1375	Rhubarb	200
Orange	8	Strawberry	15-35
Plum	1-12	**Purple Tomato**	**500**

Figure 1. Structure of the major anthocyanins produced in tomato leaves of WT control plants and in the fruit of Purple Tomatoes.
Delphinidin 3-*O*-(coumaroyl)rutinoside-5-*O*-glucoside also known as Nasunin: R1 and R2 = H;
Petunidin 3-*O*-(coumaroyl)rutinoside-5-*O*-glucoside: R1 = CH3 and R2 = H.



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Data Submitted: USDA

Figure 2. Genetic map of the pDEL.ROS plant transformation vector with the T-DNA component containing the *Del* and *Ros1* genes. pDEL/ROS was constructed by inserting the *Del*/*Ros1* T-DNA cassette shown at the top of the figure into bacterial plasmid pRK290 (shown at the bottom). A description of the individual genetic elements is presented in Table 2.

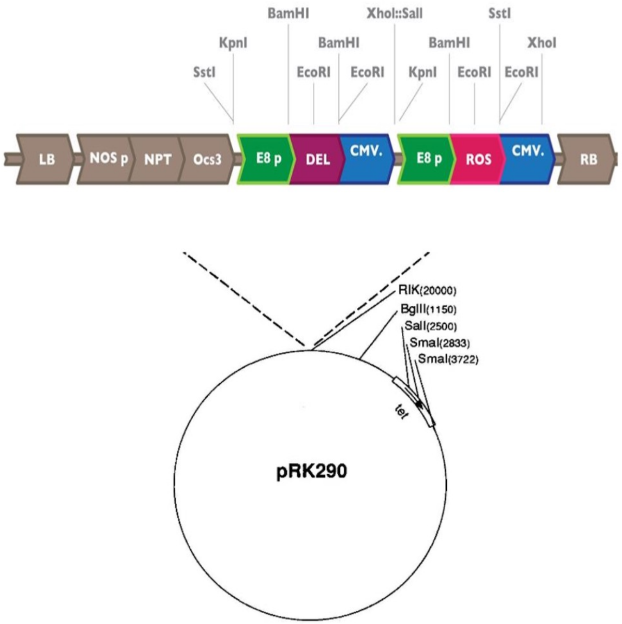

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Figure 3. Schematic representation of the *Del/Ros1* T-DNA insert in the chromosome of the Purple Tomato.

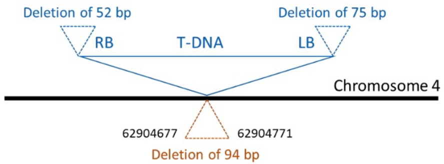

Figure 4. The Annotated Nucleotide Sequence of the Del/Ros1 T-DNA Locus in Tomato.

A. Annotation of the *Del/Ros1* T-DNA insertion. Abbreviations are as listed in Table 2. An overall representation and orientation of the *Del/Ros1* T-DNA insert is represented in Figure 3.

Nucleotide position		Genetic Element
Start	End	
1	332	Tomato chromosomal DNA ("chr4") position 62904346…62904677 (Build SL 3.0). 94 bp of genomic sequence after position 62904677 have been deleted
333	864	RB region of the T-DNA insert 52 bp at the end of the RB region have been deleted (including the RB)
865	1502	**CMV** (Cauliflower mosaic virus termination region)
1615	2277	**ROS** (*Rosea1* cDNA from snapdragon)
2284	4470	**E8p** (E8 promoter region from tomato)
4500	5227	**CMV** (Cauliflower mosaic virus termination region)
5287	7221	**DEL** (*Delila* cDNA from snapdragon)
7305	9493	**E8p** (E8 promoter region from tomato)
10535	11242	**Ocs 3** (Octopine synthase termination region)
11269	12063	**NPT II** (Neomycin phosphotransferase gene conferring resistance to Kanamycin)
12150	12329	**NOSp** (Nopaline synthase promoter region)
12330	12915	LB region of the T-DNA insert 75 bp at the end of the LB region have been deleted (including the LB)
12916	13098	Tomato chromosomal DNA ("chr4") position 62904771…62904953 (Build SL 3.0)

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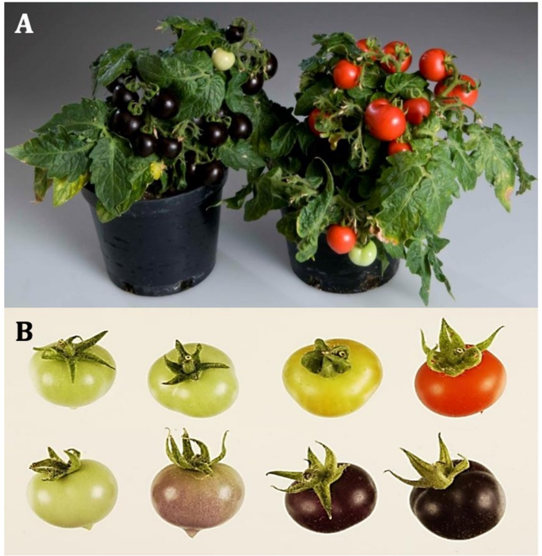
Figure 5. (A) Comparison of the growth and development of a *Del/Ros1* tomato (left) in the MicroTom variety with a nontransgenic wildtype MicroTom tomato (right). (B) Fruit derived from the wildtype MicroTom (top) compared to fruit from the Purple Tomato (bottom) harvested at the mature green (left), breaker (second from left), breaker plus 2 days (third from left), and red ripe (right) stages.



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Regulatory Timeline

March, 2020	FDA: BNF178 Submitted for Juice as intended use, following pre-submission consultations
August 28, 2020	USDA: RSR pre-submission consultation
April 5, 2021	USDA: Secure Rule goes into effect for Tomatoes
August 4, 2021	USDA: Norfolk Plant Sciences RSR Submission
August 19, 2021	USDA: Norfolk Plant Sciences RSR Completion Check
December, 2021	Company: Norfolk Healthy Produce, Inc. established (US-based commercial subsidiary)
February, 2022	FDA: Question on intended use of purple tomatoes
March 2, 2022	Company: Tomato seeds received in US (USDA import permit 121-SP4MESD)
June, 2022	Company: Growing and trialing in contained greenhouse; Awaiting USDA decision
June-August, 2022	FDA: Consultation on expanded use scope
September 7, 2022	**USDA Published RSR decision: Purple Tomato may be grown and used in breeding in the US**
June 20, 2023	**FDA Completes BNF178, issues letter of "no further questions" for human food (No feed response, because intended use for human food)**

Lessons Learned

- **Consult** with agencies at early stage of development to understand data requirements
  - Large and small questions, listen, learn, share and adapt
  - Helpful Advice: FDA suggested early to test whole tomatoes, not just juice
- Develop a market plan that encompasses present and possible future uses
- Review previously submitted dossiers to understand data requirement needs and quality

  (FOIA and/or work with experienced regulatory people)
- Check data well for accuracy
- Write the story - don’t just share data, explain your conclusions
- Respond to questions as soon as practicable
- **Consult** with agencies to clarify what is needed in responses to questions raised
  - Constructive conversations, with lots of listening, were very helpful
- Understand any requirements for product claims made and labeling