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Carotenoids in mature green and ripe red fruits of tomato (*Solanum lycopersicum* L.) grown under different levels of irrigation

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Abstract: The effect of water deficit on concentrations of carotenoids was investigated in ripening tomatoes using HPLC-PDA. Fifteen different tomato cultivars were grown under three levels of water supply and unripe and fully-ripe fruits were harvested at different stages. Water deficit significantly affected several morphological and fruit yield-related parameters. In unripe tomato fruits, the relative concentrations of xanthophyll cycle carotenoids, e.g., violaxanthin and antheraxanthin, were significantly increased at the expense of β-carotene upon limiting the water supply. In ripe fruits, nutritionally-relevant lycopene, β-carotene and lutein levels were broadly independent of water deficit when considering all 15 cultivars, although significant variations were observed among fruits from different genotypes. Our study highlights the importance of careful genotype selection for the production of tomatoes rich in nutritionally-relevant compounds like lycopene and β-carotene.

Key words: antioxidants; β-carotene; lycopene; lutein; vitamin A

INTRODUCTION

Facing a rapidly growing world population, an increased production of high-quality foods with reduced inputs is urgently needed, but also highly challenging due to global environmental changes. The current breeding focus is often on traits potentially increasing yield, but the continuous selection of elite germplasm has led to a narrowing of the available genetic diversity, particularly for some crops like soybeans and peanuts [1]. With regard to tomato (*Solanum lycopersicum* L. formerly *Lycopersicon esculentum* Mill.), the availability of a large germplasm including numerous wild species served to be useful for introgressing resistances against many diseases as well as tolerances against soil salinity and drought [2]. Recently, Blanca et al. [3] provided a comprehensive overview and analysis of genomic variations in over a thousand tomato accessions, including *Solanum pimpinellifolium* L., its closest wild relative. The importance of tomato as a crop may be highlighted by its worldwide production, which has increased from 116.5 Mio metric tons in 2002 to approximately 161.8 Mio tons in 2012. The largest producers are currently China (ca. 50.0 Mio tons), India (17.5 Mio tons) and the United States (13.2 Mio tons) [4]. Germplasm improvement by breeding may become particularly important for tomato world production, since several important production regions, such as Mediterranean countries like Italy, Spain, Egypt, and Turkey, increasingly suffer from periods of drought [5]. Furthermore, the state of California in the USA has been struck by a severe drought from 2012-2015, possibly extending into 2016 and dramatically fueling the unquestionable water crisis in this region [6].

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Besides novel irrigation strategies, the availability of drought-tolerant genotypes may help to intensify or, at least, maintain the current tomato production in such arid regions. Previous studies on plants exposed to water deficit and other stress factors have reported that growth rates are often suppressed, but fruit quality was often enhanced due to increased sugar and acid levels [7-9]. In contrast, the pigment content of red ripe tomato fruits, i.e. mostly the carotenoids lycopene and β-carotene, were shown to be diminished by abiotic stress factors in several studies [10-12]. Contrary findings have also been described [13,14] and may highlight the inconsistency of these results. Nevertheless, it is widely accepted that carotenoids play an important role in the plant defense against abiotic stress due to their potent antioxidant properties and their function in the xanthophyll cycle [15]. The latter protects the photosynthetic apparatus from excessive oxidative stress, occurring in plants exposed to chilling, heat, senescence or salinity stress [16]. To date, the effect of water deficit on carotenoids involved in the xanthophyll cycle of plants has not been studied much.

Since the abovementioned carotenoids also represent potentially health-promoting micronutrients, high levels are desirable quality attributes from both horticultural and nutritional standpoint. Therefore, the present study aimed at investigating the influence of three different water supply treatments on the carotenoid patterns of fruits from 15 tomato cultivars. While several morphological features are also reported, a particular focus was on the xanthophyll cycle carotenoids, violaxanthin, antheraxanthin, and zeaxanthin, in unripe but mature green fruits. In addition, the fruits at a fully red-ripe stage mostly containing β-carotene and lycopene should be also examined in detail.

MATERIALS AND METHODS

Plant material and reagents

The seeds of 15 tomato (*Lycopersicon esculentum* Mill.) cultivars were kindly provided by the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany. Cultivar names, their botanical name and their geographical origins are shown in Table 1. All experiments were carried out from September 2014 to March 2015 in greenhouses of the Suez Canal University.

The plants were prepared by sowing the seeds in a nursery (beginning of September 2014) in a special agricultural flinty loam, with 40 holes (10-cm deep) per cultivar filled with peat moss and vermiculite at a ratio of 1:1. After 28 days (mid October 2015), the

Table 1. Commercial Name, IPK Accession Code, Botanical Name, and Origin of the 15 Studied Tomato Genotypes

Cultivar Name	IPK Accession Code*	Botanical Name#	Origin
Anna Aasa	LYC4112	*Lycopersicon esculentum* Mill. convar. *infiniens* Lehm. var. *flammatum*	Russia
Australische Fruehe	LYC192	*Lycopersicon esculentum* Mill. convar. *infiniens* var. *commune* L.H.Bailey	Australia
Australische Rosen	LYC3152	*Lycopersicon esculentum* Mill	Australia
California	LYC2987	*Lycopersicon esculentum* Mill.	USA
California Red Cherry	LYC4113	*Lycopersicon esculentum* Mill. convar. *parvibaccatum* Lehm. var. *cerasiforme* (Dunal) Alef	USA
Dedication	LYC3912	*Lycopersicon esculentum* Mill.	Russia
Florida MH-1	LYC2937	*Lycopersicon esculentum* Mill. convar. *fruticosum* Lehm. var. *finiens* Lehm	USA
Gelbfruechtig	LYC2019	*Lycopersicon esculentum* Mill. convar. *infiniens* Lehm. var. *cordiforme*	Germany
Petomech	LYC4242	*Lycopersicon esculentum* Mill. convar. *fruticosum* Lehm. var. *speciosum* Lehm	Italy
Sandpoint	LYC2493	*Lycopersicon esculentum* Mill. convar. *fruticosum* Lehm. var. *pygmaeum* Lehm.	USA
Sankt Ignatius	LYC4079	*Lycopersicon esculentum* Mill. convar. *infiniens* Lehm. var. *commune*	Italy
Sintesti	LYC1346	*Lycopersicon esculentum* Mill. convar. *esculentum* var. *esculentum*	Romania
Tiganesti	LYC359	*Lycopersicon esculentum* Mill. convar. *infiniens* Lehm. var. *flammatum* Lehm	Romania
Vencal	LYC2431	*Lycopersicon esculentum* Mill. convar. *fruticosum* Lehm. var. *speciosum* Lehm	Netherlands
Zevat	LYC2432	*Lycopersicon esculentum* Mill. convar. *fruticosum* Lehm. var. *speciosum* Lehm	Netherlands

*: Accession code of the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK)
#: The botanical name *Lycopersicon esculentum* is used in the database of IPK and, thus, being used here.

tomato plants were transplanted to the greenhouse, i.e., 27 plants per cultivar were grown in pots of 30 cm diameter (total volume 10 L) filled with a mixture of peat moss and quartz sand at a ratio 1:3. The pots were set up in rows and a split-plot combination of treatments was performed following a randomized complete block design (RCBD) with three replicates. Three levels of water supply were applied to the main plots, and tomato cultivars were assigned to subplots. Each subplot consisted of three pots with one plant each. In the control experiment (T1, no water deficit), plants were supplied with 600 mL of water, twice a week during the first month in the greenhouse and subsequently every other day until the end of the experiment. After two weeks with full water supply (600 mL per watering), two levels of water deficit were induced by reducing the water amount to 400 and 200 mL (T2 and T3, respectively), following the same schedule as described above. Upon starting the water deficit treatment, all genotypes were still in the vegetative stage before flowering, having reached a height of 30-40 cm with at least the 8th leaf in the main stem unfolded (BBCH Code for solanaceous plants according to Feller et al. [17] was >108, growth stage 1). The moisture contents of the freshly watered soils, as measured by gravimetric determination after drying for 24 h at 100°C, were 19.6, 11.1, and 5.9% (v/v) for T1, T2, and T3, respectively. After 110-130 days from transplanting, mature green fruits were harvested and prepared for analyses. After 130-150 days from transplanting, ripe red fruits were harvested and the plants' root length (RL), shoot length (SL), root fresh weight (RFW), shoot fresh weight (SFW), root dry weight (RDW), shoot dry weight (SDW), shoot/root length (S/RL), root/shoot dry weight (R/SDW), number of leaves (NL), leaf fresh weight (LFW), leaf dry weight (LDW), number of branches (NB), number of inflorescences (NI), number of fruits (NF), fruit fresh weight (FFW), and yield (Y) were determined. All ripe fruits were stored at 25°C for up to 2 weeks before analysis.

Extraction of carotenoids

Tomato fruits were both frozen and ground with liquid nitrogen prior to extraction. All procedures described below were carried out under dim light. An aliquot of 200-250 mg of ground tomato fruit sample was combined with 250 mg of calcium carbonate ($CaCO_3$) and extracted with 2 mL of extraction solvent (acetone with 1 g/L butylated hydroxytoluene, BHT) using the ultrasonic homogenizer Sonopuls HD 3100 with an MS 72 microtip (Bandelin electronic, Berlin, Germany). After centrifugation, the extraction solvent was collected and extraction was repeated 2-4 times until the solid residue was colorless. After drying with Na_2SO_4, the combined organic extracts were evaporated to dryness under a gentle nitrogen stream and stored at -80°C until high-performance liquid chromatography (HPLC) analysis. Prior to HPLC, the dried extracts were redissolved in 250 µL of methyl *tert*-butyl ether (MTBE). Subsequently, the samples were briefly sonicated in a water bath to enhance dissolution, followed by the addition of 250 µL of methanol and membrane-filtration (PTFE, 0.45 µm) into amber HPLC vials.

HPLC-PDA analyses

For HPLC analyses, a Waters separation module 2695 (Waters, Milford, MA, USA) with a Waters 2996 photodiode array detector (PDA) was equipped with a YMC C30 reversed phase column (150×3.0 mm i.d., 5 µm particle size, YMC Europe, Dinslaken, Germany) protected by a YMC C30 guard column of the same material. As previously used by Kopec et al. [18], HPLC solvents consisted of methanol/water (80:20, v/v, eluent A) and methanol/MTBE/water (20:78:2, v/v/v, eluent B), both containing 0.4 g/L of ammonium acetate. The elution gradient was as follows: from 100% to 0% A for 24 min, isocratic at 0% A for 1 min, from 0% to 100% A in 1 min, and isocratic at 100% A for 2 min. Total run time was 28 min at a flow rate of 1.2 mL/min and a column temperature of 35°C. Injection volume was 20 µL. Carotenoids were monitored at 450 nm.

Individual carotenoids were identified by comparing retention times and UV/Vis absorption spectra to those of authentic standards. Standards of (*all-E*)-α-carotene, (*all-E*)-antheraxanthin, (*all-E*)-β-carotene, (*all-E*)-lutein, (*all-E*)-mutatoxanthin (mixture of two isomers), (*all-E*)-neoxanthin, (*all-E*)-violaxanthin, and (*all-E*)-zeaxanthin were obtained from CaroteNature (Ostermundingen, Switzerland). Chlorophylls *a* and *b* were from Sigma-Aldrich (Steinheim, Germany).

β-carotene and violaxanthin (*Z*)-isomers were identified according to their D_B/D_{II} ratios, which were obtained as described by Britton [22] and then compared to those found in literature [19-21].

Prior to quantitation by HPLC-PDA, concentrations of stock solutions of the authentic standards mentioned above were verified spectrophotometrically. The specific absorption coefficients reported by Britton [22] were used to establish linear calibration curves. Linear calibration curves of authentic standards were used, except for the quantitation of the detected violaxanthin (*Z*)-isomer and the β-carotene (*Z*)-isomer, where the violaxanthin and β-carotene calibrations were used, respectively.

Statistical analyses

All analyses were carried out in duplicate. Analysis of variance (ANOVA), Tukey's HSD (honestly significant difference), and Duncan's test were used for determination of significantly different means ($P<0.05$) using the procedure GLM of SAS 9.1 (SAS Institute, Cary, USA). All values are reported as means±standard deviation. Correlations (Pearson's correlation coefficient) were calculated using the software "R" version 2.10.0 (R Foundation for Statistical Computing, 2012, available at www.r-project.org).

RESULTS AND DISCUSSION

Influence of water deficit on morphological growth parameters

In our study, 15 tomato cultivars were grown under normal water supply, i.e. providing 600 mL per watering and plant (T1), and under two levels of water deficit (400 mL (T2) or 200 mL (T3) per watering and plant). Fig. 1 shows the cultivar 'Vencal' grown under T1, T2 and T3.

The shoot length of five cultivars was significantly affected when grown under intermediate water deficit (T2) as shown in Table 2, while differences were insignificant at T2 for most cultivars. The severe water deficit (T3) had a more pronounced impact on shoot length, leading to significantly smaller plants for most cultivars, except for the cultivars California,

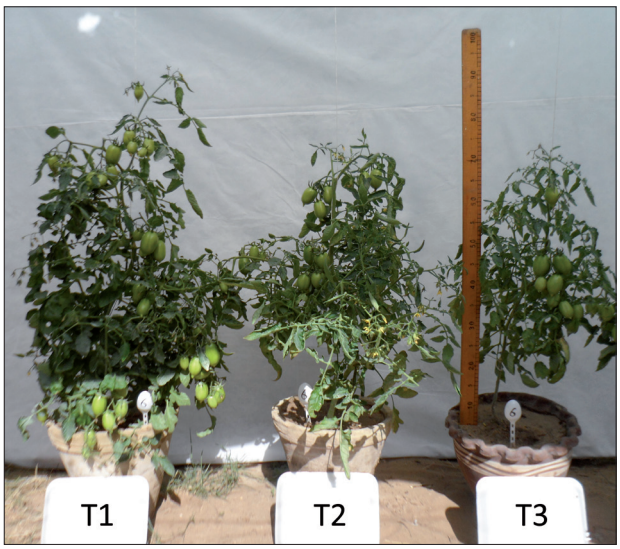

Fig. 1. The cultivar 'Vencal' grown under different levels of water deficit (T1, T2 and T3).

Florida MH-1, Petomech, and California Red Cherry ($P<0.05$; Table 2). By analogy, water deficit at level T3 resulted in significantly smaller fruits for most cultivars (except for California, Sandpoint, Anna Aasa, and Vencal). Although being inconsistent for a few cultivars, a weak correlation between the mean reduction in shoot length and the mean reduction in fruit weight was observed ($r=0.59$). Similar findings were previously obtained by Mitchell et al. [8] and De Pascale et al. [10] who reported a negative effect of water deficit on tomato plant growth and fruit yield. In contrast, Atkinson et al. [12] found that a comparably mild water deficit did not influence individual fruit weights. For inducing the water deficit, the authors supplied 80% of the water received by their well-watered control plants, while the water deficit applied in our study was substantially more rigid, providing only 66% (T2) and 33% (T3) of the water supply of the well-watered control (T1). Plant growth and yield parameters such as fruit weight and plant height were apparently only affected when a certain level of water deficit was reached.

Influence of water deficit on carotenoids in mature green and ripe red fruits

In our study, carotenoids were analyzed in mature green and ripe red tomato fruits. In agreement with previous reports, the carotenoid profile drastically changed when fruit color turned from green to red

Table 2. Morphological Traits of 15 Tomato Cultivars Grown Under Normal Water Supply (T1) and Different Levels of Water Deficit (T2, T3).

	Shoot Length in [cm / plant]				Fruit Weight in [g / fruit]				Number of Fruits per Plant		
Cultivar Name	600 mL per watering (T1)	400 mL per watering (T2)	200 mL per watering (T3)	mean reduction from T1 to T3 [%]	600 mL per watering (T1)	400 mL per watering (T2)	200 mL per watering (T3)	mean reduction from T1 to T3 [%]	600 mL per watering (T1)	400 mL per watering (T2)	200 mL per watering (T3)
California	70 ± 5 a	65 ± 5 a	76 ± 18 a	-8	33 ± 4 a	23 ± 1 b	28 ± 1 ab	14	16 ± 2 a	14 ± 0 a	13 ± 1 a
Florida MH-1	63 ± 3 a	53 ± 8 a	58 ± 1 a	8	51 ± 3 a	50 ± 4 ab	44 ± 2 b	14	16 ± 1 a	15 ± 1 ab	14 ± 1 b
Petomech	67 ± 2 a	68 ± 7 a	59 ± 2 a	12	50 ± 13 a	32 ± 3 b	33 ± 4 b	35	15 ± 1 a	15 ± 1 a	15 ± 1 a
Gelbfruechtig	98 ± 2 a	93 ± 5 b	86 ± 1 c	12	83 ± 11 a	66 ± 1 b	58 ± 0 b	29	16 ± 1 a	14 ± 1 a	15 ± 2 a
California Red Cherry	91 ± 10 a	85 ± 5 a	79 ± 2 a	13	12 ± 1 a	13 ± 1 a	9 ± 0 b	24	16 ± 0 a	15 ± 1 a	15 ± 1 a
Sandpoint	76 ± 1 a	68 ± 3 b	61 ± 4 b	20	19 ± 2 a	17 ± 1 a	18 ± 1 a	6	17 ± 1 a	15 ± 0 a	16 ± 1 a
Tiganesti	75 ± 6 a	70 ± 6 a	56 ± 1 b	25	57 ± 8 a	45 ± 2 b	36 ± 0 b	6	16 ± 0 a	14 ± 1 a	15 ± 1 a
Anna Aasa	93 ± 10 a	77 ± 5 b	73 ± 5 b	26	16 ± 2 a	13 ± 0 b	15 ± 0 ab	37	16 ± 1 a	15 ± 1 a	14 ± 2 a
Australische Rosen	82 ± 7 a	77 ± 1 a	60 ± 2 b	26	17 ± 4 a	7 ± 0 b	9 ± 0 b	46	15 ± 1 a	14 ± 1 a	14 ± 2 a
Australische Fruehe	85 ± 7 a	81 ± 6 a	63 ± 3 b	26	50 ± 10 a	33 ± 1 b	26 ± 1 b	49	15 ± 1 a	14 ± 3 a	15 ± 1 a
Sintesti	79 ± 1 a	72 ± 2 b	58 ± 3 c	27	94 ± 13 a	79 ± 2 ab	59 ± 5 b	62	15 ± 1 a	15 ± 1 a	14 ± 1 a
Sankt Ignatius	72 ± 4 a	67 ± 3 a	53 ± 1 b	27	74 ± 21 a	37 ± 3 b	28 ± 2 b	37	13 ± 1 a	13 ± 1 a	12 ± 1 a
Vencal	80 ± 6 a	64 ± 4 b	48 ± 2 c	40	23 ± 11 a	20 ± 6 a	8 ± 3 a	65	15 ± 1 a	13 ± 1 ab	11 ± 1 b
Zevat	68 ± 10 a	66 ± 6 a	40 ± 1 b	40	26 ± 9 a	14 ± 1 ab	9 ± 1 b	63	15 ± 1 a	13 ± 2 a	11 ± 2 a
Dedication	66 ± 3 a	61 ± 5 a	35 ± 6 b	47	20 ± 3 a	15 ± 0 b	14 ± 1 b	27	16 ± 0 a	15 ± 1 ab	13 ± 1 b

Different letters indicate significant differences of means within a row of a parameter ($P < 0.05$).

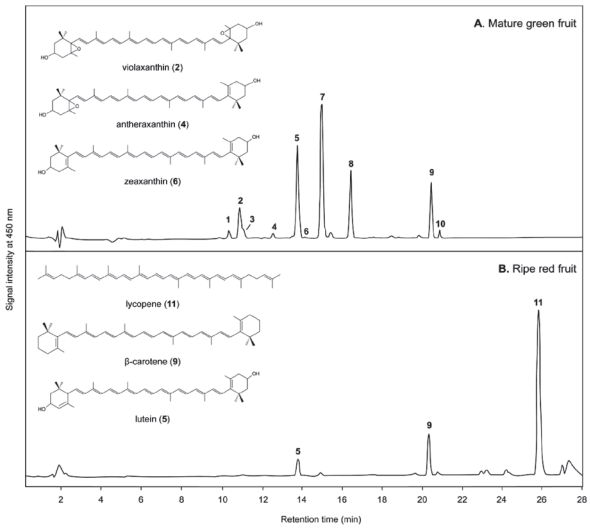

Fig. 2. HPLC chromatograms of carotenoids from a mature green fruit (**A**) and ripe red fruit (**B**) monitored at 450 nm. Peak assignment: 1 – violaxanthin (Z)-isomer; 2 – (*all-E*)-violaxanthin, 3 – neoxanthin; 4 – antheraxanthin; 5 – lutein; 6 – zeaxanthin; 7 – chlorophyll *b*; 8 – chlorophyll *a*; 9 – (*all-E*)-β-carotene; 10 – β-carotene (*Z*)-isomer; 11 – lycopene.

(Fig. 2A and B). The predominant carotenoids present in green tomato fruits were lutein (39-49% of total carotenoids), violaxanthin and neoxanthin (20-29%) and β-carotene (6-30%), while ripe red tomatoes contained mostly lycopene (59-91% of total carotenoids), β-carotene (6-32%) and lutein (3-18%). This change in the carotenoid profile was previously observed during tomato fruit ripening [23-26]. It is noteworthy that the carotenoid profile found in green tomato fruits is typical for those of green tissues in higher plants, being specific for photosynthetically active chloroplasts [27]. During color-break in the course of tomato ripening, chloroplast-specific plastid structures like grana and stroma thylakoids disintegrate and large crystalloid chromoplasts appear due to the massive accumulation of lycopene [28,29].

Regarding mature green fruits, significant variations in total carotenoid concentrations were detected among the different cultivars when well-watered (T1). The cultivars standing out by their high total xanthophyll concentrations in mature green fruits were cvs. Dedication (10.7 μg/g FW), Florida MH-1 (7.8 μg/g FW), Sintesti (7.6 μg/g FW) and Anna Aasa (7.1 μg/g FW), as shown in Table 3.

Table 3. Concentrations of Total Xanthophylls (Antheraxanthin, Lutein, Neoxanthin, Violaxanthin, Zeaxanthin), Total Carotenes (β-Carotene, α-Carotene), and Total Carotenoids (Sum of Total Xanthophylls and Total Carotenes) in Mature Green Fruits of 15 Tomato Cultivars Grown under Full Water Supply (T1) and at Different Levels of Water Deficit (T2, T3).

Cultivar Name	Total Xanthophylls [µg/g FW]			Total Carotenes [µg/g FW]			Total Carotenoids [µg/g FW]		
	600 mL per watering (T1)	400 mL per watering (T2)	200 mL per watering (T3)	600 mL per watering (T1)	400 mL per watering (T2)	200 mL per watering (T3)	600 mL per watering (T1)	400 mL per watering (T2)	200 mL per watering (T3)
Dedication	10.7 ± 1.4 a	5.0 ± 0.4 b	6.4 ± 1.0 ab	3.0 ± 0.5 a	1.8 ± 0.0 a	1.9 ± 0.5 a	13.7 ± 1.9 a	6.8 ± 0.4 b	8.4 ± 1.5 ab
Anna Aasa	7.1 ± 0.3 a	8.6 ± 1.2 a	6.9 ±0.0 a	2.1 ± 0.1 a	2.6 ± 0.3 a	0.4 ± 0.0 b	9.2 ± 0.4 a	11.3 ± 1.5 a	7.5 ± 0.1 a
Gelbfruechtig	5.3 ± 0.6 b	2.9 ± 0.3 b	9.6 ± 0.6 a	1.4 ± 0.1 b	0.9 ± 0.1 b	2.9 ± 0.2 a	6.8 ± 0.5 b	3.8 ± 0.4 b	12.6 ± 0.8 a
Australische Fruehe	5.9 ± 0.9 a	6.4 ± 2.4 a	5.2 ± 0.9 a	1.8 ± 0.3 a	1.9 ± 0.5 a	0.9 ± 0.3 a	7.6 ± 1.2 a	8.3 ± 2.9 a	6.2 ± 1.2 a
Australische Rosen	6.2 ± 0.4 a	4.4 ± 0.5 a	n.a.	2.8 ± 0.9 a	1.5 ± 0.1 a	n.a.	9.0 ± 0.5 a	5.9 ± 0.7 a	n.a.
Vencal	4.5 ± 0.9 a	2.9 ± 1.4 a	4.0 ± 0.3 a	1.5 ± 0.3 a	1.0 ± 0.4 a	0.5 ± 0.1 a	6.0 ± 1.2 a	3.9 ± 1.9 a	4.5 ± 0.4 a
Zevat	4.9 ± 0.8 a	5.7 ± 1.3 a	5.4 ± 0.1 a	1.5 ± 0.3 a	1.7 ± 0.4 a	1.4 ± 0.0 a	6.4 ± 1.1 a	7.5 ± 1.7 a	6.8 ± 0.0 a
Petomech	6.0 ± 0.2 a	7.1 ± 0.3 a	n.a.	2.0 ± 0.1 a	2.2 ± 0.2 a	n.a.	7.9 ± 0.2 a	9.5 ± 0.5 a	n.a.
Sankt Ignatius	5.3 ± 0.3 b	8.5 ± 0.1 a	n.a.	1.8 ± 0.1 a	2.7 ± 0.1 a	n.a.	7.1 ± 0.5 b	11.3 ± 0.3 a	n.a.
Sintesti	7.6 ± 0.3 a	8.9 ± 0.3 a	5.1 ± 0.3 b	2.4 ± 0.1 a	2.3 ± 0.1 a	0.8 ± 0.3 b	10.0 ± 0.4 a	10.7 ± 0.4 a	6.0 ± 0.1 b
Tiganesti	5.4 ± 2.6 a	7.7 ± 0.3 a	5.8 ± 0.3 a	1.7 ± 0.7 a	2.0 ± 0.0 a	1.0 ± 0.1 a	7.1 ± 3.3 a	9.8 ± 0.3 a	n.a.
Florida MH-1	7.8 ± 1.9 a	6.3 ± 0.6 a	2.8 ± 0.4 a	2.1 ± 0.4 a	1.8 ± 0.2 ab	0.7 ± 0.1 b	9.9 ± 2.3 a	8.1 ± 0.8 a	6.9 ± 0.2 a
Sandpoint	2.9 ± 0.4 b	5.2 ± 0.8 b	9.1 ± 0.9 a	9.7 ± 1.1 a	4.8 ± 0.7 b	1.7 ± 0.5 c	12.6 ± 1.5 a	11.4 ± 1.9 a	10.8 ± 0.5 b
California	4.2 ± 0.4 a	8.5 ± 2.2 a	6.2 ± 0.1 a	1.5 ± 0.2 a	2.7 ± 0.5 a	1.7 ±0.0 a	5.6 ± 0.5 a	11.2 ± 2.7 a	7.9 ± 0.4 a
California Red Cherry	4.6 ± 0.2 b	6.5 ± 0.9 a	10.1 ± 3.0 a	1.3 ± 0.0 a	1.9 ± 0.1 a	2.2 ± 0.8 a	6.0 ± 0.2 b	8.5 ± 1.0 ab	12.3 ± 0.1 a

n.a.: not available.
Different letters indicate significant differences of means within a row of a cultivar and parameter ($P < 0.05$).

The lowest concentrations were found in cvs. Sandpoint (2.9 µg/g FW), California (4.2 µg/g FW) and California Red Cherry (4.6 µg/g FW). Similar rankings were found when considering total carotene and total carotenoid concentrations, except for cv. Sandpoint, which had ripened exceptionally early and, thus, had accumulated a certain amount of lycopene when harvested at the date when other cultivars were harvested at mature green stages. Although not always reaching statistical significance (Table 3), fruits of the abovementioned cultivars with low carotenoid concentrations in T1 (600 mL per watering) revealed higher carotenoid concentrations under water deficits T2 and T3, whereas an inverse relationship may be suggested for cultivars with high carotenoid concentrations in T1 (Table 2). Total xanthophylls, total carotenes and total carotenoids did not correlate with morphological parameters such as shoot length, fruit size or number of fruits.

While the influence of water deficit on total carotenoid concentrations was inconsistent and remained unclear, concentrations of several carotenoids and their variances were significantly influenced by water deficit treatments. The concentration ratios of violaxanthin, neoxanthin, antheraxanthin, lutein and β-carotene shown in Fig. 3 were previously reported to be typical for unstressed green plant tissues, commonly ranging between 20-25% for β-carotene, 40-45% for lutein, 10-15% for violaxanthin and 10-15% for neoxanthin [30]. In contrast, the mean relative concentrations of the xanthophylls violaxanthin/neoxanthin, antheraxanthin and lutein were significantly higher ($P<0.05$) when the plants had been stressed by maximum water deficit (T3). Xanthophyll concentrations apparently increased at the expense of β-carotene (Fig. 3), which was present at a significantly lower ($P<0.05$) share of ca. 15-20% of total carotenoids (25-75th percentiles). Furthermore, higher variances were observed when water stress was increased (Fig. 3). Similar effects have been previously observed when exposing tomatoes to excessive illumination, most likely being associated with the photoprotective role of violaxanthin, antheraxanthin and zeaxanthin in the course of the xanthophyll cycle [31]. Since the illumination conditions were identical for all treatments (grown

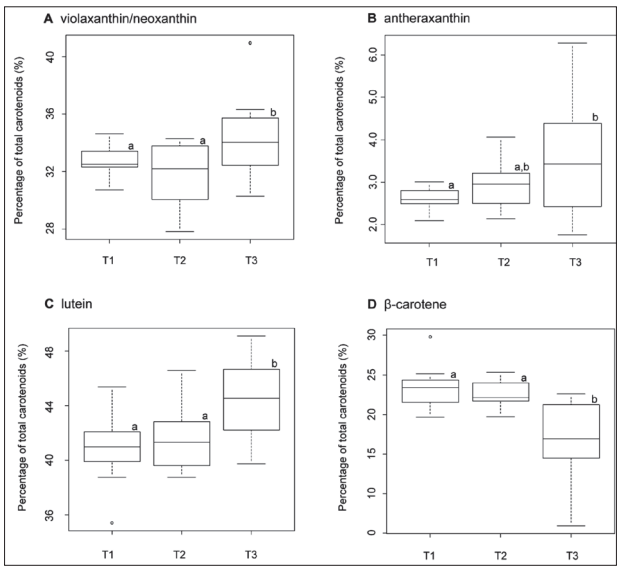

Fig. 3. Individual carotenoids of mature green tomato fruit of all cultivars as affected by the different levels of water deficit (T1-T3).

at the same location), our findings may indicate that stressed tomato plants had increased the concentrations of specific "xanthophyll cycle" carotenoids (such as violaxanthin and antheraxanthin) due to the water deficit, in order to cope with the increased oxidative stress when exposed to suboptimal growth conditions. The function of the xanthophyll cycle is believed to be mostly related to preventing oxidative damage of membranes [32]. In the course of the xanthophyll cycle, violaxanthin is enzymatically de-epoxidized to antheraxanthin and then to zeaxanthin. Under low light conditions or darkness, zeaxanthin is epoxidized to regenerate antheraxanthin and violaxanthin [33]. Notably, in our study the levels of zeaxanthin were much lower than expected, mostly being non-quantifiable. Most likely, initially present stress-induced zeaxanthin might have been rapidly epoxidized back to antheraxanthin and violaxanthin. Nevertheless, the notably higher concentration of these "xanthophyll cycle" carotenoids clearly indicated an effect of water deficit on the plant's stress response. However, a correlation of the significantly increased concentrations of xanthophyll cycle carotenoids with an increased ability of the tomato plants to cope with water deficit (e.g., as expressed by unaffected morphological parameters) should not be deduced from our study.

A correlation between the total carotenoid concentrations in mature green fruits and those of ripe red fruits was not observed. However, total carotenoid levels in red ripe fruits (on average 33.0±12.2 μg/g FW) were approximately 4-fold higher than those in green fruits (8.2±2.9 μg/g FW).

Regarding carotenoids of ripe red fruits, the cultivars Florida MH-1, Australische Fruehe, and California produced the fruits richest in total carotenoids when grown under T1, i.e. with full water supply (52.2, 49.1 and 43.1 μg/g FW, respectively). The lowest carotenoid concentrations were found in fruits of the cultivars Sankt Ignatius, Gelbfruechtig and Dedication (18.0, 24.5, and 26.6 μg/g FW, respectively). The dependence of the total carotenoid and lycopene concentrations on the cultivar is in agreement with a previous study on two tomato cultivars, Corfú and Lunarossa [34]. When comparing total and individual carotenoid concentrations in fruits of T1 with those of T2 and T3, establishing a clear-cut relationship between water deficit and carotenoid concentrations was impossible. The fruits of several cultivars (Sandpoint, Australische Fruehe) contained higher lycopene and total carotenoid concentrations when grown under water deficit (Table 4), while carotenoid concentrations in fruits of other cultivars (Florida MH-1, California) were diminished when the water supply was limited. Such inhomogeneous results were also previously described regarding the effect of water deficit on lycopene concentrations in tomato. In several studies [35-38], lycopene contents in fruits of several cultivars were shown to decrease when water deficit was more severe. In contrast, lycopene contents in two cherry tomato cultivars increased when grown under water deficit conditions [35-38]. The effect might be cultivar-dependent, which may explain the findings of our study, being as inconsistent as previous literature reports. Therefore, further studies on carotenoid concentrations in different cultivars grown in multi-year or multi-site settings might be helpful for elucidating a potential genetic factor. As shown in Fig. 4, water deficit had an impact on the variability of total carotenoids in ripe red fruits, while the effect on total lycopene was less pronounced.

To summarize, water deficit clearly exerted cultivar-dependent effects on the morphological traits of tomato plants, leading to shorter shoot lengths, slightly reduced fruit weights and lower numbers of fruits per plant. Modulating total carotenoid concen-

Table 4. Concentrations of Lutein, β-Carotene, Lycopene, and Total Carotenoids in Ripe Red Fruits of 15 Tomato Cultivars Grown Under Full Water Supply (T1) and at Different Levels of Water Deficit (T2, T3).

Cultivar Name	Lutein [µg/g FW]			β-Carotene [µg/g FW]			Lycopene [µg/g FW]			Total Carotenoids [µg/g FW]		
	600 mL per watering (T1)	400 mL per watering (T2)	200 mL per watering (T3)	600 mL per watering (T1)	400 mL per watering (T2)	200 mL per watering (T3)	600 mL per watering (T1)	400 mL per watering (T2)	200 mL per watering (T3)	600 mL per watering (T1)	400 mL per watering (T2)	200 mL per watering (T3)
Dedication	3.1 ± 0.1 a	4.6 ± 0.4 a	2.6 ± 0.3 a	5.8 ± 0.3 a	3.9 ± 0.3 b	3.8 ± 0.0 b	17.7 ± 2.9 b	0.2 ± 0.5 c	32.6 ± 1.5 a	26.6 ± 3.3 b	8.6 ± 1.2 c	38.9 ± 1.3 a
Anna Aasa	3.3 ± 0.1 a	2.8 ± 0.1 ab	2.1 ± 0.2 b	11.2 ± 0.2 a	9.5 ± 2.9 a	8.0 ± 0.7 a	20.5 ± 0.4 b	23.8 ± 3.7 b	38.2 ± 2.3 a	34.9 ± 0.2 b	36.2 ± 0.7 b	48.3 ± 1.4 a
Gelbfruechtig	2.0 ± 0.3 b	1.9 ± 0.1 b	3.7 ± 0.1 a	4.4 ± 0.8 a	3.7 ± 0.1 a	3.4 ± 0.0 a	18.2 ± 4.9 a	26.2 ± 1.7 a	8.0 ± 0.3 b	24.5 ± 5.9 a	31.8 ± 1.8 a	15.1 ± 0.4 a
Australische Fruehe	2.0 ± 0.0 b	3.8 ± 0.1 a	3.7 ± 0.7 a	7.3 ± 0.0 a	7.3 ± 0.1 a	6.1 ± 0.8 a	33.8 ± 0.8 a	31.5 ± 1.3 a	40.1 ± 9.8 a	43.2 ± 0.8 a	42.6 ± 1.1 a	50.0 ± 11.3 a
Australische Rosen	1.8 ± 0.2 a	1.5 ± 0.1 a	1.4 ± 0.2 a	6.4 ± 0.4 a	5.3 ± 0.4 a	5.3 ± 0.6 a	29.0 ± 1.0 a	36.9 ± 2.4 a	35.0 ± 0.6 a	37.3 ± 0.3 a	43.8 ± 2.8 a	41.7 ± 1.4 a
Vencal	1.9 ± 0.1 a	2.7 ± 0.2 a	2.2 ± 0.2 a	5.9 ± 0.2 a	4.5 ± 0.1 b	2.7 ± 0.2 c	34.2 ± 0.5 a	14.5 ± 0.3 b	12.9 ± 0.7 b	42.0 ± 0.3 a	21.7 ± 0.0 b	17.8 ± 1.1 c
Zevat	1.9 ± 0.2 a	1.3 ± 0.1	1.5 ± 0.3 a	5.0 ± 0.5 a	3.6 ± 0.2 b	3.8 ± 0.6 b	27.6 ± 1.1 a	31.6 ± 1.5 a	27.3 ± 0.4 a	34.6 ± 1.9 a	36.5 ± 1.8 a	32.5 ± 1.3 a
Petomech	1.3 ± 0.1 a	2.2 ± 0.1 a	1.9 ± 0.4 a	4.3 ± 0.2 ab	5.0 ± 0.2 a	3.2 ± 0.3 b	36.4 ± 1.9 a	36.6 ± 1.7 a	15.2 ± 1.8 b	42.0 ± 1.7 a	43.8 ± 2.0 a	20.3 ± 2.5 b
Sankt Ignatius	1.7 ± 0.2 a	2.1 ± 0.2 a	2.0 ± 0.1 a	4.0 ± 0.4 a	4.3 ± 0.2 a	3.2 ± 0.0 a	12.4 ± 1.6 a	16.1 ± 0.4 a	9.7 ± 0.1 b	18.0 ± 2.1 a	22.4 ± 0.8 a	14.9 ± 0.0 a
Sintesti	2.4 ± 0.0 a	2.8 ± 0.3 a	2.6 ± 0.1 a	6.1 ± 0.1 a	5.3 ± 0.3 a	5.0 ± 0.4 a	29.6 ± 0.4 a	26.6 ± 0.7 a	12.4 ± 0.8 b	38.1 ± 0.5 a	34.7 ± 0.1 a	20.0 ± 1.3 b
Tiganesti	2.9 ± 0.2 a	3.0 ± 0.3 a	2.1 ± 0.0 a	6.5 ± 0.3 a	4.6 ± 0.1 b	4.0 ± 0.0 b	26.1 ± 1.6 a	9.0 ± 0.0 b	8.1 ± 0.1 b	35.6 ± 2.1 a	16.6 ± 0.4 b	14.2 ± 0.1 b
Florida MH-1	1.5 ± 0.1 a	1.4 ± 0.3 a	1.2 ± 0.1 a	3.4 ± 0.1 a	2.7 ± 1.0 a	3.2 ± 0.2 a	47.4 ± 3.6 a	21.7 ± 8.7 b	29.9 ± 0.0 b	52.2 ± 3.8 a	25.9 ± 9.9 b	34.3 ± 0.4 ab
Sandpoint	2.7 ± 0.1 b	3.5 ± 0.2 a	2.3 ± 0.1 b	9.1 ± 0.3 a	8.6 ± 0.2 a	6.4 ± 0.0 b	29.2 ± 2.9 a	40.4 ± 6.7 a	47.1 ± 4.3 a	40.9 ± 3.3 a	52.5 ± 6.7 a	55.7 ± 4.5 a
California	4.7 ± 0.3 a	2.7 ± 0.1 b	2.3 ± 0.0 b	8.8 ± 0.3 a	5.3 ± 0.2 b	4.6 ± 0.1 b	35.6 ± 1.5 a	19.5 ± 0.3 b	16.2 ± 0.6 b	49.1 ± 0.8 a	27.5 ± 0.4 b	23.1 ± 0.7 b
California Red Cherry	n.a.	2.7 ± 0.0 a	2.2 ± 0.1 a	n.a.	8.0 ± 0.1 a	7.1 ± 0.2 a	n.a.	21.4 ± 1.9 a	22.0 ± 0.7 a	n.a.	32.1 ± 2.0 a	31.3 ± 1.0 a

n.a.: not available.
Different letters indicate significant differences of means within a row of a cultivar and carotenoid ($P < 0.05$)

Fig. 4. Total carotenoid content and lycopene percentage of ripe red tomato fruits of all cultivars as affected by the different levels of water deficit (T1-T3).

trations in ripe red tomato fruits by providing full or limited water supply seems to be unpromising due to our highly heterogeneous findings, and the selection of appropriate cultivars appears to be crucial. A comprehensive review on tomato breeding for increased lycopene contents was recently published by Baranksi et al. [39]. Due to the effect of water deficit on relative carotenoid concentrations in green fruits, altered concentration ratios might allow estimating the stress level of tomato plants in the future, requiring further study prior to deducing potential clear-cut relationships. Several genotypes were found to increase their xanthophyll cycle carotenoid concentrations more than others when exposed to water deficit, and, thus, further investigations in a larger growing set-up should clarify if these genotypes also adapt better to abiotic stress conditions, such as water deficit. In the future, grow-

ing high-quality tomato fruits with high carotenoid concentrations might become economically feasible in arid countries such as Egypt and Saudi Arabia when selecting appropriate cultivars and granting a sufficient minimum water supply.

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