Journal of Applied Botany and Food Quality 93, 34 - 43 (2020), DOI:10.5073/JABFQ.2020.093.005

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Effect of potassium fertilization on the concentration of antioxidants in two cocktail tomato cultivars

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(Submitted: July 25, 2019; Accepted: December 27, 2019)

Summary

Tomatoes are an important source of beneficial phytochemicals, which act as antioxidants. These include ascorbic acid, phenolic compounds, carotenoids, and tocopherols. The concentration of antioxidants is influenced, among others, by abiotic stress factors like nutritional status. Potassium (K) is a macronutrient, which is essential for several physiological functions in plants – for example, translocation of assimilates, activation of enzymes, maintenance of turgescence, and stomata regulation. This study aims to investigate the effect of increasing K fertilization on the concentration of antioxidants in cocktail tomatoes. Therefore, two tomato cultivars (Primavera and Resi) grown in an outdoor pot experiment were fertilized with increasing K doses for two consecutive years. It has been confirmed that antioxidants in tomato fruit can be affected by the K regime, but it is also shown that other factors may reduce or even reverse those effects when cultivation takes place in an uncontrolled outdoor environment. The most consistent K fertilization effects were found for naringenin, *p*-coumaric acid, and caffeic acid. However, the enrichment of tomatoes with antioxidants by K fertilization is cultivar-dependent and therefore general statements should be avoided.

Keywords: *Solanum lycopersicum* L.; potassium; ascorbic acid; phenols; carotenoids; tocopherols

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetables worldwide. It is consumed not only fresh and raw, but also in various processed forms such as in sauces, pastes, and powders. About 177 million tons of tomatoes were globally produced in 2016, accounting for 16.5% of the global vegetable market (FAOSTAT, 2019). Within the group of tomatoes, cocktail tomatoes (small sized-fruit) have been gaining in popularity for fresh consumption in western countries (Sinesio et al., 2010).

Tomato fruits are rich in antioxidants such as phenolic compounds, carotenoids, and ascorbic acid, which have important physiological functions in plants and humans (Dumas et al., 2003). In plants, antioxidants control the concentrations of intracellular reactive oxygen species (ROS), as they reduce ROS to their non-reactive forms (Cruz de Carvalho, 2008). Being highly toxic and reactive, ROS can cause severe plant cell damage (Gill and Tuteja, 2010). They are produced during photosynthesis in the chloroplasts as well as in the peroxisomes and the mitochondria. Ascorbic acid is one of the major quenchers of ROS due to its high concentration in the plant cytoplasm, rather than because of being a highly effective antioxidant (Gill and Tuteja, 2010). In the plant, next to its function as an antioxidant, ascorbic acid acts as an enzyme cofactor – for example, during photosynthesis or in the synthesis of anthocyanidins – and controls cell growth (Smirnoff and Wheeler, 2000). Unlike many mammals, humans cannot synthesize ascorbate, but it is essential for the hydroxylation of proline and lysine during the production of collagen (Du et al., 2012).

Phenolic compounds are the most abundant secondary metabolites in plants (Dai and Mumper, 2010). They have several different functions in plants, such as providing resistance and defense against microbial infections (Grassmann et al., 2002). These functions are connected with stress-induced ROS formation by their quenching capacity. This has especially been shown for flavonoids (Agati et al., 2013). The proposed health effects are, for example, anti-atherogenic, anti-inflammatory, antimicrobial, cardioprotective, and vasodilatory in nature (Shahidi and Ambigaipalan, 2015).

Carotenoids can protect plant cells by quenching triplet chlorophylls and ROS under excessive light energy conditions (Bramley, 2002). In tomatoes, the major carotenoid is lycopene (Gautier et al., 2008; Egea et al., 2010), while the concentration of other common carotenoids, such as β-carotene, is much lower (Gautier et al., 2008). Lycopene is cyclized by lycopene cyclase to form other carotenes (DellaPenna and Pogson, 2006). During the ripening of tomatoes, the activity of lycopene cyclase is reduced, which is why lycopene is enriched at the cost of, for example, stagnating β-carotene levels (Bramley, 2002). In humans, dietary lycopene has been shown to have preventive properties against different chronic diseases (Rao and Rao, 2007), whereas β-carotene is important for vision and cell growth (Bramley, 2002).

The main function of tocopherols in plants is the stabilization of membranes (Pongracz et al., 1995). Germination and seedling growth are negatively affected in tocopherol-deficient plants (Falk and Munné-Bosch, 2010). In humans, tocopherols and tocotrienols are important due to their Vitamin E activity, the best availability being provided by α-tocopherol (Wagner et al., 2004).

The concentration of antioxidants in tomato fruit is, however, strongly influenced by biotic and abiotic stress factors such as plant water status, irradiation, and nematodes (Gautier et al., 2008; Atkinson et al., 2011). Moreover, the availability of macronutrients and micronutrients to the plant has a major impact on the chemical composition of tomato fruit (Wright and Harris, 1985; Kaur et al., 2018). The macronutrient potassium (K) is essential for several physiological functions in plants, including translocation of assimilates, activation of enzymes, maintenance of turgescence, and stomata regulation (Mengel and Viro, 1974; Zörb et al., 2014; Zhao et al., 2018). K fertilization has a positive effect on crop yield in general (Cakmak, 2005; Zörb et al., 2014), and some studies have shown a positive effect on tomato yield (Taber et al., 2008; Amjad et al., 2014). Contradictory studies have shown a cultivar-dependence (Hartz et al., 2005; Sonntag et al., 2019) or even no effect (Asri and Sönmez, 2010; Constán-Aguilar et al., 2015). In addition, the resistance to biotic and abiotic stresses – for example, drought, salinity, cold, and pests, as well as pathogens – can directly and indirectly be positively influenced by an increased level of K supply (Cakmak, 2005; Zörb et al., 2014). Diverse studies also showed an effect of K fertilization on the concentration of certain plant antioxidants such as carotenoids (Constán-Aguilar et al., 2015; Kaur et al., 2018; Tavallali

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et al., 2018), tocopherols (Caretto et al., 2008), phenolic compounds (Fanasca et al., 2006; Tavallali et al., 2018), and ascorbic acid (Kaur et al., 2018). However, contradictory results have been reported: Some studies demonstrated increasing levels of antioxidants with rising K fertilization (Constán-Aguilar et al., 2015; Tavallali et al., 2018), while others showed no effect or even a decrease in antioxidant levels (Fanasca et al., 2006; Taber et al., 2008). These diverse results might be due to varying cultivation environments – for example, greenhouse (Constán-Aguilar et al., 2015) or open field (Taber et al., 2008) – along with alternating abiotic factors or even due to different cultivars. Nonetheless, K is the main cation in the cell cytoplasm and acts as a co-enzyme in several metabolic processes (Mengel and Viro, 1974; Zörb et al., 2014; Zhao et al., 2018). Consequently, the fruits antioxidants deriving from different pathways of the secondary metabolism show an effect due to an increasing level of K supply. Therefore, a hypothesis can be made that rising K application influences the main antioxidants in the two cocktail tomato cultivars.

Four different antioxidant groups – ascorbic acid, phenolic compounds, carotenoids, and tocopherols – were analyzed in tomato fruits grown in an outdoor pot experiment over two consecutive years. As carotenoids change during the ripening process and share a precursor with tocopherols (Hirschberg, 1999), the potential interactive effects of ripening on lipophilic antioxidants under different K regimes were studied as well.

Materials and methods

Growth conditions

The study was conducted over two consecutive years at the University of Goettingen. In both years (2014 and 2015), two cocktail tomato cultivars – namely Primavera and Resi – were planted. The sowing in both years took place in early April and the first transplantation into 1 L pots happened in late April. A peat mixture ('Anzuchtsubstrat organisch' from Kleeschulte, Rüthen, Germany) was used as the substrate in the starter trays (volume 0.1 L), while pure peat soil (A 400 from Stender, Schermbeck, Germany) was used as the substrate in the subsequent 1 L pots. Temperature and light (long daylight conditions: 16 h, 22 °C and 18 °C during day and night, respectively) were controlled until the final transplantation. In late May, the final transplantation to the outdoor location at the University of Goettingen (coordinates: 51.54° N, 9.94° E) took place. The tomato plants were arranged in a randomized block design with four replications (Fig. S1). The plants were pruned to one shoot. All necessary minerals were applied twice during the growing season to the pot ('Mitscherlich vessels', 6 L volume) of each plant (more details in Tab. S1), and only phosphorus was fully integrated at the final transplantation into the substrate (peat, 'Gartentorf' from Naturana, Vechta, Germany). K and nitrate fertilization took place on a weekly basis in liquid form. In 2014, five increasing K levels – K1 to K5 (0.37 g, 0.73 g, 1.09 g 1.47 g, and 2.20 g K_2SO_4 weekly fertilization) – were applied. In week 16 (July 11 in 2014), the application of the levels K3, K4, and K5 (K3 to 1.47 g K_2SO_4; K4 to 2.20 g K_2SO_4; K5 to 3.66 g K_2SO_4) was raised in order to strengthen the K fertilization effect. In 2015, only two increased levels were applied (K1 and K5 as used in 2014).

Sampling

In both years, tomatoes were harvested starting in mid-July on a weekly basis. Each week, the fruits of a plant group (comprising five plants in 2014 and eight in 2015) were harvested (Fig. S1). A plant group consisted of tomato plants of the same cultivar and K treatment. The ripe fruit of Harvest No. 4 (August 7) in 2014 and of Harvest No. 6 (August 17) in 2015 were used for all analysis, except for tocopherols and carotenoids in 2015. Here, the development stages of breaker, orange, and ripe red were sampled to determine the concentrations of carotenoids and tocopherols during tomato fruit ripening. The harvest of fruit at the three developmental stages was done for each K fertilization treatment and lasted from August 24 until September 18. The classification of fruit into the ripening stages was done visually and checked with a Chroma Meter CR-400 (Konica Minolta, Inc., Marunouchi, Japan) (Tab. S7).

All fresh fruit were quartered, separated, and shock-frozen in liquid nitrogen and then stored at -80 °C. To analyze tocopherols, phenolic compounds, and K, a part of the quarters was freeze-dried (Christ, Epsilon 2-40, Osterode, Germany). The dried samples were ground with a ball mill (30 s at 30 Hz; Retsch, model: MM 400, Haan, Germany) and stored at -80 °C until analysis.

Determination of potassium

Subsamples of the lyophilized and ground samples were dried at 60 °C to constant weight. The K concentration in the fruit was analyzed according to the method used by Koch et al. (2019).

Determination of ascorbic acid

To determine the concentration of ascorbic acid, 5 g of frozen quarters were crushed by an Ultra-Turrax (T18 digital Ultra Turrax, IKA, Staufen, Germany) with 20 ml of 5% meta-phosphoric acid. Subsequently, the suspension was filled up to 50 ml with demineralized water and filtered (Filter paper MN 616 ¼, Macherey-Nagel GmbH & Co. KG, Düren, Germany). Next, 10 ml of the filtrate was titrated twice against the 2,6-Dichlorophenolindophenol (DIP) solution (0.21 g of DIP in 1,000 ml distilled water) until the solution changed from colorless to light pink. The ascorbic acid concentration was calculated per 100 g of fresh weight.

Determination of phenolic compounds

100 mg of the freeze-dried and ground samples were used for duplicate analyses of phenolic compounds using a slightly modified version of the method developed by Eggert et al. (2010). Following the addition of 2 ml of extraction solution (methanol/water/acetic acid, 80:19:1, v/v/v), the samples were homogenized and shaken for 12 h at room temperature with 300 rpm. The samples were centrifuged at 21,801 g at 4 °C for 10 min (Heraeus Megafuge 16R, Thermo Scientific, Waltham, MA USA), and the supernatant was collected. This extraction was repeated twice. The water was evaporated from the combined extracts with a rotational vacuum concentrator (RVC 2-25 CD plus, Christ, Osterode am Harz, Germany) for 17 h at 20 °C. Afterwards, acid hydrolyses were performed by dissolving the pellet in 1 ml 0.1 M H_2SO_4 and incubated for 1 h at 100 °C. Subsequently, the samples were subjected to the first enzymatic hydrolysis by adding 0.5 ml 1 M CH_3COONa of α-amylase (>375 units, Sigma-Aldrich, St. Louis, Missouri, USA) and incubated for 2 h at 30 °C. Later, a second enzymatic hydrolysis with 0.5 ml of 0.1 M CH_3COONa and cellulase (>12 units, Sigma-Aldrich, St. Louis, Missouri, USA) was done for 18 h at 30 °C. After the incubation, 0.5 ml of 25% NaCl solution was added and the samples were centrifuged with 5,450 g at 4 °C. Liquid extraction with 1 ml of ethyl acetate was carried out three times, and the supernatants were combined and evaporated in a rotational vacuum concentrator for 18 h at 20 °C. The pellet was re-dissolved in 400 µl extraction solution (methanol/water/acetic acid, 80:19:1, v/v/v) and filtered through a 0.45 µm PTFE filter (VWR, Darmstadt, Germany) into high-performance liquid chromatography (HPLC) vials. A HPLC system from Jasco (auto sampler: AS-2051 Plus, UV/VIS detector: MD-2015Plus, pump: LG-2080-04, column oven: CO-2060 Plus, Jasco, Pfungstadt, Germany) was used. The separation of phenolic compounds was performed on a PerfectSil Target ODS-3 HD column (125×3.0 mm,

5 µm, MZ Analysentechnik, Mainz, Germany) with a matching pre-column (MZ) as follows – injection volume: 20 µl; column temperature: 40 °C; flow rate: 0.8 mL/min; gradient elution with water/acetic acid (99:1, v/v; eluent A) and methanol/acetic acid (99:1, v/v; eluent B): 0-35 min 10-30% B, 35–50 min 30-90% B, 50–52 min 90–100% B, and 52–60 min 100% B. The detection wavelengths were 280 nm and 206 nm. For the purposes of quantification and identification, external calibrations were prepared for *p*-coumaric acid, caffeic acid, ferulic acid, sinapinic acid, naringenin, and quercetin. The chromatograms were analyzed using the software ChromPass (version 1.8.6.1, Jasco, Pfungstadt, Germany). The limit of detection (LOD) was three times the noise level and the limit of quantification (LOQ) was 10 times the noise level.

Determination of carotenoids

Fresh samples were milled with liquid nitrogen for 30 s at 30 Hz (Retsch, model: MM 400, Haan, Germany). Next, 600 mg of the ground and frozen samples were weighed in a 50 ml centrifuge tube (Carl Roth, Karlsruhe, Germany). Carotenoids were analyzed using the method of Serio et al. (2007), with the following modifications: The non-polar n-hexane/carotenoid layer was evaporated using a rotational vacuum concentrator for 13 h at 20 °C and dissolved in a 1,250 ml solution of ethyl acetate/dichloromethane/n-hexane (80:16:4, *v:v:v*). The solution was filtrated and diluted 1:100 (v/v) with the ethyl acetate/dichloromethane/n-hexane solution. Analyses were performed using the Jasco HPLC system described above either within a day after the extraction or samples were stored at -20 °C prior to the analysis. The LOD was three times the noise level and the LOQ was 10 times the noise level.

Determination of tocopherols

Tocopherols were extracted from freeze-dried material with acetone containing 0.025% butylhydroxytoluene as previously described (Knecht et al., 2015). HPLC analyses were carried out on a Shimadzu high-pressure gradient system consisting of a DGU-20A5 degasser, two LC-30AD pumps, a SIL-30AC autosampler, a CTO-20AC column thermostat, a SPD-M20A diode array detector, and a RF-20A XS fluorescence detector (FLD). Separation of tocopherols was carried out on a Develosil RP Aqueous C30 column (150 × 3 mm, 3 µm, Phenomenex, Aschaffenburg, Germany) as follows – injection volume: 10 µl; column temperature: 18 °C; flow rate: 0.5 mL/min; gradient elution with methanol/water (91:9, v/v; eluent A) and *tert*-methylbutylether/methanol/water (80:18:2; v/v/v; eluent B): 0–5 min 0% B, 5–25 min 0–5% B, 25–40 min 5% B, 40–46 min 5–55% B, 46–48 min 55–100% B, 48–51 min 100% B, 51–53 min 100–0% B, and 53–63 min 0% B. FLD excitation and emission wavelengths were set as previously described (Knecht et al., 2015). Tocopherols were quantified using external calibrations (0.1–10 µg/ml) and linear regression.

Statistics

The statistics were performed using the program SPSS Version 24 (IBM Corporation, New York, United States). To begin with, the data were checked for normal distribution and homogeneous variance. If both were confirmed, a one-factorial analysis of variance (ANOVA) was performed to test if there was a significant effect of the K treatments. In case of significance, Tukey's honestly significant difference was performed post hoc to test for differences between the K application levels within the two cultivars for each parameter individually. If the data were not normally distributed, the Kruskal-Wallis test was performed. The Welch test was used only if the data showed inhomogeneous variance but normal distribution. The Kruskal-Wallis test and the Welch test were both followed by the Mann-Whitney-U test to compare the means of the treatments. To analyze the relationships between fruit K concentration and the different antioxidants, a two-sided Pearson correlation was performed with a significance level of $p \leq 0.05$. In addition, a principal component analysis (PCA) was prepared with Statistica 13.0 (TIBCO, Palo Alto, California, United States). For the supplement data in addition to the above described procedure, were two-factorial and three-factorial ANOVAs (Tab. S2, S3, S4, S7, and S8) and t-tests (Tab. S5, S6, and S9) calculated with SPSS 24.

Results

In both years, the K concentration increased significantly in the tomato cultivars from K1 to K5 – this increase was cultivar-dependent and ranged between 26% and 57% (Tab. S6; Sonntag et al., 2019). Within the PCA plot, which could only be created for 2014, the K levels were lined up in the middle according to rising fertilizer treatment and the fruit K concentration was closely located below the points that represent the K levels (Fig. 1). *p*-Coumaric acid was also grouped in the lower part of the PCA. Narigenin and lycopene were positioned close to the low fertilization levels K1 and K2 in the upper part. The other antioxidants were all located in the middle of the PCA plot, closer to K3 and K4.

K fertilization resulted in diverse effects on antioxidants, which were i) cultivar-dependent, ii) not consistent in both study years, and iii) not always reflected in correlations between antioxidants and the K concentrations in the fruit. Fruit ascorbic acid concentration, for example, was only significantly influenced by K application in 2015 (Fig. 2). The plants with high K application (K5) of both cultivars had significantly higher ascorbic acid concentration in their fruit. However, the correlation between ascorbic acid and the fruit K concentration was significant for Resi in both years and in 2015 for Primavera (Tab. 1). A two-factorial ANOVA revealed a significant interaction between year and K treatment only for Primavera (Tab. S2). However, the year itself showed no significant influence on the ascorbic acid concentration according to the two-way ANOVA in both analyzed cultivars.

In 2014, there was no significant change with rising K fertilization (Tab. 2). Only Primavera showed a significant negative correlation of the fruit K concentration with naringenin in 2014 (Tab. 1). The concentration of naringenin decreased significantly from low to high K application in Primavera in 2015, but it was not negatively correlated with the fruit K concentration in Primavera in 2015. *p*-Coumaric acid rose non-significantly in both cultivars with an increasing level of K supply, but it showed a significant positive correlation with an increasing level of K concentration in Primavera (both years) and Resi (2015 only) (Tab. 1). In 2015, *p*-coumaric acid as well as caffeic acid levels increased with rising K treatment in the fruit of Resi and Primavera (Tab. 2) – in this case, it was also reflected in a significant correlation with fruit K concentration in both cultivars (Tab. 1). A two-factorial ANOVA revealed that for both cultivars, the year had a significant influence on *p*-coumaric acid, caffeic acid, ferulic acid, quercetin, and additionally for sinapinic acid in Resi (Tab. S2). The interaction of year and K treatment was significant in Primavera for caffeic acid, while for Resi this interaction was significant for caffeic acid and *p*-coumaric acid.

There were no significant differences for lycopene between the five K fertilization levels in both years (Fig. 3). However, as expected, lycopene increased during the ripening of both Resi and Primavera in 2015 (Fig. 3). If averaged over both K levels, this effect was significant (Tab. S4). The β-carotene levels decreased with rising K application only in the fruit of Primavera in 2014 (Fig. 3). In 2015, the β-carotene concentrations of the higher K treatment (K5) increased in both cultivars and all ripening stages. These differences were significant in the breaker and orange ripening stages

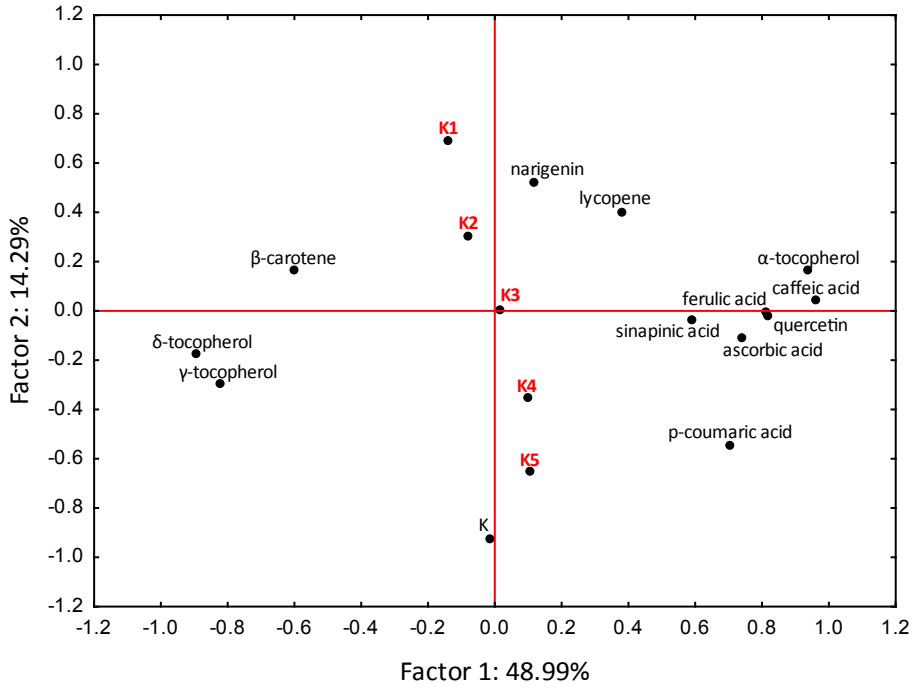

Fig. 1: Principal component analysis of the antioxidants in relation to the increasing K levels in 2014. K levels increase from K1 to K5 (0.37 g, 0.73 g, 1.47 g, 2.2 g, and 3.66 g K_2SO_4 per week). K represents the K concentration in the tomato fruit.

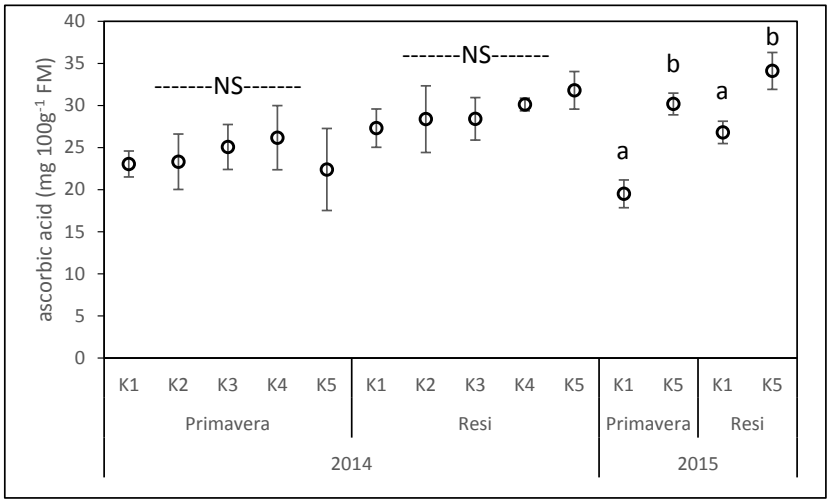

Fig. 2: K fertilization differently affects the ascorbic acid concentration of the cocktail tomato cultivars. K levels increase from K1 to K5 (0.37 g, 0.73 g, 1.47 g, 2.2 g, and 3.66 g K_2SO_4 per week) for each cultivar. The mean values and standard deviations were determined from four biological replicates. Letters indicate statistically significant differences and NS indicates no significant difference, according to a Mann-Whitney-U or Tukey-HSD test.

of Primavera but only in the breaker stage of Resi. For Resi, this relationship between K concentration and β-carotene concentration was confirmed by a positive significant correlation (Tab. 1). A two-factorial ANOVA revealed that there was a significant interaction between year and K treatment for β-carotene in Primavera but not in Resi (Tab. S2). Within the different ripening stages and averaged over both K levels, β-carotene concentration rose only until the orange ripe stage in both cultivars (Tab. S4).

In 2014, both α- and β-tocopherol were below LOD in Primavera, as for β-tocopherol in Resi. Also, α-tocopherol showed no significant tendency in Resi (Tab. 3). Though γ-tocopherol increased in both Primavera and Resi, it was significant only in the latter, and a positive significant correlation with the K concentration in the fruit was detected for γ-tocopherol in Resi (Tab. 1). In Resi, the values of δ-tocopherol were below LOQ and in Primavera the values were not significantly affected by K fertilization. In 2015, tocopherols were analyzed in the ripe stage, like in 2014, as well as in breaker and orange ripe stages. α- and β-tocopherol were again mostly below the detection limit in Primavera. Additionally, β- and δ-tocopherol were not detectable or below LOD in Resi in 2015. At all ripening stages, the concentrations of α-, γ-, and δ-tocopherol of a low K treatment were higher than those at the high K level in both cultivars, if measurable. This tendency was significant in both cultivars for γ- and δ-tocopherol and in Resi for α-tocopherol in the ripening stages of orange and ripe (Tab. 3). For both cultivars, a significantly negative correlation was detected between K level and γ-tocopherol in 2015 (Tab. 1). Additionally, the K concentration was negatively correlated with α-tocopherol in Resi and with δ-tocopherol in Primavera.

Tab. 1: Pearson correlation between the concentration of K and antioxidants in tomatoes.

		2014		2015	
		Primavera	**Resi**	**Primavera**	**Resi**
ascorbic acid	correlation	0.028	0.477*	0,978**	0,904**
	n	19	20	8	8
***p*-coumaric acid**	correlation	0.666**	0.375	0,923**	0,979**
	n	19	20	8	8
caffeic acid	correlation	-0.221	0.392	0,769*	0,829*
	n	19	20	8	8
ferulic acid	correlation	-0.326	0.293	0.326	0.471
	n	19	20	8	8
sinapinic acid	correlation	0.014	-0.067	-0.039	-0.395
	n	19	20	8	8
quercetin	correlation	0.198	0.048	-0.606	0.259
	n	19	20	8	8
narigenin	correlation	-0.489*	-0.220	-0.700	-0.174
	n	19	20	8	8
β-carotene	correlation	-0.686**	0.255	0.357	0,513*
	n	19	20	24	24
lycopene	correlation	-0.307	-0.229	0.187	0.135
	n	19	20	24	24
α-tocopherol	correlation		0.198		-0,596**
	n		20		24
β-tocopherol	correlation				
	n				
γ-tocopherol	correlation	0.313	0.696**	-0,553**	-0,601**
	n	19	20	24	24
δ-tocopherol	correlation	0.006		-0,778**	
	n	19		24	

Two-tailed Pearson correlations are significant at the level of $p<0.05$ (*) or 0.01 (**). n is the number of observations and if there is no value, the concentration of the antioxidant was below the limit of quantification. The correlation for β-carotene, lycopene, α-, β-, γ-, and δ-tocopherol in 2015 was performed for all ripening stages.

Tab. 2: Potassium (K) fertilization differentially affects the individual phenolic compounds of the cultivars.

			***p*-coumaric acid (µg 100 g^{-1} FM)**		**caffeic acid (mg 100 g^{-1} FM)**		**ferulic acid (µg 100 g^{-1} FM)**		**sinapinic acid (µg 100 g^{-1} FM)**		**quercetin (mg 100 g^{-1} FM)**		**naringenin (µg 100 g^{-1} FM)**	
2014	**Primavera**	K1	2.0 ± 1.0	NS	3.9 ± 1.7	NS	3.0 ± 1.1	NS	1.4 ± 0.5	NS	0.8 ± 0.3	NS	8.8 ± 5.0	NS
		K2	2.9 ± 0.8	NS	3.1 ± 0.5	NS	2.8 ± 0.5	NS	1.4 ± 0.2	NS	0.7 ± 0.1	NS	2.9 ± 1.0	NS
		K3	4.3 ± 1.2	NS	3.0 ± 0.7	NS	2.8 ± 0.6	NS	1.4 ± 0.3	NS	0.7 ± 0.2	NS	1.9 ± 1.8	NS
		K4	5.4 ± 0.9	NS	4.0 ± 0.5	NS	3.2 ± 0.4	NS	1.6 ± 0.4	NS	0.9 ± 0.1	NS	1.0 ± 0.8	NS
		K5	5.6 ± 2.5	NS	3.3 ± 1.3	NS	2.5 ± 0.7	NS	1.5 ± 0.4	NS	0.8 ± 0.3	NS	2.2 ± 1.9	NS
	Resi	K1	4.4 ± 1.4	NS	5.9 ± 1.3	NS	3.8 ± 0.8	NS	1.6 ± 0.1	NS	1.0 ± 0.2	NS	5.9 ± 4.0	NS
		K2	5.2 ± 1.0	NS	6.6 ± 0.5	NS	4.2 ± 1.1	NS	1.6 ± 0.2	NS	1.2 ± 0.1	NS	2.2 ± 1.3	NS
		K3	5.9 ± 1.5	NS	7.7 ± 1.2	NS	3.8 ± 0.9	NS	1.8 ± 0.3	NS	1.4 ± 0.3	NS	8.5 ± 5.6	NS
		K4	5.7 ± 1.2	NS	7.4 ± 1.2	NS	4.2 ± 0.5	NS	1.6 ± 0.2	NS	1.5 ± 0.5	NS	2.8 ± 1.5	NS
		K5	7.6 ± 2.4	NS	8.1 ± 0.8	NS	4.9 ± 1.4	NS	1.7 ± 0.3	NS	1.2 ± 0.2	NS	2.8 ± 3.4	NS
2015	**Primavera**	K1	3.0 ± 1.4	a	4.9 ± 1.1	a	3.8 ± 0.7	NS	1.3 ± 0.3	NS	2.2 ± 0.8	NS	3.3 ± 1.0	a
		K5	10.8 ± 2.5	b	6.9 ± 1.0	b	4.1 ± 0.5	NS	1.2 ± 0.2	NS	1.4 ± 0.1	NS	1.9 ± 0.5	b
	Resi	K1	6.4 ± 1.1	a	8.3 ± 1.3	a	6.1 ± 1.1	NS	1.3 ± 0.1	NS	2.2 ± 0.6	NS	1.8 ± 0.5	NS
		K5	14.4 ± 1.7	b	11.2 ± 1.4	b	7.0 ± 0.9	NS	1.3 ± 0.1	NS	2.4 ± 0.7	NS	1.7 ± 0.3	NS

Mean values and standard deviations were determined from four biological replicates. K levels increase from K1 to K5 (0.37 g, 0.73 g, 1.47 g, 2.2 g, and 3.66 g K_2SO_4 per week) for each cultivar. Letters indicate statistically significant differences and NS indicates no significant difference, according to a Mann-Whitney-U or Tukey-HSD test.

A two-way ANOVA revealed a significant year effect for γ- and δ-tocopherol in both cultivars, and additionally, for α-tocopherol in Resi. Interactions between year and K treatment were significant in Primavera for γ- and δ-tocopherol, and in Resi for γ-tocopherol (Tab. S2). The ripening stage had an influence on the tocopherol concentration in only two cases: the γ-tocopherol concentration in the red ripe stage was significantly higher than in the other two stages in case of Primavera; in Resi, the orange ripe stage had a significantly lower concentration of γ-tocopherol than that in the red ripe stage (Tab. S4).

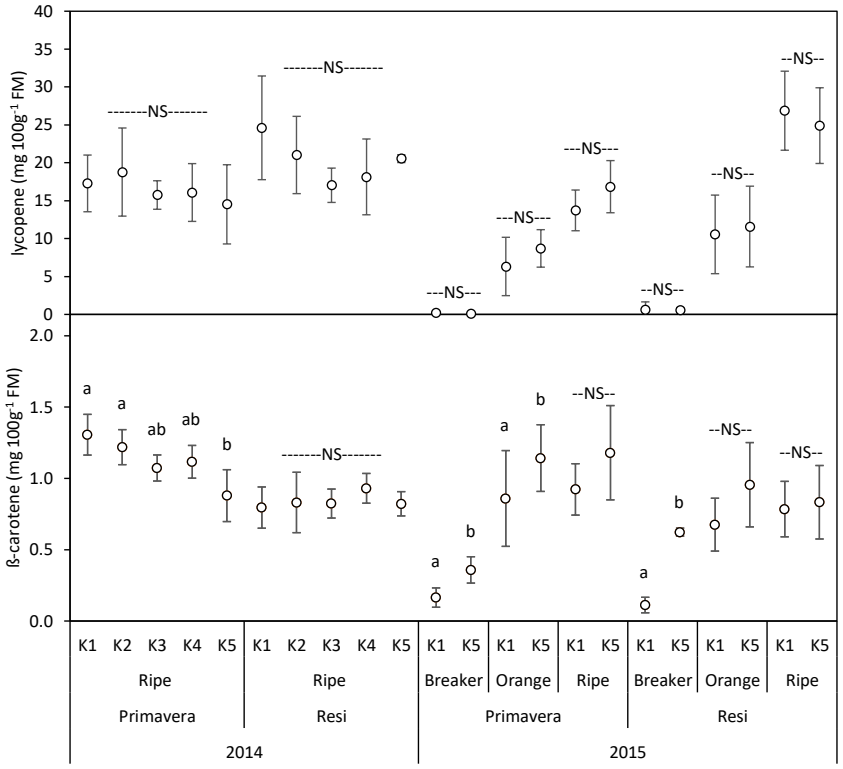

Fig. 3: Potassium (K) fertilization differentially affects the carotenoids lycopene and β-carotene in the cultivars. Mean values and standard deviations were determined from four biological replicates. K levels increased from K1 to K5 (0.37 g, 0.73 g, 1.47 g, 2.2 g, and 3.66 g K_2SO_4 per week) for each cultivar. Letters indicate statistically significant differences and NS indicates no significant difference, according to a Mann-Whitney-U or Tukey-HSD test.

Tab. 3: Potassium (K) fertilization differentially affects the tocopherols (α, β, γ, δ).

				α-tocopherol (mg 100g^{-1} FM)		β-tocopherol (mg 100g^{-1} FM)	γ-tocopherol (mg 100g^{-1} FM)		δ-tocopherol (mg 100g^{-1} FM)	
2014	Primavera	Ripe	K1	< LOD		< LOD	1.60 ± 0.25	NS	0.06 ± 0.01	NS
			K2	< LOD		< LOD	1.40 ± 0.26	NS	0.05 ± 0.01	NS
			K3	< LOD		< LOD	1.49 ± 0.25	NS	0.05 ± 0.01	NS
			K4	< LOD		< LOD	1.65 ± 0.25	NS	0.06 ± 0.01	NS
			K5	< LOD		< LOD	1.72 ± 0.16	NS	0.05 ± 0.01	NS
	Resi	Ripe	K1	2.13 ± 0.21	NS	< LOQ	0.62 ± 0.04	a	< LOQ	
			K2	2.04 ± 0.30	NS	< LOQ	0.59 ± 0.11	a	< LOQ	
			K3	2.40 ± 0.25	NS	< LOQ	0.68 ± 0.05	ab	< LOQ	
			K4	2.41 ± 0.26	NS	< LOQ	0.67 ± 0.04	ab	< LOQ	
			K5	2.29 ± 0.26	NS	< LOQ	0.73 ± 0.10	b	< LOQ	
2015	Primavera	Ripe Breaker	K1	< LOD/LOQ		< LOD	2.36 ± 0.61	a	0.05 ± 0.02	a
			K5	< LOD		< LOD	1.50 ± 0.25	b	0.03 ± 0.00	b
		Ripe Orange	K1	0.12 ± 0.24		< LOD/LOQ	1.77 ± 0.22	a	0.07 ± 0.01	a
			K5	< LOD		< LOD	1.21 ± 0.15	b	0.03 ± 0.01	b
		Ripe	K1	0.14 ± 0.29		< LOD/LOQ	1.25 ± 0.16	a	0.06 ± 0.01	a
			K5	< LOD		< LOD	0.87 ± 0.06	b	0.03 ± 0.00	b
	Resi	Ripe Breaker	K1	1.02 ± 0.17	NS	< LOQ	0.42 ± 0.10	a	< LOD/LOQ	
			K5	0.85 ± 0.13	NS	< LOQ	0.21 ± 0.08	b	< LOD/LOQ	
		Ripe Orange	K1	1.23 ± 0.02	a	0.02 ± 0.00	0.45 ± 0.03	a	< LOQ	
			K5	0.89 ± 0.01	b	< LOQ	0.30 ± 0.01	b	< LOD/LOQ	
		Ripe	K1	1.10 ± 0.20	a	< LOQ	0.26 ± 0.05	a	< LOD/LOQ	
			K5	0.94 ± 0.10	b	< LOQ	0.23 ± 0.04	b	< LOD/LOQ	

Mean values and standard deviations were determined from four biological replicates. K levels increase from K1 to K5 (0.37 g, 0.73 g, 1.47 g, 2.2 g, and 3.66 g K_2SO_4 per week) for each cultivar. Letters indicate statistically significant differences, according to Mann-Whitney-U or Tukey-HSD test. NS indicates no significant difference. Below the limit of detection (< LOD). Below the limit of quantitation (< LOQ). If a tocopherol concentration was < LOQ for one or more of the biological replicates, < LOQ was given as the mean.

Discussion

In the two cultivars, a rising level of K fertilization increased K accumulation in the tomato fruit, showing that the plants successfully absorbed the nutrient. This confirms the results of other studies showing an increasing response to the K concentration in the tomato fruit with rising K fertilization levels (Fanasca et al., 2006; Taber et al., 2008). Compared with the soilless system used by Fanasca et al. (2006) the fruit K concentrations in our experiment (data presented in Sonntag et al., 2019) were lower, presumably as we cultivated the plants in a substrate without continuous supply of nutrient solution. Taber et al. (2008) used a better comparable system with sandy soil provided with daily fertigation. With 1.5 to 3.2 g kg^{-1} in 2014 and 1.1–3.4 g kg^{-1} in 2015 calculated on fresh matter basis (data not shown) we reached higher fruit K concentrations than Taber et al. (2008). The habitus of the whole plants from the K5 treatment did not show any deficiency symptom (Fig. S1). Moreover, even in the low fertilized plants, yellow shoulder symptom was an exception. One can conclude, that the nutritional status of plants ranged from (i) deficient in K nutrition for all cultivars (K1 and K2), (ii) slight deficient K nutrition especially in Primavera (K3), (iii) sufficiently nourished with K (K4), and (iv) sufficiently to high nourished with K especially for Resi (K5).

As expected and based on previous studies, antioxidant accumulation varied between the cultivars, as it was shown for tocopherols (Caretto et al., 2008) and carotenoids, ascorbic acid, and total phenolics (Bhandari et al., 2016). In both years, Resi accumulated higher levels of ascorbic acid and lycopene, while Primavera had higher concentrations of β-carotene, γ-tocopherol, and δ-tocopherol (Fig. 1 and 2, Tab. 3). Notably, Primavera did not contain detectable amounts of α- and β-tocopherol, whereas α-tocopherol was the main tocopherol in Resi (Tab. 3). Since γ- and δ-tocopherol are converted into α- and β-tocopherol by tocopherol methyltransferase in the plant (Wagner et al., 2004), our data suggests that any variation in the γ-/δ-tocopherol methyltransferase genes leads to a downregulation of α-/β-tocopherol biosynthesis in Primavera.

The antioxidants investigated in this study were differently affected by increasing K fertilization. For some compounds, such as ferulic acid, sinapinic acid, quercetin, and lycopene, no significant correlations with fruit K level were determined (Tab. 1), indicating that those substances are either less affected by K fertilization or that their concentration in the tomato fruit is dominated by other factors. Other antioxidants, such as ascorbic acid, *p*-coumaric acid, caffeic acid, naringenin, β-carotene, and tocopherols, were more correlated with the K concentration in the tomato fruit. However, consistently significant correlations were not observed throughout the study period (Tab. 1). For example, a significant positive correlation of K fruit level and ascorbic acid was shown in both years for Resi and in 2015 also for Primavera. Yet, this trend was not observed in Primavera in 2014. However, a t-test revealed a significant difference in the ascorbic acid concentration between K1 and K5 for Resi in 2014 (Tab. S5). In addition, for both Resi and Primavera, the fertilization treatment was significant, while the year had no effect, as shown by a two-factorial ANOVA (Tab. S2). Several earlier studies had also shown a positive relationship between K application and ascorbic acid concentration in tomatoes (El-Nemr et al., 2012; Constán-Aguilar et al., 2015; Tavallali et al., 2018), while others did not observe this effect (Fanasca et al., 2006) or found it to be cultivar-dependent (Schwarz et al., 2013). The results from our study indicate that the effect of K fertilization on the accumulation of ascorbic acid is first of all cultivar-dependent but not climate-dependent (Tab. S2, S3). Overall, the levels of ascorbic acid in Primavera and Resi were positively influenced by K fertilization.

p-Coumaric acid was the only antioxidant investigated in this study that consistently showed positive relations with the tomato fruit K concentration across the cultivars and years (Tab. 1). However, those correlations were not always significant and a t-test between K1 and K5 also did not consistently show significant differences across cultivars and years (Tab. S5). In case of caffeic acid, the t-test revealed a significant difference between K1 and K5 for Resi from 2014 (Tab. S5), while no significant effects were observed for ferulic and sinapinic acids. The four phenolic acids investigated in this study belong to the group of hydroxycinnamic acids, which are synthesized in the phenylpropanoid pathway (Shahidi and Ambigaipalan, 2015). Notably, the K treatment only affected the biosynthetic stages of caffeic acid and *p*-coumaric acid but not the subsequent stages, thereby resulting in ferulic acid and sinapinic acid. However, a two-factorial ANOVA showed that for two hydroxycinnamic acids, besides the K application effect, a year effect and an interaction of these two factors were present (Tab. S2). This indicates that other abiotic factors such as weather conditions, may have played a role in the formation of these compounds. Between the two analyzed flavonoids, only naringenin accumulated in the cultivars and in both years under low K supply, this tendency was only significant in 2015 for Primavera. Yet, the t-tests revealed a significant difference between K1 and K5 in both years for Primavera (Tab. S5). Naringenin is one of the main flavonoids in tomato peels (Navarro-González et al., 2011) and most likely has a defensive function during periods of stress. A study by Fanasca et al. (2006) demonstrates that K treatment was of minor importance for flavonoids and caffeic acid. However, in this study, naringenin, *p*-coumaric acid, and partly, caffeic acid showed the same tendencies with increasing K fertilization in both years. Consequently, individual phenolic compounds were influenced by increasing levels of K application.

An effect of the year was also observed for other antioxidants such as quercetin, β-carotene, and tocopherols (Tab. S2). In case of β-carotene or γ-tocopherol, even opposite significant correlations with tomato fruit K concentration were determined in 2014 and 2015 (Tab. 1). This again indicates that other factors, such as ambient temperature or light intensity, may affect or even reverse the effects of K fertilization in tomatoes in an outdoor environment. Antioxidant formation shows a negative correlation to light and a positive correlation to temperature (Balliu and Ibro, 2000; Ehret et al., 2013; Balliu and Ibro, 2000; Ehret et al., 2013). This influence has been described for ascorbic acid (Lee and Kader, 2000; Gautier et al., 2008), phenols (Slimestad and Verheul, 2009), carotenoids (Dumas et al., 2003), and tocopherols (Lushchak and Semchuk, 2012). In addition, some of the antioxidants are located in higher concentrations near the skin of the fruit (Vinha et al., 2014), where the influence of abiotic factors on the concentrations is higher. In 2015, there were not significantly more sunshine hours, but the mean temperature was significantly higher in 2014 within two weeks before the harvest (Tab. S9), although the difference between the months was not significant (Tab. S8). Nonetheless, it is possible that temperature had an influence. Also, the concentrations of many antioxidants were significantly different between the two years, according to an ANOVA (Tab. S2). In this study, tocopherol concentrations were about two- to three-fold higher in 2014 than in 2015. It may be hypothesized that K fertilization does not significantly affect tocopherols if they are already showing high accumulation rates, for example, due to light stress (Lushchak and Semchuk, 2012). This could explain the absence of a K-effect in 2014, while the concentrations of all tocopherols decreased in 2015 under high K treatment. It should be emphasized that β-carotene, lycopene, and tocopherols share a biosynthetic precursor (Hirschberg, 1999) and that increasing accumulation of tocopherols may result in a decrease of carotenoids and vice versa. In this study, K fertilization often affected tocopherols and carotenoids in the opposite way (Tab. 1).

Lycopene levels were not influenced by increasing K fertilization in either year (Fig. 3). In contrast, the β-carotene concentration significantly decreased with increasing K application in Primavera in the

first year. However, the opposite trend was detected in the second year, especially in the earlier ripening stages. The importance of K fertilization on the tomato fruit carotenoids has been a matter of debate. Some studies showed an increase in lycopene with rising K application (Dumas et al., 2003; Tavallali et al., 2018), whereas others showed a correlation only for high-pigment cultivars (Serio et al., 2007) or no correlation at all between K fertilization and lycopene (Taber et al., 2008; Liu et al., 2011). In the present study, a two-factorial ANOVA also revealed a significant interaction between year and K treatment for β-carotene, once again suggesting the influence of other factors. Overall, this study indicates that K application has a minor influence on the carotenoid concentrations in tomatoes.

The carotenoids are plant pigments, whereby lycopene and β-carotene are known to increase when the tomato fruit ripens (Egea et al., 2010). This was confirmed in the present study (Fig. 3, Tab. S4), whereas β-carotene concentrations increased until the orange ripening stage. As at a certain ripening stage, the biosynthesis of β-carotene is down-regulated, thereby supporting further accumulation of its precursor lycopene. The present data indicates that those ripening effects are not influenced by K supply. Ripening had less effect on α-tocopherol levels. However, γ-tocopherol significantly decreased in the course of ripening in Primavera. In Resi, the γ-tocopherol concentrations of the orange ripening stage were also significantly higher than those of the red ripening stage.

Tocopherols have exceptional antioxidant activity and therefore tend to increase during times of stress in plants (Falk and Munné-Bosch, 2010). In the present study, γ-tocopherol concentrations were influenced by K treatment in most of the ripening stages and cultivars (Tab. 3). The tomatoes with low K treatment had increased tocopherol concentrations in 2015, possibly due to the stress caused by the deficiency of K. This has been observed also for other abiotic stresses such as light, heavy metal, or drought stress (Lushchak and Semchuk, 2012). Caretto et al. (2008) detect the opposite effect, while another study by Fanasca et al. (2006) found no effect on α- and ß-tocopherol. As the tendencies differed between the years and contradicted other studies, it is likely that other abiotic factors influenced the tocopherol accumulation in tomatoes. Also, a two-way ANOVA showed significant interaction of year and K treatment for all tocopherols.

Conclusion

As a plant macronutrient, K plays a critical role in several physiological and biochemical pathways making the dependence of plants biochemical composition on K complex. Overall, it can be concluded from the results of this study that antioxidant concentrations in tomato fruit are affected by K fertilization, but other abiotic factors may reduce or even reverse those effects in an uncontrolled cultivation environment. General statements on the effects of K fertilization on tomato antioxidants should be avoided, as many results showed some kind of cultivar dependency. Nonetheless, the tendencies in changes of ascorbic acid, naringenin, *p*-coumaric acid, and caffeic acid are similar in both years for Primavera and Resi, indicating a strong K fertilization effect. The enrichment of tomatoes with certain antioxidants is possible by means of K supply, but this is dependent on the cultivar and environment.

Acknowledgments

The authors wish to thank the technical staff of the LC-MS metabolomics laboratory at the Department of Safety and Quality of Fruit and Vegetables of the Max Rubner-Institut Karlsruhe (Rita Bäuerle, Lara Frommherz) for their excellent technical assistance. We also thank the members of the Division Quality of Plant Products for their help during the pot experiment and laboratory measurements (Gunda Jansen, Evelyn Krüger, Bettina Egger) and furthermore Anna-Lena Lomberg and Eva-Maria Görk. In addition, we would like to thank Bernd Horneburg and his team for helping with the cultivation in the greenhouse.

Conflict of interest

No potential conflict of interest was reported by the authors.

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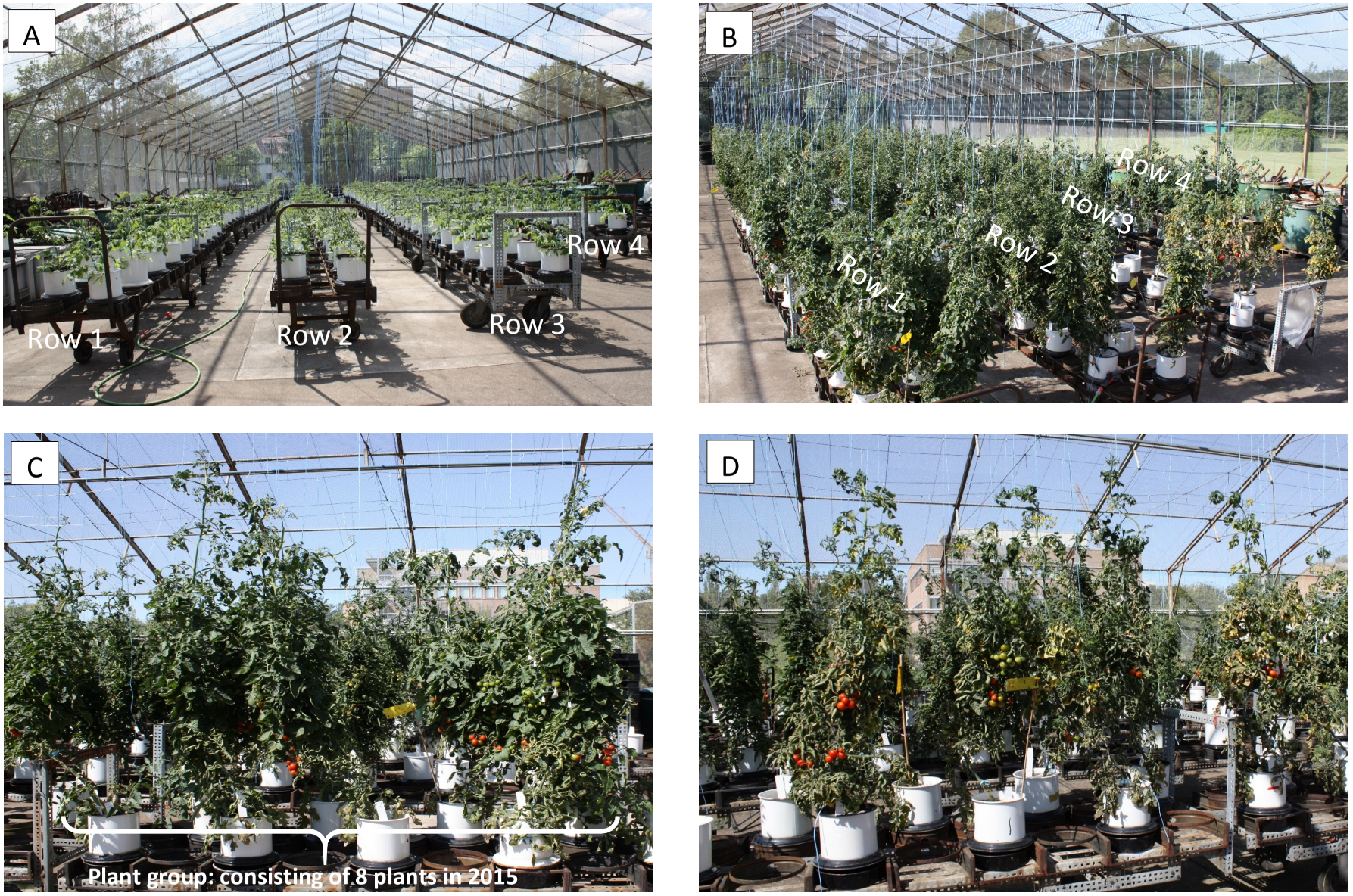

Figure S1: Randomized block design at the outdoor location in Göttingen. Plants are arranged in four blocks [(A) and (B)]. (A) shows the plants at the beginning of the outdoor experiment (May 22 in 2015) and (B), (C), and (D) towards the middle of the growing season (August 5 in 2015). Plants were grown in pots ("Mitscherlich vessels", 6 L volume) and pruned to one shoot. Eight plants comprise a plant group, as visible in picture (C). The plants of picture (C) were well-supplied with potassium (K5), while the plants of picture (D) were potassium-deficient (K1).

Table S1. Macro- and micronutrient fertilization of the cocktail tomato cultivars in the years 2014 (A) and 2015 (B).

A. Fertilization 2014

nutrients	chemical	per pot (g)	times applied during the outdoor season	time after planting
macronutrients				
N	$Ca(NO_3)_2 + NH_4NO_3$ (2:1)	110.8 + 18.8	weekly 18 times	starting week 7
K 1	K_2SO_4	7,3	weekly 20 times	starting week 7
K 2	K_2SO_4	14,7	weekly 20 times	starting week 7
K 3	K_2SO_4	26,0	weekly 20 times	starting week 7
K 4	K_2SO_4	37,4	weekly 20 times	starting week 7
K 5	K_2SO_4	60,0	weekly 20 times	starting week 7
Ca	$Ca(NO_3)_2$	sufficient supply by $Ca(NO_3)_2$		
P	$Ca(H_2PO_4)$	26,0	once	week 7
Mg	$MgSO_4 \bullet 7H_2O$	19,0	twice	week 7 and 15
S	K_2SO_4	sufficient supply by K_2SO_4		
micronutrients				
Cl	$MnCl_2 \bullet 4H_2O$	0.52	twice	week 7 and 15
Fe	Fe-EDTA	1,41	twice	week 7 and 15
Mn	$MnCl_2 \bullet 4H_2O$	sufficient supply by $MnCl_2 \bullet 4H_2O$		
Zn	$ZnSO_4 \bullet 7H_2O$	0.16	twice	week 7 and 15
B	H_3BO_3	0.21	twice	week 7 and 15
Cu	$CuSO_4 \bullet 5H_2O$	0.04	twice	week 7 and 15
Mo	$Na_2MoO_4 \bullet 2H_2O$	$4.72E^{-04}$	twice	week 7 and 15

All tomato plants received the same nutrient concentrations, except in the case of potassium (K). K levels increase from K1 to K5 for the different treatments.

B. Fertilization 2015

nutrients	chemical	per pot (g)	times applied during the outdoor season	time after planting
macronutrients				
N for K5	$Ca(NO_3)_2 + NH_4NO_3$ (2:1)	105.3 + 17.8	weekly 17 times	starting week 7
N for K1	$Ca(NO_3)_2 + NH_4NO_3$ (2:1)	61.0 + 10.3	every other week 11 times	starting week 7
K1	K_2SO_4	7,0	weekly 19 times	starting week 8
K5	K_2SO_4	69.6	weekly 19 times	starting week 8
Ca	sufficient supply by $Ca(NO_3)_2$			
P	$Ca(H_2PO_4)_2$	13.0	once	week 7
Mg	$MgSO_4 \bullet 7H_2O$	38.0	twice	week 7 and 16
S	sufficient supply by K_2SO_4			
micronutrients				
Cl	$MnCl_2 \bullet 4H_2O$	0.52	twice	week 7 and 15
Fe	Fe-EDTA	1,41	twice	week 7 and 15
Mn	sufficient supply by $MnCl_2 \bullet 4H_2O$			
Zn	$ZnSO_4 \bullet 7H_2O$	0.11	twice	week 7 and 15
B	H_3BO_3	0.43	twice	week 7 and 15
Cu	$CuSO_4 \bullet 5H_2O$	0.04	twice	week 7 and 15
Mo	$Na_2MoO_4 \bullet 2H_2O$	$9.45E^{-04}$	twice	week 7 and 15

All tomato plants received the same nutrient concentrations, except for potassium (K). K1 and K5 were weekly fertilized, but K1 received only one tenth. Nitrogen was applied in similar amounts but in different forms: $(NH_4)_2SO_4$ were given to K1 as sulfur compensation.

Table S2: Results of two-factorial analysis of variance (ANOVA) performed for the categorical variables "year" and "fertilization level" of each cultivar as well as each measurement variable.

		Primavera			Resi		
		year	fertilization	year * fertilization	year	fertilization	year * fertilization
ascorbic acid	F	2,35	13,17	16,82	0,77	33,24	1,88
	significance	0,151	0,003	0,001	0,396	0,000	0,195
***p* -coumaric acid**	F	10,09	33,91	4,71	26,16	42,54	8,06
	significance	0,008	0,000	0,051	0,000	0,000	0,015
caffeic acid	F	207,93	6,39	7,23	377,88	11,20	8,35
	significance	0,000	0,027	0,020	0,000	0,006	0,014
ferulic acid	F	10,26	0,08	1,16	17,92	3,45	0,05
	significance	0,008	0,784	0,303	0,001	0,088	0,824
sinapinic acid	F	1,08	0,00	0,02	12,58	0,00	0,45
	significance	0,319	0,998	0,886	0,004	0,980	0,515
quercetin	F	80,00	4,09	4,26	79,71	0,24	0,19
	significance	0,000	0,066	0,061	0,000	0,636	0,675
narigenin	F	4,64	8,80	3,75	3,88	1,47	1,30
	significance	0,052	0,012	0,077	0,072	0,249	0,276
β-carotene	F	0,14	0,59	9,59	0,00	0,17	0,01
	significance	0,716	0,458	0,009	1,000	0,689	0,908
lycopene	F	0,10	0,01	2,30	1,76	1,47	0,18
	significance	0,757	0,927	0,155	0,209	0,249	0,681
α-tocopherol	F				138,82	0,00	2,51
	significance				0,000	0,996	0,139
β-tocopherol	F						
	significance						
γ-tocopherol	F	50,13	2,47	8,36	180,83	1,68	4,95
	significance	0,000	0,142	0,014	0,000	0,219	0,046
δ-tocopherol	F	10,79	15,02	5,39			
	significance	0,007	0,002	0,039			

The values of K1 and K5 in 2014 and 2015 for Primavera and Resi were compared. The level of significance was p≤0.05. If there is no number, the concentration of the antioxidant was below the limt of quantification (LOQ).

Table S3: Results of multi-factorial analysis of variance (ANOVA) performed for the categorical variables "fertilization level", "cultivar", and "year"as well as each measurement variable.

		fertilization	cultivar	year	fertilization* cultivar	fertilization * year	cultivar* year	fertilization* cultivar * year
ascorbic acid	F	9,07	40,98	2,49	0,76	13,66	0,41	4,96
	significance	0,000	0,000	0,122	0,555	0,001	0,528	0,031
***p* -coumaric acid**	F	26,72	26,92	45,34	0,58	16,77	1,31	0,08
	significance	0,000	0,000	0,000	0,677	0,000	0,258	0,782
caffeic acid	F	7,64	95,63	1015,53	0,43	26,98	59,03	0,47
	significance	0,000	0,000	0,000	0,788	0,000	0,000	0,497
ferulic acid	F	0,776	53,682	34,477	1,076	0,249	2,880	0,819
	significance	0,547	0,000	0,000	0,381	0,620	0,097	0,371
sinapinic acid	F	0,21	3,25	7,06	0,42	0,21	0,46	0,03
	significance	0,934	0,079	0,011	0,791	0,652	0,499	0,860
quercetin	F	0,35	5,50	275,26	1,18	1,58	3,35	4,61
	significance	0,843	0,024	0,000	0,332	0,215	0,074	0,038
narigenin	F	5,04	0,99	8,77	3,59	4,91	0,03	0,35
	significance	0,002	0,326	0,005	0,013	0,032	0,871	0,555
β-carotene	F	0,47	26,46	0,11	0,59	8,10	0,11	7,15
	significance	0,758	0,000	0,741	0,671	0,007	0,741	0,011
lycopene	F	1,19	22,67	0,76	1,01	1,67	1,61	0,38
	significance	0,329	0,000	0,389	0,413	0,203	0,212	0,543
α-tocopherol	F	1,74	4,03	102,94		1,86		
	significance	0,180	0,058	0,000		0,187		
β-tocopherol	F							
	significance							
γ-tocopherol	F	2,06	356,03	87,22	1,33	8,26	2,55	2,54
	significance	0,103	0,000	0,000	0,275	0,006	0,118	0,119
δ-tocopherol	F	3,86		9,69		4,84		
	significance	0,017		0,005		0,039		

The values of the ripe fruits for Primavera and Resi were compared. The level of significance was p≤0.05. If there is no number, the concentration of the antioxidant was below the limt of quantification (LOQ).

Table S4: Lipophilic antioxidants are differentially affected by ripening stages in the two cocktail tomato cultivars grown in 2015.

cultivar	ripening stage	β-carotene (mg/100g FM)	lycopene (mg/100g FM)	α-tocopherol (mg/100g FM)	β-tocopherol (mg/100g FM)	γ-tocopherol (mg/100g FM)	δ-tocopherol (mg/100g FM)
Primavera	breaker	0,37 a	0,14 a			1,79 a	0,04 NS
	orange	1,03 b	8,18 b			1,40 a	0,04 NS
	ripe	1,12 b	15,57 c			1,04 b	0,04 NS
Resi	breaker	0,32 a	0,47 a	0,91 NS		0,30 ab	
	orange	0,84 b	11,92 b	1,04 NS		0,35 a	
	ripe	0,85 b	24,77 c	1,02 NS		0,25 b	

Mean values were determined from four biological replicates. Letters indicate statistically significant differences and NS indicates no significant difference according to a Mann-Whitney-U or Tukey-HSD test. The level of significance was p≤0.05. If there is no value, the concentration of the antioxidant was below the LOQ.

Table S5: t-test between K1 and K5 of the antioxidants for each cultivar in 2014 and 2015.

					Levene-test for equal variances			t-test significance (2-sided)
		K level	mean value	standard error	F	significance	decision	
2014	Primavera	ascorbic acid	K1	23,05	0,77	11,07	0,016	Variances are not equal	0,812
			K5	22,40	2,44				
		p -coumaric acid	K1	1,99	0,49	6,31	0,046	Variances are not equal	0,056
			K5	5,57	1,24				
		caffeic acid	K1	3,93	0,85	0,39	0,556	Variances are equal	0,582
			K5	3,31	0,63				
		ferulic acid	K1	3,01	0,57	0,73	0,426	Variances are equal	0,456
			K5	2,48	0,34				
		sinapinic acid	K1	1,43	0,26	0,13	0,732	Variances are equal	0,938
			K5	1,46	0,22				
		quercetin	K1	0,77	0,13	0,26	0,632	Variances are equal	0,698
			K5	0,85	0,15				
		naringenin	K1	8,80	2,48	2,31	0,179	Variances are equal	0,046
			K5	2,16	0,94				
		β-carotene	K1	1,31E-03	7,14E-05	0,12	0,739	Variances are equal	0,010
			K5	8,79E-04	9,10E-05				
		lycopene	K1	1,73E-02	1,87E-03	3,24	0,122	Variances are equal	0,423
			K5	1,45E-02	2,61E-03				
		α-tocopherol	K1						
			K5						
		β-tocopherol	K1						
			K5						
		γ-tocopherol	K1	1,60	0,13	3,89	0,096	Variances are equal	0,476
			K5	1,72	0,08				
		δ-tocopherol	K1	0,06	0,00	0,01	0,912	Variances are equal	0,337
			K5	0,05	0,00				
	Resi	ascorbic acid	K1	27,31	1,14	0,05	0,825	Variances are equal	0,030
			K5	31,80	1,12				
		p -coumaric acid	K1	4,41	0,71	0,51	0,502	Variances are equal	0,062
			K5	7,58	1,18				
		caffeic acid	K1	5,95	0,65	0,48	0,513	Variances are equal	0,033
			K5	8,08	0,42				
		ferulic acid	K1	3,77	0,38	4,33	0,083	Variances are equal	0,216
			K5	4,88	0,71				
		sinapinic acid	K1	1,60	0,07	0,80	0,405	Variances are equal	0,745
			K5	1,66	0,16				
		quercetin	K1	1,04	0,12	0,04	0,843	Variances are equal	0,434
			K5	1,17	0,11				
		naringenin	K1	5,91	2,01	0,01	0,917	Variances are equal	0,282
			K5	2,79	1,71				
		β-carotene	K1	7,96E-04	7,23E-05	0,79	0,408	Variances are equal	0,761
			K5	8,22E-04	4,26E-05				
		lycopene	K1	2,46E-02	3,42E-03	4,94	0,068	Variances are equal	0,281
			K5	2,06E-02	2,58E-04				
		α-tocopherol	K1	2,13	0,11	0,54	0,489	Variances are equal	0,380
			K5	2,29	0,13				
		β-tocopherol	K1						
			K5						
		γ-tocopherol	K1	0,62	0,02	2,72	0,150	Variances are equal	0,093
			K5	0,73	0,05				
		δ-tocopherol	K1						
			K5						
2015	Primavera	ascorbic acid	K1	19,51	0,82	0,16	0,705	Variances are equal	0,000
			K5	30,18	0,65				
		p -coumaric acid	K1	2,97	0,69	1,71	0,238	Variances are equal	0,002
			K5	10,82	1,26				
		caffeic acid	K1	49,01	5,53	0,00	0,992	Variances are equal	0,039
			K5	69,06	5,23				
		ferulic acid	K1	3,83	0,33	0,15	0,714	Variances are equal	0,476
			K5	4,14	0,24				
		sinapinc acid	K1	1,27	0,14	0,35	0,575	Variances are equal	0,876
			K5	1,24	0,10				
		quercetin	K1	22,47	3,90	6,57	0,043	Variances are not equal	0,130
			K5	14,42	0,54				
		naringenin	K1	3,26	0,49	1,38	0,285	Variances are equal	0,043
			K5	1,87	0,24				
	Resi	ascorbic acid	K1	26,80	0,66	2,23	0,186	Variances are equal	0,001
			K5	34,10	1,10				
		p -coumaric acid	K1	6,37	0,53	0,62	0,460	Variances are equal	0,000
			K5	14,41	0,87				
		caffeic acid	K1	83,10	6,29	0,08	0,785	Variances are equal	0,020
			K5	112,18	6,84				
		ferulic acid	K1	6,15	0,54	0,39	0,553	Variances are equal	0,261
			K5	7,02	0,44				
		sinapinc acid	K1	1,33	0,05	0,50	0,507	Variances are equal	0,318
			K5	1,27	0,03				
		quercetin	K1	21,98	3,23	0,49	0,508	Variances are equal	0,663
			K5	24,24	3,72				
		naringenin	K1	1,78	0,23	0,85	0,391	Variances are equal	0,734
			K5	1,69	0,13				

The level of significance was p≤0.05. If there is no value the concentration of the antioxidant was below the LOQ. A red background indicates a significance. K levels were low (K1) and high (K5) fertilization (0.37 g and 3.66 g per week).

Table S6: Potassium values are differentially affected by fertilization in the two cocktail tomato cultivars grown in 2015.

Cultivar	ripening stage	fertilization level	K (% in DM)	
Primavera	breaker	K1	1,39	0,11 a
		K5	2,93	0,14 b
	orange	K1	1,55	0,10 a
		K5	3,10	0,22 b
	ripe	K1	1,46	0,06 a
		K5	3,04	0,24 b
Resi	breaker	K1	1,67	0,03 a
		K5	2,54	0,16 b
	orange	K1	1,63	0,03 a
		K5	2,72	0,23 b
	ripe	K1	1,67	0,13 a
		K5	2,89	0,12 b

Mean values and standard deviation were determined from four biological replicates. Letters indicate statistically significant differences (statistical test: t-test). The level of significance was p≤0.05.

Table S7: Color values of the three ripening stages breaker, orange, and ripe in the two cocktail tomato cultivars grown in 2015.

Cultivar	ripening stage	L*	a*	b*
Primavera		58,43 ± 8.04 A	10,78 ± 13.47 NS	25,84 ± 4.47 A
	breaker	69,07 ± 1.35 a	-7,42 ± 1.84 a	30,47 ± 1.52 a
	orange	55,50 ± 1.49 b	17,81 ± 2.43 b	26,35 ± 1.99 b
	ripe	50,73 ± 1.22 c	21,95 ± 2.96 c	20,69 ± 2.10 c
Resi		64,88 ± 8.98 B	16,73 ± 16.38 NS	31,24 ± 4.10 B
	breaker	76,42 ± 2.69 a	-5,40 ± 3.19 a	33,14 ± 3.43 a
	orange	62,58 ± 0.72 b	24,23 ± 1.09 b	33,96 ± 1.64 a
	ripe	55,64 ± 1.13 c	31,36 ± 1.42 c	26,61 ± 1.90 b

Mean values and standard deviation were determined from four biological replicates. Upper-case letters indicate statistically significant differences (statistical test: t-test) between the cultivars. Lower-case letters show the statistical difference between the three ripening stages (ANOVA followed by Tukey-HSD test). The level of significance was p≤0.05.

Table S8: Averaged results of sunshine duration, average daily temperature, relative humidity, and precipitation along with results of two-sided analysis of variance (ANOVA) performed for the categorical variables and "year".

		sunshine duration (h)	average daily temperature (°C)	relative humidity (%)	precipitation (mm)	sunshine duration (h) * year	average daily temperature (°C) * year	relative humidity (%) * year	precipitation (mm) * year
overall year	2014	5,541	15,576	78,397	2,802				
	2015	6,162	15,764	74,707	1,988				
	F					1,724	0,184	15,649	1,803
	Significant					0,190	0,668	0,000	0,180
May	2014	5,541	15,576	78,397	2,802				
	2015	6,162	15,764	74,707	1,988				
	F					1,724	0,184	15,649	1,803
	Significant					0,190	0,668	0,000	0,180
June	2014	5,541	15,576	78,397	2,802				
	2015	6,162	15,764	74,707	1,988				
	F					1,724	0,184	15,649	1,803
	Significant					0,190	0,668	0,000	0,180
July	2014	5,541	15,576	78,397	2,802				
	2015	6,162	15,764	74,707	1,988				
	F					1,724	0,184	15,649	1,803
	Significant					0,190	0,668	0,000	0,180
August	2014	5,541	15,576	78,397	2,802				
	2015	6,162	15,764	74,707	1,988				
	F					1,724	0,184	15,649	1,803
	Significant					0,190	0,668	0,000	0,180
September	2014	5,541	15,576	78,397	2,802				
	2015	6,162	15,764	74,707	1,988				
	F					1,724	0,184	15,649	1,803
	Significant					0,190	0,668	0,000	0,180

"Overall year" includes the months May until September which represents the outdoor cultivation period of the plants. ANOVA was performed to evaluate the effect of year on climate. The data was provided from German Weather Service and values were edited. The level of significance was p≤0.05.

Table S9: t-test between the years 2014 and 2015 of the climate variables sunshine, temperature, relative humidity, and precipitation calculated as mean values for the period May until September.

				Levene-test for equal variances			t-test
	year	mean value	standard error	F	significance	decision	significance (2-sided)
sunshine duration (h)	2014	5,60	1,07	0,03	0,867	Variances are equal	0,082
	2015	8,40	1,13				
average daily temperature (°C)	2014	19,15	0,41	1,18	0,287	Variances are equal	0,005
	2015	21,42	0,63				
relative humidity (%)	2014	79,13	1,75	3,86	0,060	Variances are equal	0,110
	2015	73,43	2,97				
precipitation (mm)	2014	2,78	1,22	4,42	0,045	Variances are not equal	0,363
	2015	6,26	3,52				

The data was provided by German Weather Service and values were edited. The level of significance was $p \leq 0.05$.