Research Article

Ekin
Journal of Crop Breeding and Genetics

www.ekinjournal.com

2(2):1-10, 2016

Ekin International biannual peer-reviewed journal

Genetic Improvement of Tomato (*Solanum lycopersicum* L.) for Phytonutrient Content at AVRDC - The World Vegetable Center

Peter M. Hanson* **Ray-Yu Yang**

AVRDC - The World Vegetable Center, P.O. Box 42, Shanhua, Tainan, Taiwan.

* Corresponding author e-mail: peter.hanson@worldveg.org

Citation:
Hanson P.M, Yang R.Y, 2016. Genetic improvement of tomato (*Solanum lycopersicum* L.) for phytonutrient content at AVRDC - the World Vegetable Center. Ekin J. 2(2):1-10.

Received: 10.12.2015 ***Accepted: 09.01.2016*** ***Published Online: 28.07.2016*** ***Printed: 30.07.2016***

ABSTRACT

Tomato is a widely consumed global vegetable and a major source of the phytonutrients vitamin C, beta-carotene (provitamin A), lycopene, and flavonoids. Tomato cultivars with increased fruit phytonutrient density could help overcome micronutrient malnutrition and contribute to better human health. It is important for plant breeders to understand the genetic diversity and genetic control of targeted phytonutrients, the extent to which environmental factors such as temperature or light intensity affect phytonutrient content, and whether altered phytonutrient content significantly affects yield and horticultural or fruit quality traits. Tomato breeding at AVRDC - The World Vegetable Center has included phytonutrient objectives in its breeding programs and has developed tropically adapted, high yielding and multiple disease resistant lines with increased content of beta-carotene, lycopene, flavonoids, or anthocyanin in different fruit types. Increased content of some phytonutrients such as lycopene is associated with better fruit quality. On the other hand, high beta-carotene content results in orange-fleshed fruit, which is not readily accepted by many consumers. AVRDC seeks to popularize high phytonutrient tomato cultivars through linkages with organizations promoting nutrition and health.

Keywords: Nutrition, beta-carotene, lycopene, flavonoids.

Introduction

Micronutrients including vitamins and minerals are essential components of healthy diets and are required for proper physical and mental growth and immune system functions. However, it is estimated that more than two billion people worldwide are stunted, anemic, and or vitamin A deficient due to one more micronutrient deficiencies a condition also known as 'hidden hunger' (Muthayya *et al*., 2013). Vegetables are major dietary sources of micronutrients and provide antioxidant phytochemicals that act to prevent or neutralize free radical chain reactions, which may lead to development of cardiovascular diseases or cancers (Keatinge *et al*., 2013). AVRDC-The World Vegetable Center (AVRDC) is the leading international center for vegetable research and development with the mission of alleviating poverty and malnutrition in developing countries. Conventional plant breeding to increase density of phytonutrients (micronutrients and antioxidant phytochemicals) of selected vegetables crops is among AVRDC's multiple strategies to improve access of the poor to adequate supplies of safe and affordable vegetables (Yang *et al*., 2007).

Tomato is one of the most economically important, widely grown and consumed vegetables in the world (FAO, 2013). Tomato fruit contains significant amounts of beta-carotene (provitamin A carotenoid), lycopene, ascorbic acid, some phenolic acids and flavonoids, all of which can be phytonutrients. Although phytonutrient levels in tomato are small compared to some other vegetables (Keatinge *et al*., 2011), daily consumption of tomatoes is high in many countries and even modest increases in tomato phytonutrient content could contribute to better human health (Hanson *et al*., 2004).

Genetic variation for some phytonutrients exists in tomato. More than 20 genes have been described affecting the types and concentrations of carotenoids in tomato (Stommel, 2007). Some of these include the alleles *Beta* (B) and *crimson* (og^c) mapped to the Beta locus on chromosome 6 (Ronen *et al*., 2000); *high pigment-1* (*hp-1*) and *high pigment-2* (*hp-2*) mapped to chromosomes 2 and 1, respectively (Yen *et al*., 1997), as well as $hp\text{-}2^{dg}$, which is allelic to *hp-2* (Bino *et al*., 2005). Additional genes or quantitative trait loci (QTLs) have been identified that increase flavonoids such as the gene *Anthocyanin fruit* (*Aft*) mapped to chromosome 10, and a major QTL increasing rutin content mapped to chromosome 5 (Hanson *et al*., 2014). Tomato also produces phenolic acids including caffeic acid and chlorogenic acid that act as antioxidants (Bravo, 1998). Although there is potential to increase fruit nutrient content, tomato breeding programs have largely emphasized yield, disease resistance, and fruit quality objectives (Stommel, 2007). AVRDC tomato breeding assigns a high priority to increasing the content of some fruit phytonutrients, particularly carotenoids and flavonoids. Phytonutrients to include as breeding objectives are selected for their potential to make a significant improvement, the extent to which nutrients levels are affected by environmental factors, and prospects for acceptance by consumers and markets. This paper outlines some of the work on genetic improvement of tomato nutrition traits by AVRDC, the strategies used, and current progress.

Materials and Methods

Season-Year Effects on Phytonutrient Contents

Twelve tomato entries (Table 1) representing different fruit market types or carrying genes affecting fruit carotenoids, vitamin C, and anthocyanin content were grown at AVRDC Taiwan in two seasons for two years. Mean temperatures, rainfall, and solar intensities during trials are given in Table 2. Seasons 1 and 2 are early and late dry seasons, and conditions in season 1 usually favor tomato production compared to season 2. Plots included four or nine plants and entries were replicated three times and arranged in a randomized complete block design (RCBD). Plants were staked and pruned and grown using recommended management practices. Full red ripe fruit were harvested from the inner two or seven plants and provided to AVRDC Nutrition for analysis of phytonutrients except for vitamin C, which was not analyzed in year 1-season 1. Methods for phytonutrient analysis are given in Hanson *et al*., (2004). Data were subjected to analysis of variance for individual environments and over seasons and years using the general linear models (PROC GLM) procedure of SAS Online Version 9.4 software (SAS Institute, Inc., Cary, N.C.). For the combined analysis of variance, a mixed effects model was applied in which entry was considered a fixed effect and year, season, and replications were deemed random effects. The significance of mean squares was determined using appropriate error terms.

Dual-Purpose Tomato Trials

Two preliminary yield trials (PYT) were carried out at AVRDC-Taiwan in the first half of 2015. Both PYT included 8 test lines (coded CLN) and checks 'Tanya,' 'UC204A', and T5020. 'Tanya' and 'UC204A' are processing tomato cultivars and T5020 is a fresh market line homozygous for *hp-1* and og^c. PYT1 entries were sown and transplanted, respectively, on 2 and 30 December 2014. PYT2 entries were sown and transplanted, respectively, on 6 February and 10 March 2015. PYT1 plots consisted of two 1.5 m-wide beds with one 5.0-m-long row per bed (24 plants). PYT2 plots included 24 plants but consisted of one 1.5 m-wide bed with two 5.0-m-long rows per bed. Plants in PYT1 were not staked while PYT2 plants were staked and pruned. Entries were replicated twice and plots were arranged in a RCBD. PYT1 plots were harvested three times (9, 20, 30 April 2015). PYT2 plots were harvested on 20 May, 28 May, and 8 June. Fruit samples were taken to the AVRDC Nutrition lab for analysis of vitamin C, lycopene and beta-carotene. A separate and a combined analysis of variance were performed. Average daily maximum, minimum, and mean temperatures from transplanting to final harvest during PYT1 were 26.2 °C, 15.9 °C, and 20.2 °C respectively; total rainfall and average relative humidity during PYT1 were 109 mm and 68.1%, respectively, and average daily solar intensity was 5087 watt-hours per m^2. During PYT2, average daily maximum, minimum, and mean temperatures from transplanting to final harvest were 30.2 °C, 20.9 °C, and 24.8 °C respectively. Total rainfall and average relative humidity during the trial were 491 mm and 68.9%, respectively. Average daily solar intensity was 5798 watt-hours per m^2. Data were subjected to analysis of variance using SAS software.

Results

Season-Year Effects on Phytonutrient Content

The analyses of variance revealed highly significant entry means squares for all phytonutrients and quality traits except caffeic acid (Table 3). Season affected phytonutrient contents and are indicated by highly significant or significant season means squares for all traits except beta-carotene. All entry-season interactions were nonsignificant for all traits except for beta-carotene; entry-year interactions were also nonsignificant except for lycopene and caffeic acid.

Carotenoids and Vitamin C

Mean lycopene content over entries was about 18% lower in season 2 compared to season 1 (Table 4a). Entries T5020 (*hp-1+og*^{c}) and ASVEG20 produced relatively high lycopene content in most trials. The three entries homozygous for *og*^{c} produced higher than average lycopene but were not outstanding compared to processing check 'UC204A.' Lycopene content of high anthocyanin line CLN3339FA was slightly lower but similar to other fresh market entries. Mean beta-carotene content was about 20% lower in season 1 versus season 2. As expected, the two entries homozygous for *Beta* developed about 5-8 times more beta-carotene content than the fresh market (Savior, CLN2498D) and processing ('UC204A') checks. T5020 and ASVEG20 developed about twice as much beta-carotene compared to the checks. Significant differences between entries were found for vitamin C content although only a two-fold range in entry means was found. ASVEG20 ranked as highest or second highest in vitamin C content in each trial.

Phenolics and Rutin (flavonoid)

Average caffeic acid content (Table 4b)varied greatly by both year and season; for example, mean caffeic acid content over entries was six times greater in year 1-season 1 compared to year 2-season 1. Similarly, entry means varied greatly between trials and were often inconsistent: CLN3339FA, for example, produced the highest caffeic content in two trials but levels were average or below-average in the other trials. Mean chlorogenic acid content was about 33% greater in season 2 versus season 1. Mean rutin content was highest in year 1-season 2 with relatively high mean temperatures and rainfall that were more stressful and likely induced higher flavonoids. Rutin content of entry BMZ51 varied among trials but was four or five times greater than other entries. CLN-3339FA, CLN2366A, T5020 and ASVEG20 also developed relatively high rutin content.

Dual-Purpose Tomato Trial

Highly significant differences among entries were detected for all traits except lycopene content (Table 5). Optimal weather for tomato production and low TYLCD incidence likely accounted for the high mean marketable yield in PYT1 (95 t/ha). Mean yield in PYT2 (18 t/ha) was sharply lower because of strong tomato yellow leaf curl disease (TYLCD) pressure coupled with higher temperatures and rainfall. Even though there were no significant differences among entries in lycopene content in both trials, entries homozygous for the *og*^{c} allele usually produced high color values sometimes exceeding 2.0 (data not shown); this indicated presence of a deep red fruit color desirable for many consumers and by tomato processors. The three entries homozygous for *hp-1* showed relatively higher levels of beta-carotene and vitamin C.

Discussion

Consumption of vegetables and fruit provide essential micronutrients and phytochemicals that reduce risks of micronutrient deficiencies as well as chronic diseases such as atherosclerosis and cancers. Unfortunately, vegetable consumption in most of the tropics and subtropics falls far short of the minimum of 200 g per person per day (Keatinge *et al*., 2011). Action must be taken to increase vegetable supplies through better production and postharvest practices and to raise consumer demand for and consumption of vegetables. AVRDC-The World Vegetable Center has developed technologies to increase supplies of diverse and affordable vegetables, such as diversification of vegetable crops, especially indigenous vegetables; off-season vegetable production; intensive home gardening; improved postharvest practices; and optimized food preparation methods, all which ultimately contribute to better diets and human nutrition (Yang *et al*., 2007). Tropically adapted, high yielding vegetable cultivars coupled with sound management practices play an essential role in boosting vegetable supplies. Successful cultivars must meet the requirements of different value chain stakeholders and combine multiple traits, including high yield, multiple disease resistance, and long shelf-life, and be safe, nutritious and good tasting for consumers. Development of phytonutrient-dense cultivars is a worthy objective pursued by all AVRDC breeding programs but the breeding strategy must be designed and carried out with all end-users in mind.

Tomato is a globally popular vegetable consumed fresh, cooked, or in processed products. Consumption is high, and thus tomato fruit is an important source of carotenoids, vitamin C and flavonoids in diets. Manipulating carotenoid content in tomato is relatively straightforward because major genes affecting the types and levels of particular carotenoids are known and characterized (Ronen *et al*., 2000; Stommel, 2007). Vitamin A deficiency is a preventable cause of blindness in children and increases risks of some infectious diseases. In response, AVRDC initiated breeding to increase tomato beta-carotene content in some tropical tomato lines using the *Beta* allele almost 20 years ago with funding from the Thrasher Foundation, USA. Presence of *Beta* boosted beta-carotene content by 3-9 times compared to normal red-fruited tomato (Yang *et al.*, 2007). Beta-carotene contents of some early AVRDC *Beta* lines reached 9.0 mg/100 FW but such high levels resulted in a bitter aftertaste. Targets shifted to beta-carotene levels 3-5.0 mg/100g FW, which are still relatively high but do not noticeably affect taste. AVRDC *Beta* lines in fresh market and cherry tomato fruit types have been distributed internationally and have been officially released as cultivars in Mali, Taiwan, and Bangladesh (unpublished data). However, adoption so far has not been high because markets and consumers are unaware, unfamiliar, or reluctant to accept the orange fruit color. With low demand, financial incentives for seed companies or public organizations to produce of *Beta* tomato lines are weak. AVRDC *Beta* cherry tomato lines are tasty, productive, easy to grow, and perhaps best targeted for tropical home gardens. More partnerships with health and nutrition public organizations are needed to increase awareness and use of *Beta* tomato by gardeners.

Tomato cultivars originally developed for processing such as 'Roma VF', 'Rio Grande', 'UC82' and others are widely grown in the tropics for the fresh market and sometimes for processing (dual-purpose). Besides fruit firmness, dual-purpose tomato cultivars typically develop deep red internal color due to high lycopene content. The *hp-1* allele is interesting because it acts to elevate total carotenoid content without changing the proportions of lycopene and beta-carotene, and it also increases content of vitamin C and flavonoids; full-ripe fruit becomes red or sometimes red-orange. Gains in beta-carotene content with *hp-1* are not as great as *Beta,* but market and consumer acceptance would be far easier, and such lines could become popular if incorporated into firm fruit types and combined with TYLCD and other disease resistances with moderate to high levels of heat tolerance. High pigment lines CLN3669A (AVTO1418) and CLN3670B (AVTO1420) are TYLCD resistant and available through the AVRDC seed catalog (*http://avrdc.org/seed/improved-lines/*). AVRDC will continue to develop tomato lines with *hp-1* alone and in combination with *og*^{c}.

Evidence suggests that flavonoids are powerful antioxidants that can benefit human health (Ross and Kasum, 2002). Rutin is the major flavonoid in tomato and AVRDC identified a major QTL in a breeding line derived from an introgression line that increased rutin content by 4-5 times (Hanson *et al*., 2014). AVRDC is incorporating this QTL into elite tomato lines by marker-assisted selection and by developing near-isogenic lines (NIL) with or without the QTL to test whether high rutin content enhances disease resistance and/or abiotic stress tolerance. AVRDC trials indicated that levels of caffeic acid and chlorogenic acid were strongly affected by the environment and large entry rank changes were common; breeding to enhance these phenolics is not warranted. Vitamin C is important both as an essential nutrient and as an enhancer of iron bioavailability (Teucher *et al*., 2004). Presently, AVRDC characterizes its inbred lines for vitamin C content but has not actively bred to improve this trait, mainly because analysis is time-consuming and AVRDC has not yet methodically screened its genebank accessions for sources of high vitamin C. Genetic variation for vitamin C content was found in cultivated tomato, but environmental effects were also large (Leiva-Brondo *et al*., 2012). Availability of cheap, accurate, and fast vitamin C screening methods would facilitate breeding.

There is significant scope to genetically improve tomato through conventional breeding for a number of important phytonutrients, including carotenoids, vitamins, and flavonoids. Many major genes affecting nutrient content in tomato are well-known, but often not used in breeding. Breeding to increase phytonutrient content is most active when phytonutrient and fruit quality objectives are in alignment: tomato breeders have selected for increased lycopene content, which deepens internal red fruit color, a trait appreciated by processors and many consumers; lycopene was later determined to be a powerful antioxidant (Stommel, 2007). Few tomato breeding programs overtly include nutrition objectives. AVRDC breeding programs pursue nutrition objectives to support the Center's mandate to alleviate poverty and malnutrition. Mounting consumer demand for nutritious and safe vegetables may create market incentives to drive more tomato breeding programs to take up nutrition.

Acknowledgements

The authors thank Rose Chan for formatting assistance and Maureen Mecozzi for editing the manuscript. Financial support from the Thrasher Foundation and the United States Agency for International Development (USAID) project "Improving Income, Nutrition and Health in Bangladesh through Potatoes, Sweet Potatoes and Vegetables" is gratefully acknowledged.

Table 1. Entry names and genes affecting fruit phytonutrient contents evaluated at AVRDC Taiwan.

Entry name	Origin	Allele (symbol) or comment	Effect of fruit phytonutrient gene	Reference
CLN3339FA	AVRDC	Anthocyanin fruit (*Aft*)	Increased anthocyanin in fruit skin is expressed if fruit not shaded	Jones *et al.*, 2003 Sapir *et al.*, 2008
CLN2366A	AVRDC	Beta (B)	Elevated fruit beta-carotene content at expense of lycopene	Tomes *et al.*, 1956
CLN2070A	AVRDC	Beta (B)	Elevated fruit beta-carotene content at expense of lycopene	Tomes *et al.*, 1956
BMZ51F2-67-1-30 (BMZ51)	AVRDC	High flavonoid QTL	Elevated rutin in fruit pericarp	Hanson *et al.*, 2014
CLN3070J	AVRDC	*crimson* (*og^c*)	Elevated fruit lycopene content	Ronen *et al.*, 2000
CLN3125P	AVRDC	*crimson* (*og^c*)	Elevated fruit lycopene content	Ronen *et al.*, 2000
NCEBR-6	USA	*crimson* (*og^c*)	Elevated fruit lycopene content	Ronen *et al.*, 2000
T5020	USA	*crimson* (*og^c*) and high pigment (*hp-1*)	Elevated carotenoids, vitamin C, and flavonoids	Wann, 1997
ASVEG20	AVRDC	Dark green fruit pericarp, presumed to be due to action of *dark-green* (*dg*)	Elevated carotenoids, vitamin C	Levin *et al.*, 2003 Konsler, 1973 Bino *et al.*, 2005
Savior	Syngenta	Fresh market hybrid (check)		
CLN2498D	AVRDC	Fresh market line (check)		
UC204A	USA	Processing cultivar (check)		

Table 2. Weather data summary of tomato trials conducted to assess phytonutrient content and other fruit traits of selected tomato lines over two seasons and two years, AVRDC Taiwan, 2011-2013.

Trial	Date			Mean daily air temperature (°C)		Total precipitation	Mean daily solar intensity
	Sowing	Transplant	Harvest	Max	Min	mm	(w-h W ·m^2)
Year 1 - Season 1	26 Oct 2011	26 Nov	3 Apr	24.2	15.4	59	3633
Year 1 - Season 2	9 Feb 2012	20 March	8 June	32.2	21.7	431	5572
Year 2 - Season 1	10 Sept 2012	9 Oct	26 Dec	28.9	17.7	103	4172
Year 2 - Season 2	24 Jan 2012	27 Feb	9 May	29.5	18.4	136	4838

Table 3. Significance of means squares from the combined analysis of variance of phytonutrients over two years and two seasons, AVRDC Taiwan.

Source of Variation	Degrees of Freedom	Lycopene	Beta-carotene	Caffeic acid	Chlorogenic acid	Rutin
Year (Yr)	1	–	–	–	–	–
Season (Yr)	2	**	ns	**	**	**
Rep (Yr*Seas)	8	–	–	–	–	–
Entry	11	**	**	ns	**	**
Entry*Yr	11	**	ns	**	ns	ns
Entry*Seas	11	ns	**	ns	ns	ns
Entry*Yr*Seas	11	ns	ns	**	**	**
Pooled	88	–	–	–	–	–

Source of Variation	Degrees of Freedom	Vitamin C
Environments (Env)	2	**
Reps (Env)	6	-
Entry	11	**
Entry*Env	22	**
Pooled	66	–

**=significant at P<0.01; ns=not significant

Table 4a. Entry means for carotenoids and vitamin C by season and year, AVRDC Taiwan.

Trial	Lycopene				Beta-carotene				Vitamin C			
	Season 1		Season 2		Season 1		Season 2		Season 1		Season 2	
	------mg/100 g fresh weight-----				------mg/100 g fresh weight-----				------mg/100 g fresh weight-----			
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
CLN3339FA	4.86 e	5.87 a	4.95 cd	3.34 bc	0.22 e	0.39 cd	0.33 f-h	0.26 ef	-	24.9 b-d	19.0 c-e	17.8 de
CLN2366A	1.14 f	1.49 b	0.88 e	0.83 de	2.48 b	2.27 a	2.21 b	2.07 a	-	23.9 de	15.7 fg	18.5 c-e
CLN2070A	1.03 f	1.15 b	0.26 e	0.25 e	3.16 a	2.12 a	3.62 a	2.00 a	-	28.0 b	24.9 a	20.8 bc
BMZ51	6.09 c-e	6.82 a	6.51 bc	4.44 ab	0.49 de	0.40 cd	0.49 de	0.46 c	-	27.7 bc	20.8 bc	19.8 cd
CLN3070J	7.76 b-d	6.22 a	8.48 ab	5.21 ab	0.30 de	0.19 d	0.21 gh	0.15 g	-	24.3 cd	14.4 g	14.7 fg
CLN3125P	6.87 b-e	7.03 a	8.13 ab	4.24 ab	0.39 de	0.31 d	0.41 d-f	0.23 e-g	-	20.6 ef	18.9 c-f	17.5 de
NCEBR-6	6.15 c-e	5.71 a	5.02 cd	4.81 ab	0.32 de	0.22 d	0.16 h	0.18 fg	-	18.6 f	16.5 e-g	13.6 g
T5020	11.15 a	6.46 a	8.98 a	5.53 a	0.59 d	0.71 bc	0.55 d	0.41 c	-	32.0 a	20.2 cd	22.8 ab
ASVEG20	9.42 ab	5.88 a	9.00 a	4.21 ab	1.09 c	1.05 b	0.73 c	0.85 b	-	33.4 a	23.7 ab	23.7 a
Savior	7.39 b-e	5.96 a	7.70 ab	4.78 ab	0.36 de	0.25 d	0.32 f-h	0.30 de	-	27.7 bc	21.5 bc	18.8 c-e
CLN2498D	5.02 de	7.01 a	4.00 d	2.20 cd	0.62 d	0.42 cd	0.47 d-f	0.43 c	-	22.8 de	19.4 c-d	16.2 ef
UC204A	8.09 bc	6.52 a	7.26 ab	5.44 a	0.44 de	0.36 cd	0.33 e-g	0.39 cd	-	20.5 ef	17.2 d-g	19.1 cd
LSD (P=0.05)	2.75	2.12	2.11	1.90	0.27	0.37	0.17	0.09	-	3.5	3.2	2.6
Mean	6.24	5.51	5.92	3.77	0.87	0.82	0.72	0.64		25.4	19.4	18.6
Entry mean square significance	**	**	**	**	**	**	**	**		**	**	**

**=significant at P<0.01

Table 4b. Entry means for phenolic acids and rutin by season and year, AVRDC Taiwan.

Trial	Caffeic Acid				Chlorogenic acid				Rutin			
	Season 1		Season 2		Season 1		Season 2		Season 1		Season 2	
	------mg/100 g fresh weight-----				------mg/100 g fresh weight-----				------mg/100 g fresh weight-----			
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
CLN3339FA	2.48 a	0.08 f	2.46 a	0.33 de	1.74 a-c	4.88 a	7.10 a	6.80 a	1.79 b-d	6.72 b	7.52 bc	5.91 b
CLN2366A	1.51 b	0.23 b	0.87 b-d	0.47 b	2.78 a	5.64 a	6.66 a	1.72 c-e	2.03 bc	4.74 c	9.00 b	4.14 bc
CLN2070A	2.29 a	0.37 a	0.79 b-e	0.44 bc	2.30 ab	4.44 a	4.07 b	0.85 e	2.06 bc	2.36 de	4.56 c-e	2.40 c-e
BMZ51	1.19 bc	0.18 cd	0.89 b-d	0.32 de	1.87 a-c	2.84 b	3.23 bc	2.42 c	9.05 a	16.3 a	37.03 a	15.68 a
CLN3070J	1.02 bc	0.14 de	0.48 d-f	0.12 g	1.25 b-d	1.95 bc	1.98 cd	2.35 c	0.69 d	0.90 e	1.61 e	0.86 e
CLN3125P	0.55 c	0.24 b	0.52 c-f	0.19 fg	1.10 b-d	1.20 c	1.87 cd	2.11 cd	1.06 cd	1.11 e	2.29 de	1.61 de
NCEBR6	0.88 bc	0.15 de	0.31 f	0.18 fg	0.99 cd	1.47 c	1.73 d	2.48 c	1.24 cd	1.78 de	2.97 de	1.37 de
T5020	0.96 bc	0.10 ef	0.93 bc	0.23 ef	0.24 d	2.19 bc	2.05 cd	1.27 de	1.87 b-d	2.51 de	6.40 bc	2.02 de
ASVEG20	1.32 b	0.39 a	1.01 b	0.35 cd	2.89 a	2.32 bc	4.35 b	3.79 b	2.65 b	2.19 de	5.01 cd	2.97 cd
Savior	0.59 c	0.26 b	0.43 ef	0.16 fg	1.20 b-d	1.63 bc	2.99 b-d	2.00 cd	1.09 cd	1.11 e	2.75 de	1.43 de
CLN2498D	1.07 bc	0.17 d	0.85 b-e	0.63 a	2.21 a-c	1.76 bc	3.00 b-d	1.22 de	2.17 bc	2.85 d	5.31 cd	2.28 de
UC204A	1.08 bc	0.10 ef	0.47 d-f	0.16 fg	2.70 a	1.67 bc	2.40 cd	2.43 c	1.22 cd	1.28 de	2.63 de	1.94 de
LSD (P=0.05)	0.65	0.06	0.43	0.10	1.22	1.31	1.42	1.06	1.29	1.68	3.25	1.79
Mean	1.24	0.20	0.83	0.30	1.77	2.67	3.45	2.45	2.24	3.65	7.26	3.55
Entry mean square	**	**	**	**	**	**	**	**	**	**	**	**

**=significant at P<0.01; ns=not significant

Table 5. AVRDC dual-purpose tomato lines evaluated for yield and fruit traits in PYT1 (December-April 2015) and/or PYT2 (March-June 2015) in Taiwan.

Entry	Genes affecting carotenoids		Marketable yield (t/ha)		Color (a/b)[1]		Vitamin C (mg/100 g FW)		Beta-carotene (mg/100 g FW)		Lycopene (mg/100 g FW)	
	high pigment (hp-1)	*crimson* (og^c)	PYT1	PYT2	PYT1	PYT2	PYT1	PYT2	PYT1	PYT2	PYT1	PYT2
Tanya	–	–	73	4	1.97	1.80	14.3	19.2	0.37	0.28	8.03	8.00
UC204A	–	–	87	10	1.81	1.63	18.2	23.1	0.36	0.40	7.49	7.28
T5020	+	+	55	4	2.14	1.65	20.6	27.6	0.56	0.53	8.67	8.37
CLN3682C	–	+	104	26	2.23	2.07	15.4	18.7	0.15	0.05	7.69	8.13
CLN3682A	–	+	89	22	2.07	1.89	19.6	20.2	0.23	0.19	7.89	7.30
CLN3682D	–	+	102	30	2.09	2.09	17.0	19.5	0.16	0.16	6.92	7.51
CLN3552B	–	+	133	21	2.18	2.01	19.4	24.0	0.59	0.34	7.18	9.85
CLN3552F	–	+	41	19	2.50	2.23	18.9	17.8	0.14	0.10	7.43	6.57
CLN3669A	+	–	130	24	1.84	1.86	33.4	42.7	0.86	0.62	7.99	9.04
CLN3670B	+	–	117	22	1.88	1.63	23.1	27.4	0.62	0.52	7.80	7.97
CLN3670F	+	–	127	19	1.72	1.77	23.3	25.9	0.77	0.45	7.83	8.27
Mean			95	18	2.02	1.87	20.1	24.2	0.43	0.33	7.55	8.02
Entry MS			**	**	**	**	**	**	**	**	ns	ns
LSD (0.05)			27	6	0.19	0.15	2.2	3.9	0.21	0.14	-	-
CV			13.0	15.6	4.28	3.70	12.1	7.3	22.6	19.05	18.3	24.45

**=significant at P<0.01; ns=not significant
[1]Values for a and b were measured with a chromometer using a red standard surface. Immature green tomatoes have a/b ratio less than 0. The a/b ratio increases to zero and above as the fruits ripen toward a dark red. Values > 2.0 have superior color.
+, – indicates presence or absence, respectively, of the allele

References

Bino RJ, de Vos CHR, Lieberman M, Hall RD, Bovy A, Jonker HH, Tikunov Y, Lommen A and Leven I (2005). The light-hyperresponsive *high pigment-2^{dg}* mutation of tomato: alterations in the fruit metabolome. New Phytologist 166: 427-438.

Bravo L (1998). Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutr. Rev. 56: 317-333.

FAO 2013. http://faostat3.fao.org/browse/rankings/commodities_by_regions/E

Hanson PM, Schafleitner R, Huang SM, Tan CW, Ledesma D and Yang RY (2014). Characterization and mapping of a QTL derived from *Solanum habrochaites* associated with elevated rutin content (quercetin-3-rutinoside) in tomato. Euphytica 200: 441-454.

Hanson PM, Yang RY, Wu J, TChen J, Ledesma D, Tsou SCS and Lee TC (2004). Variation for antioxidant activity and antioxidants in tomato. Amer J Soc Hort Sci 129: 704-711

Jones CM, Mes P, Myers JR (2003). Characterization and inheritance of the Anthocyanin fruit (*Aft*) tomato. Hered J. 94: 449-456.

Keatinge JDH, Yang RY, d'A Hughes J, Easdown WJ, and Holmer R (2011) The importance of vegetables in ensuring both food and nutritional security in attainment of the Millennium Development Goals. Food Sec 3: 491-501

Konsler TR (1973). Three mutants appearing in 'Manipal' tomato. HortScience 8: 331–333.

Leiva Brondo M, Valcárcel M, Cortés-Olmos C, Roselló S, Cebolla Cornejo and Nuez F (2012). Exploring alternative germplasm for the development of stable high vitamin C content in tomato varieties. Scientia Horticul. 133: 84-88.

Levin I, Frankel P, Gilboa N, Tanny S and Lalazar A (2003). The tomato *dark green* mutation is a novel allele of the tomato homolog of the *DEETIOLATED1* gene. Theor Appl Genet 106: 454-460.

Muthayya S, Rah JH, Sugimoto JD, Roos FF, Kraemer K and Black RE (2013). The global hidden hunger indices and maps: an advocacy tool for action. PLOS One 8: e67860

Ronen G, Carmel Goren L, Zamir D and Hirschberg J (2000). An alternative pathway to β-carotene formation in plant chromoplasts discovered by map-based cloning of *Beta* and *old-gold* color mutations in tomato. Proc Nat Acad Sci 97: 111-2-1107.

Ross JA and Kasum CM (2002). Dietary flavonoids: bioavailability, metabolic effects, and safety. Annu. Rev. Nutr. 22: 19-34.

Sapir M, Oren Shamir M, Ovadia R, Reuveni M, Evenor D, Tadmor Y, Nahon S, Shlomo H, Chen L, Meir A, Levin I (2008). Molecular aspects of anthocyanin fruit tomato in relation to *high pigment-1*. J. Hered 99: 292-303.

Stommel JR (2007). Genetic enhancement of tomato fruit nutritive value. In Razdan, M.K, Mattoo, A.K., (Eds.) Genetic Improvement of Solanaceous Crops Vol 2: Tomato. Science Publishers, Enfield NH, USA. pp. 193-238.

Teucher B, Olivares M and Cori H (2004). Enhancers of iron absorption: ascorbic acid and other organic acids. Int. J. Vitam. Nutr. Res. 74: 403-419.

Wann EV (1997). Tomato germplasm lines T4065, T4099, T5019, and T5020 with unique genotypes that enhance fruit quality. HortScience 32: 747–748.

Yang RY, Hanson PM, Lumpkin TA (2007). Better health through horticulture - AVRDC's approach to improved nutrition of the poor. Acta Hort. 744:71-77.

Yen HC, Shelton BA, Howard LR, Lee S, Vrebalov J, Giovannoni JJ (1997). The tomato *high-pigment* (*hp*) locus maps to chromosome 2 and influences plastome copy number and fruit quality. Theor Appl Genet 95: 1069-1079.