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Carotenoid, polyphenol and folate content of heritage tomatoes: 2023

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June 2023

Confidential report for:

Heritage Food Crops

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Executive summary

Carotenoid, polyphenol and folate content of heritage tomatoes: 2023

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June 2023

The Heritage Food Crops Research Trust (HFCRT), provided 25 tomato (*Solanum lycopersicum*) samples for measurement of carotenoids, polyphenol metabolites, and folate.

Results for the carotenoid concentrations were in alignment with those reported for in previous years. Carotenoid concentrations varied between cultivars. Twenty-two of the provided tomato cultivars were considered golden tomatoes due to the presence of tetra-cis-lycopene and the corresponding carotenoids phytoene, phytofluene and zeta carotene, which are biosynthetic precursors to tetra-cis-lycopene. Three tomato samples were considered orange due to their beta-carotene profile.

Chlorogenic acids (both mono-caffeoyl and di-caffeoyl) were the major polyphenols in these tomatoes. Similar to previous years, relatively low concentrations of flavonoids were detected. Polyphenolic concentrations varied between cultivars.

Folate was observed in all tomato samples at varying concentrations, and ranged from 18.4 to 55.0 µg/100 g.

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1 Introduction

The Heritage Food Crops Research Trust (HRCFT) is interested in the health benefits of its tomatoes (*Solanum lycopersicum*) for consumers. Identifying tomato cultivars and selections that have greater concentrations of nutritional and bio-active compounds such as carotenoids and polyphenols than conventionally grown commercial tomatoes is of interest to HRCFT. The New Zealand Institute for Plant and Food Research Limited (PFR) has measured carotenoids and polyphenols for several years (McGhie et al. 2022; McGhie & Cordiner 2021, Cordiner 2020). During this time improvements to the identification and confidence of these compounds have been undertaken. This year a further step to improve the identity of polyphenol compounds with similar structure and the same molecular formula (same mass) was utilised by mass spectrometry (MS) with broadband collision-induced dissociation (bbCID) capability. These compounds can fragment differently in the MS leading to unique fragmentation patterns and hence increase confidence in identification. Additionally, this year folate (vitamin B9) analysis was also included utilising a PFR in-house method. Unlike plants, humans are not able to synthesise folate so require a plant source, such as tomato, to acquire folate. Here we report the results for tomato samples collected during the 2023 summer.

2 Materials and methods

Twenty-five samples of tomato, along with their descriptions, were provided by Mark Christensen, Heritage Food Crops (HFC); and details are provided in Table 1. Tomato samples were couriered fresh, and once received at PFR, Palmerston North, were immediately stored at -20°C until analysis. Photos of the 25 tomato samples are shown in the Appendix, Figure A1.

Lab reference: HX7

Table 1. Details of the tomato samples provided for analysis by Heritage Food Crops in 2023.

Sample name	Description
Best Optical M V.2	A 2023 selection from 2022 seed of a selection of 'Optical' with high concentrations of beta-carotene.
Celia Geary P1	A 2023 selection from a 'Geary' plant, with potato leaf. (The original had a normal tomato leaf).
Celia Geary P3	A 2023 selection from a 'Geary' plant, with potato leaf. (The original had a normal tomato leaf).
Eye Drop V.2	A 2023 selection from an 'Eye Drop' plant.
Gold Dust	Determinate, deep orange, medium-sized tomato.
Golden Bell V.2	A 2023 selection from a 'Golden Bell' plant that originated from a NZ natural cross from 'Oxheart' and small orange tomato. Bright orange bell shaped tomato.
Golden Light V.2	A 2023 selection from a 'Golden Light' plant (originally a HFCRT selection from Orange Teardrop. Very similar to Orange Roma).
Golden Light V.3	A 2023 selection from a 'Golden Light' plant (originally a HFCRT selection from Orange Teardrop. Very similar to Orange Roma).
Mini Orange V.2	A 2023 selection from a 'Mini Orange' plant.
Moonbeam V.2	A 2023 selection from a 'Moonbeam' plant.
Moonglow V.2	A 2023 selection from a 'Moonglow' plant.
Moonglow V.3	A 2023 selection from a 'Moonglow' plant.
Oley V.2	A 2023 selection from an 'Oley' plant, that was a 2022 selection from 'Wally's Spanish'.
Oley V.3	A 2023 selection from an 'Oley' plant, that was a 2022 selection from 'Wally's Spanish'.
Olga's Round Golden Chicken Egg V.2	A 2023 selection from an 'Olga's Round Golden Chicken Egg' plant.
Optical (end of row) V.2	A 2023 selection from an 'Optical' plant, that fruited very well in 2022.
Optical (end of row) V.3	A 2023 selection from an 'Optical' plant, that fruited very well in 2022.
Optical (end of row) V.4	A 2023 selection from an 'Optical' plant, that fruited very well in 2022.
Optical (end of row) V.5	A 2023 selection from an 'Optical' plant, that fruited very well in 2022.
Optical S1	A 2023 selection from an 'Optical' plant, that fruited very well in 2022.
Optical SPS (small tunnel) V.2	A 2023 selection from seed from an 'Optical' plant selection in 2022.
Oracle 2 SBO V.2	A 2023 selection from an 'Oracle' in 2022. The 2022 selection contained tetra-cis-lycopene rather than beta-carotene as well as good polyphenol concentrations.
Sirius	A 2023 selection from an 'Amish Yellowish Orange Oxheart' plant, that has none of the oxheart characteristics.
Spirited	A 2023 selection from an 'Amish Yellowish Orange Oxheart' plant, where the fruit looks like 'Optical'.
Wally's Spanish V.2	A 2023 selection from a 'Wally's Spanish' plant.

2.1 Carotenoid analysis

The analytical method used for the measurement of carotenoids in this study was similar to the 2020 tomato study (McGhie & Cordiner 2020). In brief, analysis was performed using ultra high-pressure liquid chromatography (UHPLC) with a photo diode array detector (PDA), providing retention time and unique UV/Vis absorption profile for each carotenoid. One change to the method was the use of liquid nitrogen instead of dry ice to grind the samples, as dry ice was not available. Additionally, samples were analysed on a newly installed UHPLC (Thermo Vanquish Horizon). Details of the carotenoids measured are shown in Table 2.

Where authentic standards were not available, the concentrations for these carotenoids were calculated using surrogate (equivalence) standards. Corrections were based on relative extinction coefficients at the detection wavelength (nm) used for each carotenoid. Tetra-cis-lycopene concentrations were calculated using an all-trans-lycopene standard, whereas phytoene, phytofluene and zeta-carotene concentrations were calculated using a beta-carotene standard. The accuracy of the absolute concentration values should be treated with caution and comparison with quantitative data from other studies should be made with care.

Table 2. Carotenoid details measured in tomatoes provided by Heritage Food Crops (2023).

Compound	CAS#	Formula	Exact mass	Detection wavelength (nm)	Equivalence
Lutein	127-40-2	C ₄₀ H ₅₆ O ₂	568.428	450	lutein
all-trans-lycopene	502-65-8	C ₄₀ H ₅₆	536.4382	470	all-trans-lycopene
tetra-cis-lycopene	2361-24-2	C ₄₀ H ₅₆	536.4382	436	all-trans-lycopene
Phytoene	13920-14-4	C ₄₀ H ₆₄	544.5008	286	beta-carotene
phytofluene	540-15-6	C ₄₀ H ₆₂	542.4852	348	beta-carotene
zeta-carotene	13587-06-9	C ₄₀ H ₆₀	540.4695	400	beta-carotene
beta-carotene	7235-40-7	C ₄₀ H ₅₆	536.4382	450	beta-carotene

2.2 Polyphenol analysis

The method used for the analysis of polyphenols was similar to the 2020 tomato study (McGhie & Cordiner 2020). In brief, extraction analysis was by UHPLC-MS. However, this year an additional capability was added to the MS method to increase confidence in identifying compounds. This capability was broadband collision-induced dissociation (bbCID). Details of the polyphenols measured are shown in Table 3.

Where authentic standards were not available, the concentrations for these polyphenols were calculated using surrogate (equivalence) standards, which are noted in Table 3. Again, the accuracy of the absolute concentration values generated from equivalent standards should be treated with caution and comparison with quantitative data from other studies should be made with care.

Table 3. Polyphenol details measured in tomatoes provided by Heritage Food Crops (2023).

Compound	Abbreviation	CAS#	Formula	Exact mass	Equivalence
(E)-caffeoyl 3-glucoside	E-caf3glu	143729-78-6	C15H18O9	342.0951	chlorogenic acid
(E)-caffeoyl 4-glucoside	E-caf4glu	147511-61-3	C15H18O9	342.0951	chlorogenic acid
3,4-dicaffeoylquinic acid	3,4-dicafQA	14534-61-3	C25H24O12	516.1268	chlorogenic acid
3,5-dicaffeoylquinic acid	3,5-dicafQA	2450-53-5	C25H24O12	516.1268	chlorogenic acid
4,5-dicaffeoylquinic acid	4,5-dicafQA	57378-72-0	C25H24O12	516.1268	chlorogenic acid
catechin	Cat	154-24-4	C15H14O6	290.079	epicatechin
chlorogenic acid	CGA	327-97-9	C16H18O9	354.0951	chlorogenic acid
cryptochlorogenic acid	cryCGA	905-99-7	C16H18O9	354.0951	chlorogenic acid
epicatechin	epiCat	490-49-0	C15H14O6	290.079	epicatechin
kaempferol 3-rutinoside	K-rut	17650-84-9	C27H30O15	594.1585	quercetin 3-rutinoside
naringenin chalcone	NarC	73692-50-9	C15H12O5	272.0685	naringenin
neochlorogenic acid	neoCGA	202650-88-2	C16H18O9	354.0951	chlorogenic acid
procyanidin B1	ProCy B1	20315-25-7	C30H26O12	578.1424	procyanidin B2
procyanidin B2	ProCy B2	29106-49-8	C30H26O12	578.1424	procyanidin B2
procyanidin B5	ProCy B5	12798-57-1	C30H26O12	578.1425	procyanidin B2
procyanidin B7	ProCy B7	12798-59-3	C30H26O12	578.1425	procyanidin B2
quercetin 3-arabinopyranoside	Q-arapy	72581-59-0	C20H18O11	434.0849	quercetin 3-rutinoside
quercetin 3-galactoside	Q-gal	482-36-0	C21H20O12	464.0955	quercetin 3-rutinoside
quercetin 3-glucoside	Q-glu	482-35-9	C21H20O12	464.0955	quercetin 3-rutinoside
quercetin 3-rhamnoside	Q-rha	522-12-3	C21H20O11	448.1006	quercetin 3-rutinoside
quercetin 3-rutinoside	Q-rut	153-18-4	C27H30O16	610.1534	quercetin 3-rutinoside
quercetin 3-xylopyranoside	Q-xyl	549-32-6	C20H18O11	434.0849	quercetin 3-rutinoside
quercetin 3-xylosylrutinoside	Q-xylrut	129235-39-8	C32H38O20	742.1956	quercetin 3-rutinoside
trans-5-p-coumaroyl quinic acid	t5pCouQA	5746-55-4	C16H18O8	338.1002	chlorogenic acid

2.3 Folate analysis

Folate was measured in the tomato samples using a PFR in-house method. It is a high-throughput, homogeneous, fluorescence polarisation, and fluorescence intensity assay. Further details on the method can be found in the research article by Martin et.al. (2010). Table 4 makes note of folate chemical detail.

Table 4. Folate details measured in tomatoes provided by Heritage Food Crops (2023)

Compound	CAS#	Formula	Exact mass
Folate	59-30-3	C19H19N7O6	441.1397

3 Results and discussion

The following results are on the tomatoes as received. It is prudent to note that factors affecting the concentrations of compounds could have an impact on results and this should be taken into consideration. Factors could include maturity of fruit at harvest, postharvest storage conditions and environmental factors.

3.1 Carotenoid concentrations

Carotenoid concentrations (measured as mg/100 g fresh weight) for the 25 tomato samples are shown in Table 5.

As in previous years, three general profiles have emerged:

- When tetra-cis-lycopene is present, the intermediates phytoene, phytofluene and zeta-carotene are elevated, and beta-carotene and all-trans-lycopene are lowered or not detected.
- When all-trans-lycopene is present, tetra-cis-lycopene and the intermediates phytoene, phytofluene, and zeta-carotene are lowered or not detected.
- When beta-carotene is present, tetra-cis-lycopene and the intermediates phytoene, phytofluene, and zeta-carotene are lowered or not detected.

To reiterate the note in previous studies: the differences between tetra-cis-lycopene, all-trans-lycopene and beta carotene concentrations tend to align with the colour of the tomato. For example, red tomatoes contain all-trans-lycopene, orange tomatoes often contain beta-carotene, and golden tomatoes contain tetra-cis-lycopene. According to the above statement this would align the majority of the 2023 tomatoes to be classed as golden tomatoes. However, there are three tomato samples falling into the orange bracket ; these are Eye Drop V.2, Optical (end of row) V.2, and Optical (end of row) V.4. Photos of the 25 tomato samples provided can be found in Appendix A.

Phytoene, phytofluene and zeta-carotene have lesser contribution to colour due to their lower intensity of colour which is noted by their absorption wavelength. There is a growing interest in these compounds in their contribution to health (Mapelli-Brahm & Melendez-Martinez 2021).

In this study the concentrations of the carotenoids were comparable to previous studies.

It should also be noted, the carotenoid data were generated on a newly installed UHPLC, which may contribute to slight changes in the chromatography, increased sensitivity of the instrument, and therefore the data produced.

Table 5. Carotenoid concentrations (mg/100 g fresh weight) for tomatoes provided by Heritage Food Crops (2023).

Sample name	tetra-cis-lycopene	phytoene	Phytofluene	zeta-carotene	beta-carotene	all-trans-lycopene	lutein
Best Optical M V.2	3.14	11.99	13.05	17.70	n.d.	n.d.	0.04
Celia Geary P1	2.63	13.19	14.63	16.00	n.d.	n.d.	0.04
Celia Geary P3	1.80	9.43	10.52	10.91	n.d.	n.d.	0.04
Eye Drop V.2	n.d.	0.54	0.38	n.d.	5.02	0.19	0.15
Gold Dust	1.76	9.46	11.15	0.47	n.d.	0.06	0.09
Golden Bell V.2	1.36	5.59	6.82	7.96	n.d.	0.06	0.15
Golden Light V.2	1.34	4.76	5.58	5.72	n.d.	n.d.	0.10
Golden Light V.3	1.77	3.94	4.75	4.38	n.d.	0.05	0.13
Mini Orange V.2	3.31	12.83	14.65	14.56	n.d.	n.d.	0.07
Moonbeam V.2	2.84	8.70	11.83	16.93	n.d.	n.d.	0.08
Moonglow V.2	2.95	12.34	14.13	11.55	n.d.	n.d.	0.03
Moonglow V.3	4.50	9.27	11.75	8.88	n.d.	0.06	0.03
Oley V.2	1.29	4.07	5.12	5.56	n.d.	0.10	0.08
Oley V.3	1.32	2.45	3.05	3.01	n.d.	0.13	0.11
Olga's Round Golden Chicken Egg V.2	4.21	9.20	11.13	9.32	n.d.	0.05	0.07
Optical (end of row) V.2	n.d.	1.21	0.96	0.20	4.91	0.37	0.06
Optical (end of row) V.3	2.35	8.41	8.72	10.53	n.d.	0.09	0.08
Optical (end of row) V.4	n.d.	1.07	0.76	0.12	5.09	0.13	0.07
Optical (end of row) V.5	3.46	17.94	14.66	15.83	n.d.	n.d.	0.02
Optical S1	4.26	12.67	10.85	10.40	n.d.	n.d.	0.02
Optical SPS (small tunnel) V.2	1.83	4.87	4.67	5.85	n.d.	n.d.	0.03
Oracle 2 SBO V.2	3.75	7.24	8.08	8.12	n.d.	n.d.	0.05
Sirius	2.68	6.58	8.51	9.19	n.d.	0.12	0.07
Spirited	3.31	9.88	13.50	16.80	n.d.	n.d.	0.10
Wally's Spanish V.2	1.88	11.87	14.11	13.50	n.d.	n.d.	0.02

n.d. – not detected.

3.2 Polyphenol concentrations

Polyphenol concentrations (measured as mg/100 g fresh weight), which were present in some or all of the 25 tomato samples are shown in Table 6. Not presented are polyphenols that were not detected (n.d.) in all 25 tomato samples and these were: catechin, cryptochlorogenic acid, epicatechin, procyanidin B1, procyanidin B2, procyanidin B5, procyanidin B7, procyanidin C1, quercetin 3-arabinopyranoside, quercetin 3-galactoside, quercetin 3-glucoside, quercetin 3-rhamnoside, quercetin 3-xyloside, kaempferol 3-rutinoside and trans-4-p-coumarylquinic acid. The polyphenols that were n.d. in this study are consistent with those polyphenol compounds not detected in the 2022 report.

To improve the identity of polyphenol compounds with similar structure and the same molecular formula (same mass), mass spectrometry (MS) with broadband collision-induced dissociation (bbCID) capability was utilised. These similar compounds can fragment differently in the MS leading to unique fragmentation patterns and hence improved and increased confidence in identification.

Caffeic acid conjugates with quinic acid are the major polyphenolic metabolites in tomatoes. Here we have detected the monocaffeoyl and dicaffeoyl quinic acid conjugates along with chlorogenic acid and neochlorogenic acid.

Utilising bbCID had a marked impact on the concentrations calculated for (E)-caffeoyl 3-glucoside and (E)-caffeoyl 4-glucoside compared to the 2022 study. Additionally, to identify the (E)-caffeoyl 3-glucoside and (E)-caffeoyl 4-glucoside, the work by Patras et. al. (2018) described the order of elution on a reverse phase chromatography column to be (E)-caffeoyl 3-glucoside first and (E)-caffeoyl 4-glucoside second. We have used this concept in identifying these compounds.

Due to improvements and different methodologies adopted over the years, comparisons between previous studies should be made with consideration.

Table 6. Polyphenol concentrations (mg/100 g fresh weight) for tomatoes provided by Heritage Food Crops (2023).

Sample name	CGA	neoCGA	3,4-dicafQA	3,5-dicafQA	4,5-dicafQA	E-caf3glu	E-caf4glu	NarC	Q-rut	Q-xylrut	t5pCouQA
Best Optical M V.2	4.32	0.10	0.44	0.25	1.11	1.78	2.47	0.13	0.58	0.21	0.07
Celia Geary P1	3.09	0.06	0.34	0.20	1.35	1.19	2.00	n.d.	0.15	0.15	0.11
Celia Geary P3	10.08	0.14	0.25	0.90	1.87	2.17	3.68	n.d.	1.74	1.24	3.09
Eye Drop V.2	17.29	0.20	0.09	1.13	1.36	5.51	4.07	0.01	0.80	0.13	2.96
Gold Dust	5.97	0.06	0.06	0.38	0.67	1.27	1.36	n.d.	0.66	0.59	2.64
Golden Bell V.2	5.01	0.07	0.38	0.33	3.35	2.77	3.17	0.69	0.14	0.08	0.74
Golden Light V.2	2.66	0.05	0.27	0.17	0.98	3.41	2.31	0.23	0.51	0.20	0.13
Golden Light V.3	1.55	0.05	0.22	0.10	0.42	3.66	2.72	0.38	0.67	0.24	0.07
Mini Orange V.2	6.07	0.08	0.48	0.46	5.43	2.40	2.96	0.73	0.44	0.12	0.36
Moonbeam V.2	3.19	0.08	0.42	0.23	2.04	2.49	2.89	n.d.	0.02	0.02	0.41
Moonglow V.2	2.85	0.09	0.40	0.18	1.26	2.76	3.49	0.68	0.27	0.19	0.17
Moonglow V.3	4.37	0.11	0.52	0.29	1.93	1.95	2.48	n.d.	0.18	0.20	0.57
Oley V.2	4.73	0.08	0.41	0.28	1.94	3.89	2.83	0.41	0.61	0.27	0.26
Oley V.3	14.06	n.d.	0.14	1.04	1.75	2.97	2.37	0.01	2.34	0.98	1.55
Olga's Round Golden Chicken Egg V.2	18.71	0.19	0.16	1.27	2.10	4.67	3.97	n.d.	0.88	0.55	4.79
Optical (end of row) V.2	1.24	0.06	0.14	0.06	0.27	2.77	2.17	0.05	0.18	0.04	0.08
Optical (end of row) V.3	2.45	0.05	0.32	0.17	1.64	3.79	2.21	0.30	0.76	0.24	0.11
Optical (end of row) V.4	9.63	0.22	0.40	0.99	2.42	3.51	5.05	0.01	1.26	0.57	3.03
Optical (end of row) V.5	4.94	0.07	0.11	0.34	0.68	1.95	2.63	n.d.	0.41	0.47	1.56
Optical S1	6.19	n.d.	0.58	0.42	3.71	2.23	2.71	3.84	1.04	0.54	0.38
Optical SPS (small tunnel) V.2	1.74	0.05	0.21	0.10	0.74	2.16	1.90	0.44	0.27	0.06	0.18
Oracle 2 SBO V.2	2.60	0.07	0.34	0.15	0.99	0.71	2.03	0.47	0.27	0.22	0.08
Sirius	7.03	n.d.	0.12	0.56	1.00	2.26	2.57	n.d.	0.97	0.58	2.59
Spirited	3.18	0.12	0.53	0.25	2.12	5.02	2.87	0.17	0.91	0.35	0.07
Wally's Spanish V.2	8.31	0.09	0.98	0.78	2.81	5.41	5.09	0.57	0.44	0.07	0.96

n.d. – not detected.

3.3 Folate concentrations

Folate concentrations (measured as $\mu\text{g}/100\text{ g}$ fresh weight), for the 25 tomato samples are shown in Table 7.

Folate concentrations ranged from 18.4 to 55.0 $\mu\text{g}/100\text{ g}$.

Table 7. Folate concentrations ($\mu\text{g}/100\text{ g}$ fresh weight) for tomatoes provided by Heritage Food Crops (2023).

Sample name	Folate ($\mu\text{g}/100\text{g}$)
Best Optical M V.2	32.8
Celia Geary P1	22.5
Celia Geary P3	29.0
Eye Drop V.2	55.0
Gold Dust	24.5
Golden Bell V.2	22.0
Golden Light V.2	21.5
Golden Light V.3	27.5
Mini Orange V.2	37.1
Moonbeam V.2	27.8
Moonglow V.2	19.1
Moonglow V.3	21.6
Oley V.2	23.7
Oley V.3	18.4
Olga's Round Golden Chicken Egg V.2	18.7
Optical (end of row) V.2	35.6
Optical (end of row) V.3	26.6
Optical (end of row) V.4	45.7
Optical (end of row) V.5	42.7
Optical S1	33.7
Optical SPS (small tunnel) V.2	41.5
Oracle 2 SBO V.2	37.4
Sirius	23.6
Spirited	35.2
Wally's Spanish V.2	21.3

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Appendix

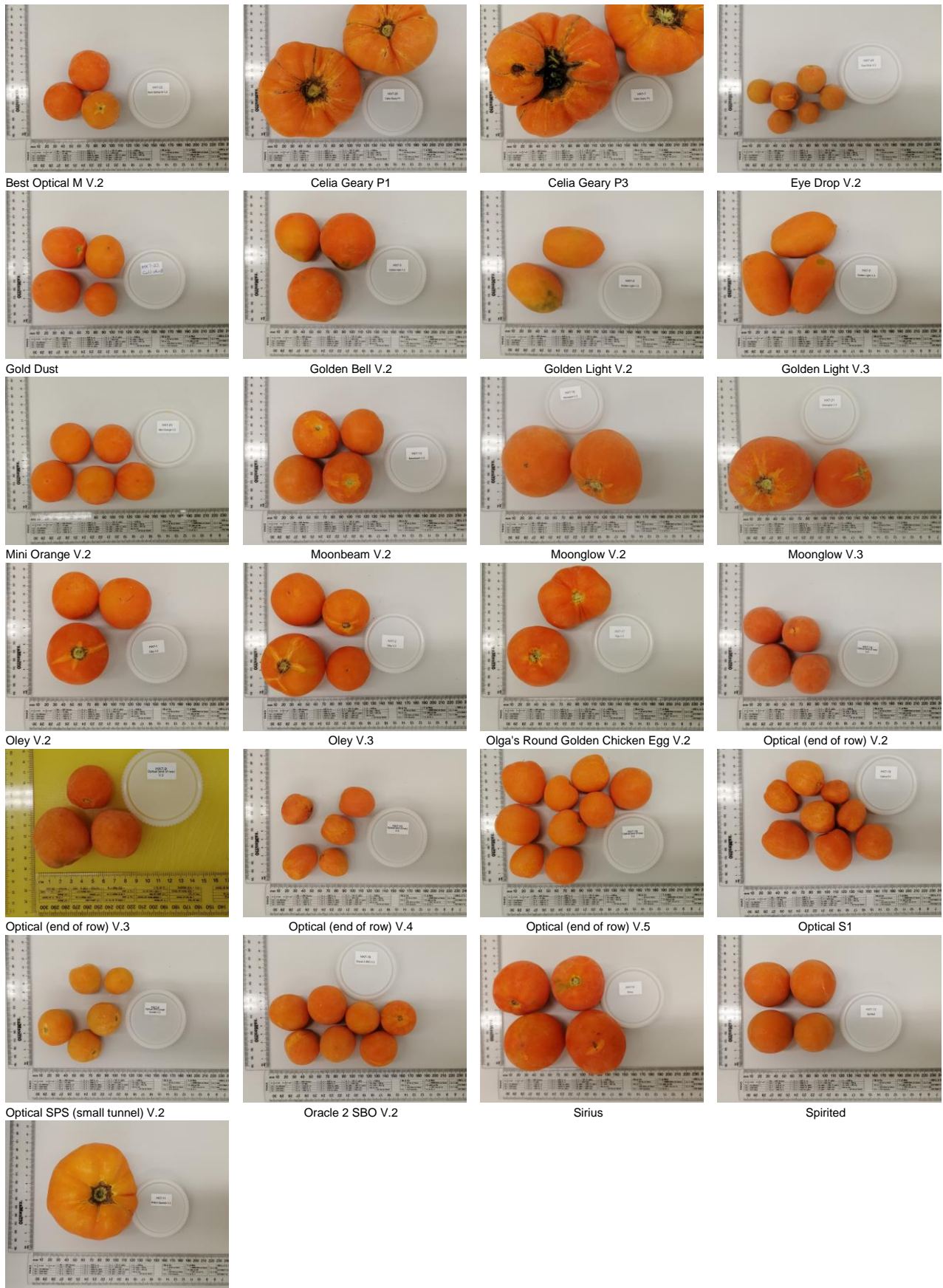


Figure A1. Photos of the 25 tomato samples provided by Heritage Food Crops (2023).

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