Influence of different potassium fertilization regimes on quality aspects and yield of cocktail tomato cultivars

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List of relevant abbreviations

Abbreviation	*Full Name*
ABA	abscisic acid
DM	dry matter
EU	European Union
FAO	Food and Agricultural Organization of the United Nations
FM	fresh matter
GABA	*gamma*-aminobutyric
GC-MS	gas chromatography - mass spectrometry
HPLC	high performance liquid chromatography
K_2SO_4	potassium sulphate
K	potassium
K1	potassium level 1 → 0.4 g K_2SO_4 weekly fertilization
K2	potassium level 2 → 0.7 g K_2SO_4 weekly fertilization
K3	potassium level 3 → 1.5 g K_2SO_4 weekly fertilization
K4	potassium level 4 → 2.2 g K_2SO_4 weekly fertilization
K5	potassium level 5 → 3.7 g K_2SO_4 weekly fertilization
Kd	potassium depletion level → 3.7 g K_2SO_4 for the first 5 weeks
MRI	Max-Rubner-Institute
P	tomato cultivar Primavera
R	tomato cultivar Resi
ROS	reactive oxygen species
TA	titratable acids
TCA	tricarboxylic acid or citric acid cycle
TSS	total soluble solids
YS	tomato cultivar Yellow Submarine

1. Introduction

1.1. Tomato background

1.1.1. Origin and history of tomato production

Tomatoes (*Solanum lycopersicum* L.) are among the most popular vegetables worldwide. They are consumed not only raw but also in a variety of processed forms, such as pasts, sauces and juices. The wild forms of the today cultivated tomatoes are native to the coast and Andes of western South America (Bai and Lindhout 2007; Paran and van der Knaap 2007; Bergougnoux 2014). In the past there were two main hypotheses discussing the beginning of domesticating, either in their native region or in Mexico (Paran and van der Knaap 2007; Bergougnoux 2014). More recent genetic studies suggest that a pre-domestication took place in the Andean region and then domestication continued in Mexico by the Aztecs. The tomatoes that were introduced to Europe by Spanish conquistadors originated from Mexico (Blanca et al. 2012). This is also evident in the term tomato, which derives from the name "tomatle" of the Aztecs language Nahuatl. Originally, this refers to fruits or berries, with many seeds and juicy flesh and was mainly used for physalis but also for tomatoes (José Díez and Nuez 2008). Already in 1544, a first written record of the cultivated tomato appeared in Italy (Paran and van der Knaap 2007). It developed into a commonly consumed vegetable around 1840, previously it was believed to be poisonous (Paran and van der Knaap 2007) and was used as an ornamental fruit (Bergougnoux 2014). Tomato cultivation in a larger scale started at the beginning of the last century, with a massive increase in consumption after the first world war (Thakur et al. 1996a). The worldwide production is still increasing and has even doubled in the time from 1994 to 2014. The most important producer in the nineties were the USA, whereas now Asia and especially China have the highest production rates (FAOSTAT 2019). However, the most productive areas in terms of yield per hectare are the under glass productions of northern Europe (Bergougnoux 2014). Tomatoes account for 14.6 % of the vegetable world market with an annual production of 170.8 million tons in 2014 (FAOSTAT 2019). With a per capita consumption of 25 kg per year is the tomato, the most consumed vegetable in the EU and in Germany (Sutor et al. 2014).

1.1.2. Taxonomic background

The tomato is part of the family of Solanaceae; this taxon includes many economic important species like potatoes, eggplants, petunias, tobacco, peppers and physalis (Paran and van der Knaap 2007; Bergougnoux 2014). The genus "tomato" comprises more than one specie. The classification of tomatoes has been a subject of debate in the past. In the last decade a major revision has taken place. The genus *Lycopersicon* was re-integrated into the *Solanum* genus (José Díez and Nuez 2008). Beforehand the cultivated tomato was referred to as *Lycopersicon esculentum* Mill.. Today the name *S. lycopersicum* L. from the Linnaeus classification is again in use. Currently eleven species have been identified next to the cultivated tomato species (Bergougnoux 2014). In this thesis, the cultivated tomato species *S. lycopersicum* is the subject of research.

1.1.3. Cultivation

Through the process of tomato cultivation various different plant and fruit forms have evolved. In general, all tomato plants form a sympodial branched shoot with compound leaves (Figure 1). Some cultivars have simpler leaves, so called potato leaves. Branching occurs by lateral drives, which appear at the leaf axes. In order to harvest more fruits, the plants are pruned to one or two shoots (Henriques da Silva et al. 2008). On an inflorescence are several flowers forming a vine. The individual vines grow successively on the plant (Heuvelink 1996; Henriques da Silva et al. 2008). At the same time the vines can have fruits of different developmental stages and blossoms (Figure 2). The flowers are self-fertile, but vibration is needed to release the pollen. There is a large variation of color, size and shape of the tomato fruit. All tomatoes are characterized by a fleshy fruit, an epidermis, a thick pericarp and the tomato typical gel like placental tissue surrounding the seeds (Bergougnoux 2014).

A major change in the tomato breeding was the introduction of the first hybrid tomato cultivar 'Single Cross' in 1946 (Bai and Lindhout 2007). Today most of the sold tomatoes derive from hybrid tomato plants, which have a high vitality and yield. The goals of the tomato breeding program have changed over the decades. In the 1970s yield increase was the major goal, whereas in the 1980s breeders aimed for a longer shelf-life, then for taste in the 1990s and currently the nutritional value is most important (Bai and Lindhout 2007).

Commonly yield is expressed as tons per hectare for field experiments (Hartz et al. 2005). In greenhouse or pot experiments the term gram or kilogram per plant is frequently used for yield (Constán-Aguilar et al. 2015). This study is a pot experiment and hence gram and number per plant were assessed.

1.1.4. Fruit ripening and formation of color and firmness

The tomato fruitification can be divided into four major processes: the floral phase, the development of the fruit by cell division, the cell enlargement and the fruit ripening (Bergougnoux 2014). Tomatoes are climacteric fruits: at the onset of ripening, respiration and the biosynthesis of ethylene increase. This induces several physiological processes such as accumulation of sugars and volatile compounds or loosening of the cell wall (Bergougnoux 2014). Visually noticeable is the color change from green to red. Responsible for this color change are the degradation of chlorophyll and simultaneously enrichment of lycopene in the tomato plastids. These plastids turn from chloroplasts into chromoplasts. Within the plastids the thylakoid membranes are disassembling and the formation of carotenoid crystals takes place (Egea et al. 2010). This color transition has been divided into different ripening phases, ranging from five (Jimenez et al. 2002) to seven (Arias et al. 2000; Gautier et al. 2008) ripening stages. All authors identify a mature green, breaker, orange, light red or pink and a mature red phase (Arias et al. 2000; Jimenez et al. 2002; Gautier et al. 2008).

Lycopene is the dominating pigment in red tomato fruits, while ß-carotene is responisbel for a yellow color (D'Souza et al. 1992; Arias et al. 2000; Egea et al. 2010). Other pigments, such as ß-carotenes or flavonoids, affect the red tone (Ballester et al. 2010). Along with the color-change, the fruit firmness decreases and the fruit becomes more deformable (Kader et al. 1978). This fruit softening is associated with cell wall modifications (Haeder and Mengel 1972; Sozzi et al. 1998), the loss of cell-to-cell adhesion, and increased contents of water-soluble pectin (Bourne 1979; Sams 1999). Further physiological mechanisms play a crucial role for the fruit firmness such as turgor, cell size and shape, and the overall fruit anatomy (Johnston et al. 2002).

1.1.5. Composition of tomato fruits

Consumers favor tomatoes that are bright red colored, firm, and medium to small in size. They should be flavorful, juicy, sweet and sour in taste, and contain only few seeds (Causse 2002; Piombino et al. 2013; Oltman et al. 2014). Consequently are color, firmness, dry matter (DM) and the primary metabolites sugar and acid important tomato fruit traits (Stevens et al. 1979; Causse 2002; Oltman et al. 2014). The nutritional value of tomatoes is rather low if only proteins, lipids and carbohydrate content are considered (Thakur et al. 1996b; Bergougnoux 2014). Nevertheless tomatoes are an important source of antioxidants and vitamins, such as ascorbic acid or carotenoids (Hernández et al. 2007; Capanoglu et al. 2008; Ehret et al. 2013; Valdez-Morales et al. 2014; Knecht et al. 2015).

The DM of a tomato fruit ranges between 5 to 10 % of the fresh weight (Wang et al. 1993; Thakur et al. 1996b). Approximately 50 % of DM are reducing sugars (Thakur et al. 1996b); organic acids account for 15 % of the DM (Yilmaz 2001). The remaining 35 % consist of proteins, cell wall cellulose, hemicelluloses, minerals, antioxidants, and lipids (Thakur et al. 1996b). The accumulation of DM in tomato fruits depends on its sink strength (Heuvelink 1996). In general, developing tomato fruits are very strong sinks for carbohydrates (Ho et al. 1987). Most photo-assimilates in the fruit are supplied by the leaves (Cocaliadis et al. 2014).

1.1.5.1. Primary metabolites

Tomato flavor comprises first a balance between the taste attributes sweetness and acidity, and low or no astringency and second the aroma or rather the concentrations of odor-active volatile compounds (Yilmaz 2001; Kader 2008).

The main sugars in ripe tomatoes are the monosaccharides glucose and fructose and they account for 2 to 4 % of the fresh weight (Klee and Giovannoni 2011). Sugars increase during the ripening process while acids are decreasing. The highest values for titratable acidity (TA) are in mature green fruits and the lowest in mature red fruits (Gautier et al. 2008). The main acids in ripe tomato fruits are citric acid and malic acid (Beckles 2012). Sugars and acids can be analyzed by HPLC. This method delivers exact quantities. However, analyzing sugars as total soluble solids (TSS) with a refractometer and acids by titration is more cost and time efficient. TSS are the dissolved solids in a solution and can be expressed as

refractometric index brix (Thakur et al. 1996b; Beckles 2012). In the past, studies showed a good correlation between sugar and TSS. Thus TSS is used as a proxy for sugar in fruits (Jones and Scott 1983). Most TSS are sugars (hexoses and sucrose; 65 %), followed by acids (citrate and malate; 13 %) and other minor components (phenols, amino acids, soluble pectins, ascorbic acid and minerals) in tomato fruits. In small tomatoes, such as the cocktail tomatoes, TSS is very high ranging from 9 to 15 %, while large beefsteak tomatoes have values of 3 to 5 % (Beckles 2012).

The acidity, described as TA, derives mostly from citric and malic acid. Beside there are certain amino acids and other organic acids contributing to the acidity (Paulson and Stevens 1974). The organic acids have several important functions in plant cells. They are part of the citric acid cycle, thus involved in energy production and in the non-cyclic mode precursor of multiple other metabolites (Etienne et al. 2013). Most of these organic acids are stored in the vacuoles and responsible for the acidic nature of the fruits (Shiratake and Martinoia 2007). Higher sugar and acid concentrations, such as in cocktail tomatoes, result in a better taste (Causse 2002).

1.1.5.2. Secondary metabolites

There are numerous secondary components in tomato plants, which have specific functions for plant survival and reproduction. Some of these components are antioxidants, such as ascorbic acid, phenolic compounds, carotenoids and tocopherols. Tomatoes are rich in antioxidants (Dumas et al. 2003), which are important for the human diet as they can prevent cardiovascular diseases and cancer (Liu et al. 2009). Hence tomatoes are considered to be healthy. In this thesis two important water-soluble antioxidants (ascorbic acid and phenolics) as well as two fat-soluble antioxidants (carotenoids and tocopherols) were analyzed.

1.1.5.2.1. Ascorbic acid

The concentration of ascorbic acid exceeds that of other antioxidants. It is therefore the major antioxidant present in plants cells and one of the most important contributor to the cellular redox state (Gallie 2013). Ascorbic acid (ascorbate) is a ketolactone with two ionizable hydroxyl groups and is therefore water soluble. As an excellent reducing agent it donates one electron to form relatively unreactive ascorbate radicals and another electron

to become dehydroascorbic acid (Du et al. 2012). The ascorbate biosynthesis via D-mannose-L-galactose in plants has been discovered in the late 1990s (Wheeler et al. 1998).

As an antioxidant, ascorbic acid is involved in the detoxification of reactive oxygen species (ROS), and serves as an enzyme cofactor for example during photosynthesis, phytohormone biosynthesis and controls the cell growth. It catalyzes the conversion of violaxanthin to zeaxanthin, proline and lysine hydroxylases or the regeneration of tocopherol from the tocopheroxyl radicals (Smirnoff and Wheeler 2000; Gallie 2013). Ascorbic acid, also known as Vitamin C, has several functions in the human body. These functions are connected to electron donation and ROS quenching or to collagen hydroxylases. But, other than most animals, humans as well as apes, guinea pigs and fruit eating bats can't synthesize ascorbic acid (Du et al. 2012) due to a mutation in the gene encoding L-gulono-1,4-lactone oxidase, the last step of the ascorbate synthesis (Naidu 2003). Vitamin C deficiency is known as the disease scurvy (Naidu 2003). The name ascorbic acid derived from its function in preventing scurvy. A sufficient ascorbic acid intake is associated with a reduced risk of chronical illnesses (Maramag et al. 1997; Naidu 2003; Mente et al. 2009).

1.1.5.2.2. Phenolics

Among the secondary metabolites, the phenolics are the largest group (Grassmann et al. 2002). There are several thousand phenolic structures discovered within the Plant Kingdom (Crozier et al. 2009; Del Rio et al. 2013) All phenolic compounds possess one or more aromatic rings and one or more hydroxyl groups (Liu et al. 2004). The group of phenolics comprises several subgroups, such as phenolic acids, acetophenones, phenylacetic acid, hydroxycinnamic acids, coumarins, xanthones, stilbenes or flavonoids (Crozier et al. 2009).

As a result of the large diversity, phenolic compounds have many different functions within the plant, such as defense mechanisms against pathogens, parasites and predators, reproduction and growth, as well as contribution to the color of plants (Liu et al. 2004). Consequently, phenolics contribute to the overall fitness of plants (Grassmann et al. 2002). In tomatoes, the most abundant phenolic compounds are chlorogenic acids and related metabolites (Martínez-Valverde et al. 2002; Slimestad and Verheul 2009). Within the group

of flavonoids naringenin (45 %) and quercetin (39 %) are the most common flavonoids in red tomatoes (Slimestad and Verheul 2009).

Flavonoids account for approximately two thirds of the phenolics in the human diet and the remaining third are manly phenolic acids (Liu et al. 2004). Phenolic compounds are not traditional vitamins and are not essential for short-term human health (Del Rio et al. 2013). However, a diet rich in phenolic compounds, e.g. the Mediterranean diet with a wide variety of vegetables, cereals, fruits, fish and others (Tripoli et al. 2007), is associated with a reduced risk of chronical diseases (Liu et al. 2004).

1.1.5.2.3. Carotenoids

A striking characteristic of all carotenoids is the color, ranging from yellow to red, which is due to the physical property of a polyene chain with several conjugated double bounds that function as a chromophore (Ruiz-Sola and Rodríguez-Concepción 2012). Their production takes place in the plastids (Bramley 2002). Tetraterpene isoprenoid molecules derived from isopentenyl diphosphate and form a 40-carbon strong phytoene (Ruiz-Sola and Rodríguez-Concepción 2012). Carotenoids are divided into the carotenes and the xanthophylls. Lycopene is precursor of the carotenes and has no ring form (DellaPenna and Pogson 2006). The xanthophylls, such as lutein or zeaxanthin, are formed by oxygenation of carotenes (Bramley 2002; Liu et al. 2009). During ripening of tomatoes, the expression of several genes coding for proteins involved in carotenogenesis changes, especially the levels of cyclases are drastically reduced. As the cyclases are responsible for the formation of ß-caroten from lycopene, this results in an accumulation of lycopene (Bramley 2002).

Carotenoids are found in all photosynthetic tissues (Bramley 2002). Xanthophylls participate in light harvesting at the photosynthetic membranes of the chloroplast. In case of excessive light they protect the photosynthetic apparatus by quenching triplet chlorophylls and superoxide anion radicals and singlet oxygen (Bramley 2002; Ruiz-Sola and Rodríguez-Concepción 2012).

ß-carotene is the most abundant carotene in chloroplasts whereas lycopene occurs in chromoplasts of some flowers and fruits (DellaPenna and Pogson 2006). Furthermore, ß-carotene is like the xanthophylls part of photosynthetic apparatus and responsible for

photoprotection by directing energy away from chlorophyll (Ruiz-Sola and Rodríguez-Concepción 2012).

Lycopene is the most abundant carotene in ripe tomatoes. Approximately 85 % of the dietary lycopene results from tomato-based products (Bramley 2000). Several studies have shown that lycopene has preventive properties against chronical illnesses, as it has one of the highest quenching capacities (Di Mascio et al. 1990). This has been shown by clinical trials (Shen et al. 2007) and rat feeding experiments (Liu et al. 2009). Beta-carotene is the most potent precursor of Vitamin A (Ruiz-Sola and Rodríguez-Concepción 2012). Vitamin A deficiency can lead to xerophthalmia, blindness and premature death and is today the most common dietary problem affecting children worldwide (Bramley 2002).

1.1.5.2.4. Tocopherols

There are four different tocopherols, specifically α-, β-, γ- and δ-tocopherol. Their structure comprises a 6-chromanol ring system and a saturated polyprenyl side chain. The four tocopherols differ by the number and position of the methyl group on the chromanol ring system (Wagner et al. 2004; Lushchak and Semchuk 2012). The four tocotrienols (α-, β-, γ- and δ-) are structurally similar, only their side change is desaturated (Lushchak and Semchuk 2012). Together they are called tocochromanols. Their tail derived from the plastidic isoprenoid synthesis, just like the carotenoids (DellaPenna and Pogson 2006). Homogentisic acid, synthesized via the cytosolic shikimate pathway, is the precursor of the chromanol ring (Lushchak and Semchuk 2012). The two parts are connected by a prenyltransferase to 2-methyl-6-phytylbenzoquinol, which is already the precursor for δ-tocopherol (Wagner et al. 2004).

The main tocopherol in green leaves is α-tocopherol, while in seeds, nuts and fruits γ-tocopherol is dominant (DellaPenna and Pogson 2006; Lushchak and Semchuk 2012). Tocopherols with their strong antioxidative power protect plants against ROS (Shao et al. 2008; Jin and Daniell 2014). As the level of ROS increases in response to abiotic stress (Cakmak 2005), the tocopherol concentration is also related to stress intensity and plant physiological state (Lushchak and Semchuk 2012). However, in tocopherol-deficient plants germination and seedling growth is damaged (Lushchak and Semchuk 2012; Falk and Munné-Bosch 2010).

Tocochromanols are lipophilic antioxidants, and as Vitamin E essential for human diet (Wagner et al. 2004). The highest vitamin E activity has α-tocopherol, because this is preferably absorbed by the human body (Hosomi et al. 1997). Further functions of tocopherol in human body are influences on lipid-derived signaling molecules, membrane-associated signaling pathways, and gene expression (DellaPenna and Pogson 2006).

1.2. Potassium in Plants

The nutritional status of a plant is a complex interaction of essential minerals and several other chemical structures that are either beneficial or harmful to the plant's metabolism. The individual need varies between different species and also between cultivars and is influenced by external factors such as climate and soil status (Passam et al. 2007). Nitrogen, phosphorus, potassium (K), calcium, sulfur and magnesium are needed in larger quantities and therefore called macro-nutrients. Boron, iron, manganese, copper, zinc, chlorine and molybdenum on the other hand are needed in smaller amounts and hence called micro-nutrients (Sainju et al. 2003).

The macro-nutrition K belongs to the first group of the periodic table of elements, the alkali metals. As an alkali metal is K relatively soft, has a low boiling and melting point and reacts fast with oxygen in water and air (Mortimer and Müller 2007). In the earth´s crust K is the seventh or eighth most abundant element with a concentration of 2.1–2.3 % (Wedepohl 1995). The soil K reserves in the world are large. However, not all agriculturally used soils have sufficient K availability, including ¾ of the paddy soils of China, and ⅔ of the soils of the Southern Australian wheat belt (Römheld and Kirkby 2010). On a global scale, the above ground phytomass contains 75, 14, and 60 million tons of nitrogen, phosphorus and K, respectively. The nutrition application for nitrogen and phosphorus are almost equal to the removal, but only 35 % of the K removal is replenished (Römheld and Kirkby 2010). In contrast to nitrogen, which can also be fixed by bacteria, K can naturally only be released by weathering of the parental rock (Coskun et al. 2015).

Potassium is vital for all plants and it fulfils several physiological functions that affect plant growth, tolerance to abiotic and biotic stress or movement of plant organelles (Ahmad and Maathuis 2014). The uptake of K is highly selective and is enabled together with transport through-out the plant by integral membrane proteins such as transporters and cation

channels (Hawkesford et al. 2012). It is not integrated into chemical structures of plant molecules but is the most abundant inorganic cation in plant tissues (Römheld and Kirkby 2010; Coskun et al. 2015). A characteristic of K is its mobility within individual cells, different plant tissue, as well as in long-distance transport via the xylem and phloem (Hawkesford et al. 2012). The cytosol concentration of K is maintained at 100– 200 mM as well as in the chloroplasts. The concentration of K within the vacuole may vary between 10 and 200 mM (Hawkesford et al. 2012). Several functions concerning osmoregulation and cell extension, stomatal movement, activation of enzymes, protein synthesis, photosynthesis, phloem loading and transport and uptake have been identified (Mengel 2009; Hawkesford et al. 2012; Zhao et al. 2018). Some of these functions depend on changing cytoplasmic K concentrations (Amtmann et al. 2008). During periods of mild or short term K deficiency plants are very efficient in redistributing K within the plant (Amtmann et al. 2008). This is controlled by several factors including the plant hormone abscisic acid (ABA) (Ahmad and Maathuis 2014). Also, the expression of genes encoding high-affinity K^+ transporters in roots increases in plants suffering under low K^+ conditions (Shin and Schachtman 2004).

Under harsh environmental conditions, such as drought, salinity, high or low temperatures, excess light and inadequate mineral nutrient supply, yield of diverse plants can decrease up to 80 % (Cakmak, 2005). Plants have developed a wide range of mechanisms to survive varying environmental conditions. The mineral status, especially K, is important for resistance against environmental stress (Cakmak, 2005). A beneficial effect of adequate to high K fertilization has also been described for biotic stresses: the damage by fungal and bacterial diseases but also by insect pests, but not viral infections, were reduced in crops (Amtmann et al. 2008).

Tomato plants specifically demand relatively high fertilizer amounts of K nutrition (Luiz et al. 2015). The specific need varies between tomato cultivars and is influenced by external factors, such as cultural practice and environmental conditions (Passam et al. 2007). During periods of plant stress, especially if K is insufficiently available, the production of ROS increases (Zhao et al. 2018). This leads to an impaired photosynthetic CO_2 fixation and reduced use of photo assimilates. Thereby the production of ROS increased in K deficient leaves, which leads to photooxidative damage (Cakmak, 2005). Subsequently leading to leaf chlorosis and necrosis as visible on the K deficient tomato leaves (Figure 1 A). On the

fruits, yellow or green areas around the fruit petiole arise when K is lacking (Figure 1 B), this is called Yellow shoulder syndrome (Hartz et al. 2005; Zhang et al. 2015). The tomatoes of Figure 1 B have only a very mild from, in severe cases the upper half of the fruit is insufficiently colored.

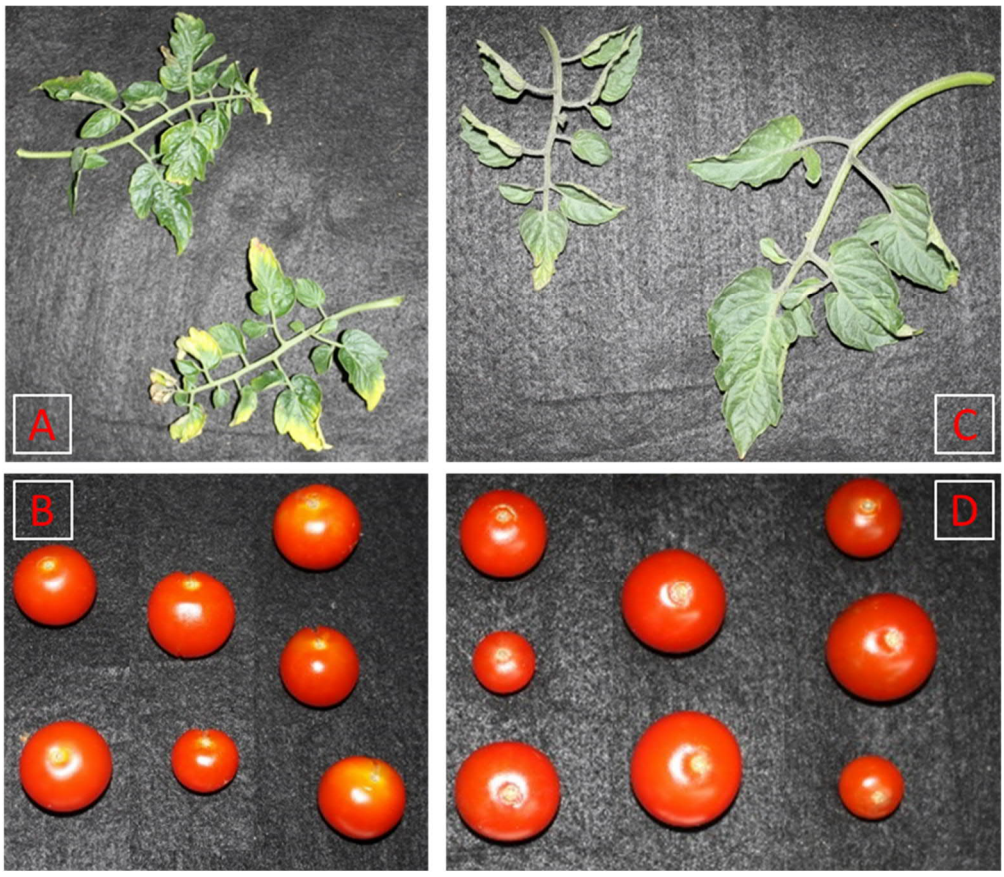

Figure 1. Leaves and fruits of the cultivar Primavera. A and B: leaves and fruits of K deficient plants; C and D: leaves and fruits of plants with K oversupply. (Picture: F. Sonntag)

1.3. Metabolomics

Beside the known effects of K concerning activation of enzymes, protein synthesis, and photosynthesis, it is interesting to evaluate further metabolic changes of rising K application. A metabolic study analyses the low-molecular-weight molecules or metabolites of a cell or organism (Osorio et al. 2011). However not all low-molecular-weight molecules can be analyzed with a single method. Different technological approaches are used today. Gas chromatography mass spectrometry (GC-MS) is a widely applied technology platform in metabolomic studies, while liquid chromatography and nuclear magnetic resonance spectroscopy are also relatively common (Osorio et al. 2011). A GC-MS covers a relatively wide range of low-molecular-weight molecules. Consequently

this technology can comprehensively characterize the untargeted metabolites of a biological system, that are affected by internal and external factors (Weinert et al. 2015). For this study a two-dimensional gas chromatography (GCxGC-MS) was used, which has an excellent separation performance and high sensitivity along with a good long-term repeatability (Weinert et al. 2015). In the past, it was used to demonstrate the influence of Alternaria alternate infections on chlorogenic acids in tomatoes and through postharvest ripening induced changes in the organic acid concentration of kiwi fruits. Overall changes of amino or organic acids, polyphenols or sugars were detected along with some metabolites of other categories (Wojciechowska et al. 2014; Mack et al. 2017). Therefore, this analytical method should be able to detect changes in tomato composition induced by differing nutritional supply.

1.4. Relation between plant metabolites and K supply and hypotheses

A sufficient mineral supply is an important abiotic factor to assure survival and reproduction of the plants. Consequently, K deficiency should, as explained above, negatively affect yield and the concentration of sugar, acids and antioxidants in tomato plants (Smirnoff and Wheeler 2000; Wuzhong 2002; Hartz et al. 2005; Taber et al. 2008; Slimestad and Verheul 2009; Tavallali et al. 2018). Nontheless, there are contradictornary studies that do not confirm this influence but rather show a opposite trend with elevated application of the macronutrient K (Fanasca et al. 2006; Serio et al. 2007; Caretto et al. 2008). Additionally, different tomato cultivars had varying metabolite concentrations (George et al. 2004; Anza et al. 2006; Slimestad and Verheul 2009; García-Valverde et al. 2013). Thus, are different cultivars responisbel for the contradictonary results?

A metabolomic study showed that several pathways were changed in K deficient tomato seedlings, mainly sugar metabolism, glycolysis, TCA cycle and nitrogen assimilation. In the same study, some components were differently affected in roots and shoots (Sung et al. 2015) so that it is very likely that tomato fruits show as well specific changes.

Small-fruited tomato cultivars, so called cocktail tomatoes, contain more antioxidants than large-fruited cultivars (George et al. 2004; Slimestad and Verheul 2009) and higher sugar and acid concentrations (Beckles 2012). As cocktail tomatoes are well liked by consumers (Laber and Lattauschke 2014), their reaction to increasing K fertilization should be tested.

To adresss thouse questions, we conducted an experiment with three different cocktail tomato cultivars (Figure 2) over two consecutive years in an outdoor environment. The plants were treated with low and high K doses in both years.

Figure 2. Tomato fruits on the vine of the three cultivars. From left to right: Primavera, Resi, and Yellow Submarine. (Picture: F. Sonntag)

2. Improvement of cocktail tomato yield and consumer-oriented quality traits by potassium fertilization is driven by the cultivar

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Abstract

The market for cocktail tomatoes is growing continuously, mainly because of their good taste. Titratable acids (TA), total soluble solids (TSS) and dry matter (DM) contents correlate positively with good taste. So far, the impact of different potassium (K) applications on yield and consumer-oriented quality traits has not been comprehensively described for cultivars of smaller fruits. To fill this gap, we tested the effect of different K fertilization regimes on three cultivars of small-sized or cocktail tomatoes. A positive impact on quality parameters (TSS, TA, DM, color and firmness) was detected by raising the K fertilizer application for the cocktail tomato cultivars Primavera and Yellow Submarine. The cultivar Resi showed no response to fertilization, except for TSS and TA. Yield increased significantly by higher K application only in Primavera, the most productive cultivar. The K concentration in tomato rose with increasing K application. Because TSS and TA increased in all cultivars, whereas only one cultivar showed an increase in yield, the results of the present study emphasize the importance of the application of cultivar-specific mineral fertilizers on yield and consumer-oriented quality traits.

Keywords

Solanum lycopersicum L.; potassium; total soluble solids; titratable acids; color; firmness

Introduction

Tomato (*Solanum lycopersicum* L.) is the most important vegetable in the world, with an annual production of 177 million tons in 2016 (FAOSTAT 2019). Likewise, in the European Union, where 17.9 million tons were produced in 2016, tomatoes are among the most produced vegetables (Sutor et al. 2014). The sales of small-sized tomatoes, comprising the so-called cocktail tomatoes, have increased because consumers describe them as tastier (Laber and Lattauschke 2014). Consumer surveys from North Carolina and Italy reveal that an attractive tomato is brightly colored (mostly red), firm and medium- to small-sized.

Furthermore, it was discovered that consumers prefer tomatoes that are full of flavor, juicy, sweet and sour at once and with only a few seeds (Causse et al. 2010; Piombino et al. 2013; Oltman et al. 2014). Tomato flavor can be defined as a combination of the taste attributes of sweetness and acidity along with concentrations of odor-active volatile compounds (Yilmaz 2001; Kader 2008). The good taste of a tomato has been positively related with rising levels of titratable acids (TA), total soluble solids (TSS) and dry matter (DM), as well as firmness and surface redness (Javaria et al. 2012). In the present study, these parameters are also referred to as consumer-oriented quality traits. There are no universal fruit quality definitions because most definitions address individual stockholders. Consumer-oriented approaches emphasize the need and behavior of consumers (e.g. include aspects such as firmness, color, soluble sugars, acids and volatile fractions) (Kyriacou and Rouphael 2018).

Among several important abiotic impact factors for yield and quality traits, such as weather or water availability (Yunis et al. 1980, p. b; Ben-Gal and Shani 2003), the nutritional status of plants plays a major role (Kirkby 2012). Several studies have found positive correlations between potassium (K) fertilization and the stress tolerance of plants to drought, salinity and cold, as well as resistance to pests and pathogens (Cakmak 2005; Amjad et al. 2014; Zörb et al. 2014). The macronutrient K improves tomato yield and quality formation in the fruit (Hartz et al. 2005; Taber et al. 2008). These positive effects of K are a result of the involvement of K in several physiological processes of plants, such as translocation of assimilates, activation of enzymes and stomata regulation (Hawkesford et al. 2012). Consequently, the yields of different crops are positively affected by K (Zörb et al. 2014), whereas K limitations reduce yields (Cakmak 2005). Our literature review showed different results regarding the relationship between the applied K amount and the increase in tomato yield. Some studies have identified an optimum fertilizer amount for K where higher application does not increase the yield, whereas others showed a linear function; (Hartz et al. 2005; Taber et al. 2008; Liu et al. 2011; Ozores-Hampton et al. 2012; Amjad et al. 2014). However, a few studies have not confirmed any relationship between the K fertilizer dose and tomato yield (Serio et al. 2007; Caretto et al. 2008). Both the productivity of tomato plants and consumer-oriented quality traits, including TSS, TA and DM, are influenced by K (Fanasca et al. 2006; Serio et al. 2007; Ozores-Hampton et al. 2012). Several studies have demonstrated that increase in K fertilization also increases TSS, TA and DM

levels (Wright and Harris 1985; Hartz et al. 2005; Serio et al. 2007a; Caretto et al. 2008; Ozores-Hampton et al. 2012; Amjad et al. 2014).

Only a few studies have analyzed the role of K in the physiological processes of color formation within tomatoes (Asri and Sönmez 2010). More frequently, either a declining effect on the color disorder, 'yellow shoulder', was observed with rising K fertilization (Hartz et al. 2005; Zhang et al. 2015) or the effects of different K fertilizer types on the color were compared (Chapagain et al. 2003). The red color in tomatoes is caused by the pigment lycopene, and there is evidence of a relationship between the red color and lycopene concentration in the fruit tissue (Hernández et al. 2007). Not all experiments consistently showed a positive relationship between rising K fertilization and fruit lycopene concentrations (Taber et al. 2008; Liu et al. 2011).

Besides color, fruit firmness is a key factor for consumer acceptance. It is important for the shelf-life and transportability of a fruit (Seymour 2002). Firmness as part of texture is a complex physical parameter that is influenced by the loss of cell-to-cell adhesion, increased content of water-soluble pectin, reduced galactose and arabinose residues, turgor, cell wall composition and fruit size (Sams 1999; Johnston et al. 2002). In this context, high K fertilization has been linked to a decrease in the firmness or crispness of snap beans (Sams 1999). However, this parameter has been studied rarely in tomatoes that were fertilized differently with K (Schwarz et al. 2013).

So far, the influence of K supply on yield, TSS, TA, DM, color and firmness has been mainly investigated in large-to-medium-sized tomatoes but not in cocktail tomatoes. However, cocktail tomatoes contain higher levels of TA, TSS and DM than medium-to-large-sized fruits (Causse 2002; Beckles 2012). As the consumption of cocktail tomatoes rises, more knowledge is needed about the influence of K on consumer-related quality traits of these tomatoes. We hypothesize that, with rising K fertilization, the above-described consumer-related quality parameters will improve. To test our hypothesis, we cultivated three cocktail tomato cultivars in an outdoor pot trial over two summer seasons. In the second year, we also studied an interrupted K fertilization to increase the K response to a sudden shortage. However, not all previous studies on tomatoes showed a linear relationship between yield and K (Serio et al. 2007; Caretto et al. 2008). Also, cultivar differences have been described for consumer-oriented quality traits (Caretto et al. 2008; Fanasca et al.

2006). Therefore, we also tested the hypothesis that cultivars differ in their responses to varying levels of K fertilization.

Material and Methods

Experimental setup

In 2014, the first year, three outdoor cocktail tomato cultivars were chosen, specifically Resi, Primavera and Yellow Submarine (CULINARIS – Saatgut für Lebensmittel, Göttingen, Germany). Next year, two among the three cocktail tomato cultivars (Resi and Primavera) were selected. In 2014, Primavera and Yellow Submarine had reacted similarly to rising K fertilizer levels in most of the analyzed parameters; therefore, only the contradictory cultivars, Resi and Primavera, were used in the subsequent year (Figure S1 and S2). On 4 April 2014, Resi, Primavera and Yellow Submarine were planted. All plants were transplanted into nursery pots on 22 April. In 2015, they were planted on 30 March and transplanted into nursery pots on 14 April. Tomato seedlings were raised under controlled conditions (long daylight conditions comprising a 16/8 h light/dark cycle; at 22°C and 18°C during the day and night, respectively), initially in seedling starter trays (capacity: 0.1 L) and then transplanted to nursery pots with a diameter of 11 cm (capacity: 1 L). The soil in the starter trays comprised different peats, flesh of coconut and perlite (Anzuchtsubstrat organisch; Kleeschulte, Rüthen, Germany), while pure peat soil (A 400; Stender, Schermbeck, Germany) was used in nursery pots. Seven weeks after sowing in 2014 and 2015 (21–23 May 2014; 19–21 May 2015), the tomato plants were transferred to their final outdoor location at the University of Göttingen, Department of Crop Sciences (coordinates as decimal degrees: latitude 51.546456; longitude: 9.944742). In 2014, five plants per round of treatment and replication were grouped together; in 2015, the plant group size was declining during the season: it started with eight and ended with three plants per group. Tomato plants were transplanted to Mitscherlich vessels (capacity: 6 L) filled with peat (Gartentorf; Naturana, Torfwerk Zubrägel, Vechta, Germany). The peat was enriched with lime ($CaCO_3$) to increase the pH to 5. Plant water requirement was evaluated by visual inspection and if needed, the plants were watered up to twice per day with deionized water. Flow-through water was collected and poured back. All macro- and micronutrients were applied twice during the season in liquid form (week 7 after planting and the second time within weeks 15 and 16). One exception was phosphorus, which was integrated in the

peat of the Mitscherlich vessels as a solid (Table S1). Nitrogen and K were applied weekly to the plants. Nitrogen application was skipped twice during early season (3 and 5 weeks after planting) to avoid over-fertilization, especially for seedlings. In 2014, the five K levels, subsequently referred to as K1, K2, K3, K4 and K5, had rising K application doses (0.36, 0.73, 1.09, 1.46 and 2.19 g K_2SO_4 per week and pot). The application of the levels K3, K4 and K5 (K3 to 1.46 g K_2SO_4; K4 to 2.19 g K_2SO_4; K5 to 3.66 g K_2SO_4) was increased in week 16 (11 July 2014) to strengthen the K fertilizer effect on the plants. In 2015, the tomato plants were treated with three different K fertilization regimes (K1, K5 and Kd). The plants received the fertilizer levels K1 (0.36 g K_2SO_4 per pot) and K5 (to 3.66 g K_2SO_4) as in 2014. To trigger a more pronounced K effect, K depletion (Kd) was introduced (Table S1). Here, the plants were fertilized only with K5 (to 3.66 g K_2SO_4) for 5 weeks at the start of the season and afterwards K fertilization was terminated. Plants of both years were continually pruned to one shoot, and plant protection was applied in accordance with good scientific practices. In both years, the experimental design was a randomized block design with four replications. The weekly harvest took place from July to October (17 July to 6 October 2014; 13 July to 6 October 2015).

Determination of yield, color and firmness

The yield and quantity of marketable fruits were determined weekly during the season. In addition, the yield and quantity of non-marketable fruits (e.g. blotchy or cracked) were recorded. To calculate, for each harvest, the 'cumulative fruit number and yield', the results of previous harvests were added up. Color was determined at two equatorial sites on each fruit using the Minolta Chroma Meter CR-400 (Konica Minolta, Inc., Marunouchi, Japan) for a set of 20 randomly chosen tomatoes. Data was reported in accordance with the L*a*b* system. Firmness was subsequently analyzed on the same 20 fruits at their equatorial sites with a texture analyzer (5 mm Staple Micro Cylinder, speed: 6 mm s^{-1}, distance: 6 mm; TA.XT2; Stable Micro System, Godalming, UK). As in 2014, yield, color and firmness were analyzed for all marketable fruits in 2015 as well. Firmness was analyzed for harvest dates 2, 3, 4 and 5. Approximately 250 g tomatoes per treatment were stored at −20°C for TTS and TA determination.

Determination of TSS, TA and DM

For harvest numbers 1, 2, 4, 6, 8 and 10 in 2014 (17 July to 16 September), TSS, TA and DM were analyzed in duplicates for each sample. The tomatoes were defrosted and mashed; they were then centrifuged for 15 min at 5450 × g (Heraeus Megafuge 16R; Thermo Scientific, Waltham, MA USA) and the supernatant was filtered (filter paper MN 616 $^1/_4$; Macherey-Nagel GmbH & Co. KG, Düren, Germany). A few drops of the filtrate were placed on the refractometer to determine TSS in °Brix (handheld refractometer; A. Krüss Optronic GmbH, Hamburg, Germany). Values were calculated based on g kg^{-1}. To determine TA, 20 mL of deionized water and 3 mL of the filtrate were combined. The solution was automatically titrated against 0.1 NaOH to an end-point of 8.1 pH by the pH titrator (Titroline 96; SCHOTT AG, Mainz, Germany). DM was determined by drying 10 g of the mashed tomato sample in a Petri dish for 1 day at 105°C. As in 2014, the TSS, TA and DM were analyzed for harvests 2, 4, 6, 8 and 10 in 2015 as well (20 July to 15 September).

Determination of yield, color, and firmness

The yield, number, and weight of marketable fruits were determined weekly during the season. The harvest values (number or weight) were added up, including the current harvest, to calculate for each harvest time the “sum of fruit number and weight” of Figure 3. Color was determined at two equatorial sites on each fruit using the Minolta Chroma Meter CR-400 (Konica Minolta, Inc., Marunouchi, Japan) for a set of 20 randomly chosen tomatoes. Data were reported in the L*, a*, b* system. Firmness was subsequently analyzed on the same 20 fruits at their equatorial site with a texture analyzer (5-mm staple micro cylinder, speed: 6 mm s^{-1}, distance: 6 mm, TA.XT2, Stable Micro System, Surrey, UK).

In the year 2015, yield, color, and firmness were analyzed like in 2014 but for all marketable fruits. Firmness was analyzed for harvest dates 2, 3, 4, and 5. Approximately 250 g tomatoes per treatment were stored at -20°C for TTS, TA, and pH determination.

Potassium determination

The fruit material for both years from harvests 2, 4 and 7 (24 July to 27 August 2014; 20 July to 28 August 2015) was used for mineral extraction in accordance with the method described by Koch et al. (2019) with minor changes. Fruits were completely dried at 105°C and ground with a ball mill (30 s at 30 Hz; model MM 400; Retsch Technology GmbH, Haan,

Germany). Next, 100 mg of the ground sample was weighed in a Teflon vessel. In the following step, 4 mL of HNO_3 and 2 mL of H_2O_2 (30 %) were added before the samples were placed in the microwave (ethos terminal 660; Milestone, Sorisole, Italy) for 75 min at 200°C and 15 bar. After microwave digestion, the samples were transferred to a volumetric flask and filled up to a total volume of 25 mL with distilled water. The K content of the samples was analyzed using inductively coupled plasma atomic emission spectroscopy (Vista-RL ICP-OES; Varian Inc., Palo Alto, USA).

Statistics

Statistical analysis was performed using the SPSS, version 24 (IBM Corp., Armonk, NY, USA). The effect of K fertilization was tested individually for each parameter within each cultivar and in each year. In advance, we tested for normal distribution and variance homogeneity; if a parameter had normally distributed data with a homogeneous variance, then a one-factorial ANOVA was performed to ascertain the fertilizer effect followed by a post-hoc test (Tukey's honest significant difference test). In rare cases, if the data were normally distributed but still had inhomogeneous variances, a Welch test was used. If the data were not normally distributed, a Kruskal–Wallis test was performed to test for the fertilizer effect. The Welch test and the Kruskal–Wallis test were followed by the Mann–Whitney U test to compare the means of the fertilizer levels (for further information see supplement of Sonntag et al. 2019).

Results

Yield

In 2014, the yield (g) per plant showed a positive relation with rising K fertilization for Primavera, which was the highest-yielding cultivar (Figures 3 and 4). Resi and Yellow Submarine did not respond significantly. In 2015, the same contrasting trends between the two varieties were noted again for Primavera and Resi: a significant increase between K1 and K5 was demonstrated for Primavera but not for Resi (Figure 3). The depletion fertilization (Kd) in 2015 was significantly different from K1 but not from K5 for Primavera. The steepest increase in the cumulative yield and fruit number was during mid-season in both years, whereas yield increase was lower at the beginning and the end of the season (Figure 3). In 2014, the number of fruits per plant was not influenced. The number of fruits

per plant was significantly lower only for Primavera in K1 than those in K5 (58 %) and in Kd in 2015 (Table 1). Regarding the non-marketable yield, we could observe a significant difference for Yellow Submarine with rising K fertilization in 2014 (Table S2). A two-way ANOVA revealed that cultivar differences were present in yield, fruit number and fruit weight. K fertilization and the interaction between K fertilization and cultivar were only significant in yield for both years and for fruit number and fruit weight in 2015.

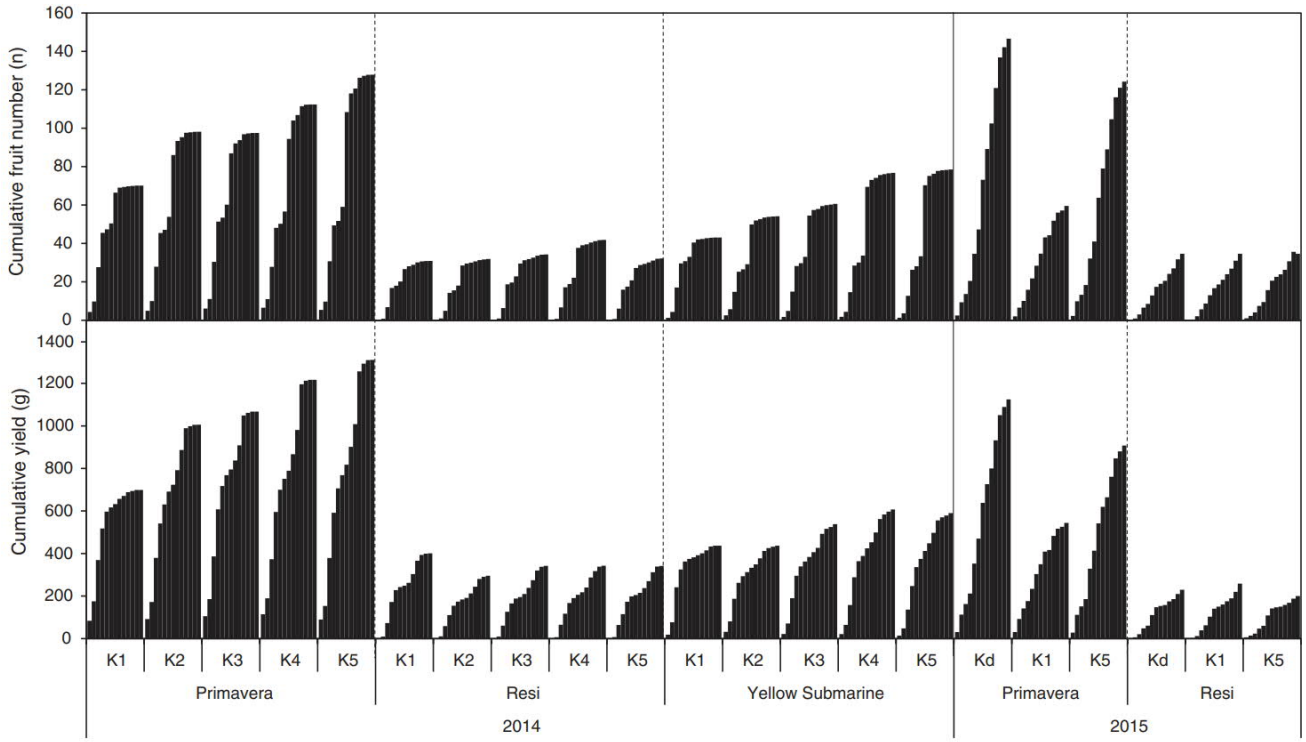

Figure 3. Potassium (K) fertilization differentially affects the cumulative fruit number (n) and yield (g) per plant over the harvest season. Fruit number and yield was determined from four biological replicates and 13 harvest dates in 2014 and 2015. Each bar represents the marketable yield of the current harvest added to the values of all harvests beforehand. K levels increase from K1 to K5 (0.37 g, 0.73 g, 1.47 g, 2.2 g to 3.66 g per week) for each cultivar. In 2015, a depletion fertilization treatment (Kd) was received only in the first five weeks K (3.66 g per week).

TSS, TA and DM

TSS and TA concentrations increased in 2014 with rising K fertilization in all cultivars (Figure 4). Also, in 2015, these parameters were significantly higher in K5 than in K1 for both cultivars. The TSS values increased by 24 % for Primavera in both years and 16 % for Resi and Yellow Submarine in 2014, whereas, in 2015, the increase was 9 % for Resi. TA values rose by 36 %, 41 % and 20 % in Primavera, Resi and Yellow Submarine in 2014, whereas increases of 33 % for Primavera and 35 % for Resi were detected in 2015. The fruits of the fertilization regime Kd reacted differently in the two analyzed cultivars: the mean values of TSS and TA in Primavera were between K1 and K5 but significantly different

from both. In Resi, the mean of Kd was only significantly different to K1, which was 10 % lower in the case of TSS and 30 % for TA. DM was positively influenced by K fertilization for Primavera and Yellow Submarine in 2014 (Table 1) but not for Resi. In 2015, Primavera and Resi showed a significant increase in the DM content with rising K doses. The values increased by 20 % for Primavera and 15 % for Resi. In both cultivars, the DM of Kd was between those of K1 and K5. However, in Primavera, it was significantly different from the low (K1) and high (K5) fertilization levels. Cultivar and K fertilization had a significant influence on the results of both years for TSS, TA and DM according to a two-way ANOVA. The interaction of cultivar and K fertilization was as well significant for TSS, TA and DM, with the exception of TA and DM in 2015.

Color and firmness

In 2014, the color value a* (red color) of the fruits showed a significant increase with rising K levels in Primavera, a significant decrease in Yellow Submarine, and no change in Resi (Table 1). The color value b* (yellow color) decreased significantly in all three cultivars with an increase in fertilization by 11 % in Primavera, 6 % in Resi and 14 % in Yellow Submarine. In 2015, the color parameters were not influenced in Resi. A significant increase for the color values a* and b* was detected between K1 and K5 for Primavera by 11 % and 5 %, respectively, whereas only values for a* showed a significant difference between Kd and K1 but not K5. A two-way ANOVA showed that cultivar differences were detectable for color values a* and b* for both years, whereas K fertilization was significant for b* in 2014 and for a* in 2015. The interaction between K fertilization was only significant for a* and b* in 2014.

In 2014, firmness increased with a rise in K contents of the nutrient solution for Primavera and Yellow Submarine. The fruits of Resi, on the other hand, showed no such tendency (Table 1). In 2015, no significant fertilizer effect was detected for the two cultivars, although a negative correlation of K with firmness was identified in Resi. Cultivar, K fertilization and the interaction of cultivar and K fertilization were significant in 2014 for firmness according to a two-way ANOVA but, in 2015, only cultivar and the interaction had a significant influence.

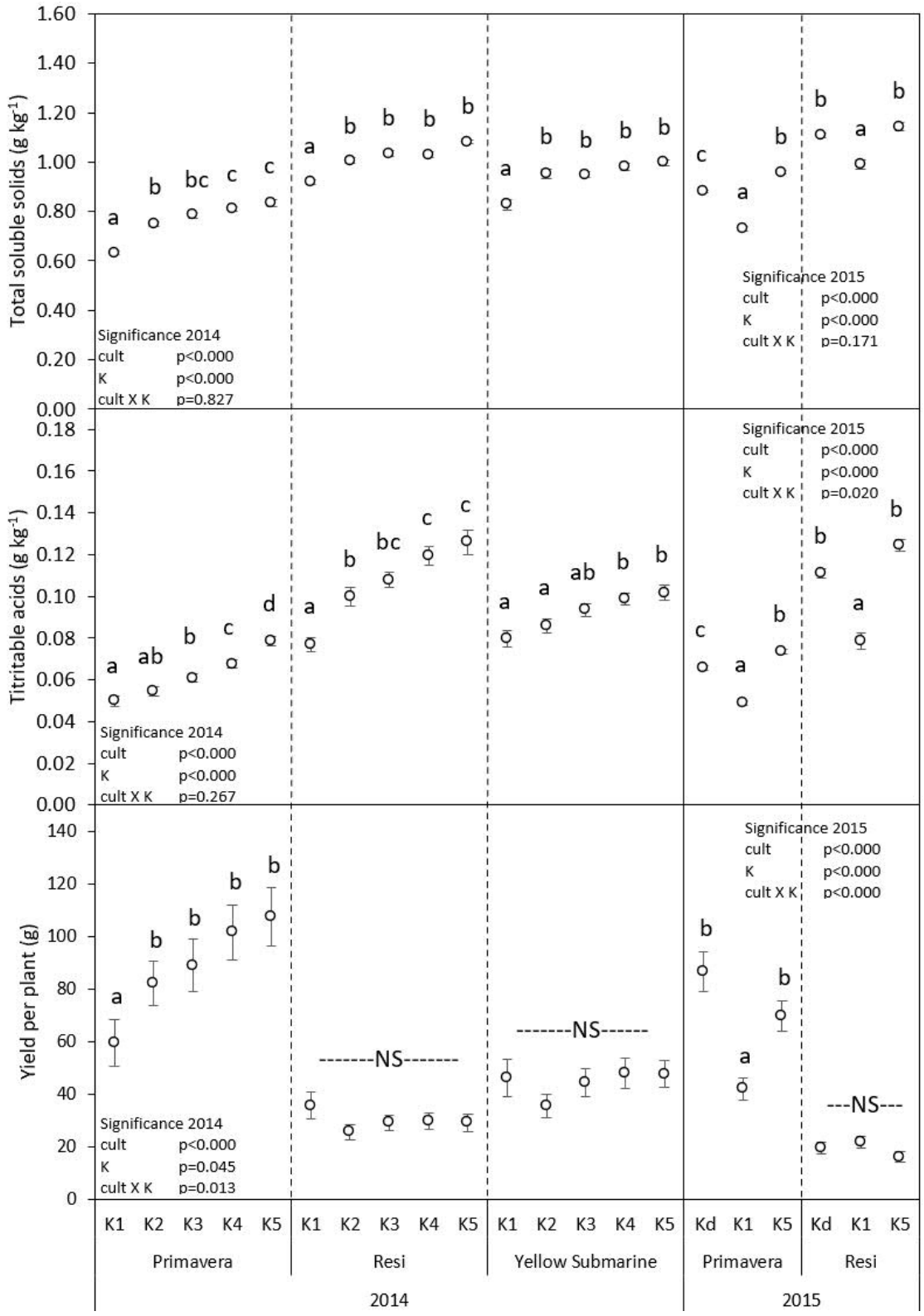

Figure 4. Potassium (K) fertilization differentially affects total soluble solids (TSS), titratable acids (TA) and the yield per plant of three cocktail tomato cultivars. K levels increase from K1 to K5 (0.37 g, 0.73 g, 1.47 g, 2.2 g to 3.66 g per week) for each cultivar. In 2015, a depletion fertilization treatment (Kd) received only in the first five weeks K (3.66 g per week). Yield was determined from four biological replicates and for 13 harvest dates (n≥50). The mean values of TSS and TA represent six harvests (1, 2, 4, 6, 8 and 10) in 2014 (n≥50) and five harvests in 2015 (2, 4, 6, 8 and 10; n≥50) with four biological replicates. The standard error of means was calculated for all mean values. The letters indicate statistically significant differences. NS = no significant difference. P-values are given for a two-way ANOVA between cultivar (cult), K fertilization (K), and the interaction (cult X K) for both years.

Table 1. Yield and quality parameters for the season 2014 and 2015.

			fruit number per plant (n)		fruit weight (g/n)		DM (g kg^{-1})		TSS / TA		color a*		color b*		firmness (N)		potassium (K, g kg^{-1})	
2014	Primavera	K1	6.0 ± 7.1	NS	10.1 ± 0.4	NS	0.77 ± 0.03	a	14.1 ± 0.3	ab	18.2 ± 0,4	a	25.1 ± 0.7	a	8.2 ± 1.0	a	0.17 ± 0.02	a
		K2	8.0 ± 10.0	NS	10.1 ± 0.4	NS	0.86 ± 0.03	b	15.0 ± 0.3	a	19.3 ± 0,4	ab	23.2 ± 0.7	b	8.7 ± 1.0	b	0.18 ± 0.03	ab
		K3	8.1 ± 9.0	NS	9.9 ± 0.4	NS	0.91 ± 0.03	bc	13.5 ± 0.3	ab	18.9 ± 0,4	ab	22.8 ± 0.7	b	9.0 ± 1.0	bc	0.21 ± 0.03	bc
		K4	9.4 ± 11.3	NS	10.1 ± 0.4	NS	0.99 ± 0.03	c	12.5 ± 0.3	b	19.9 ± 0,4	b	22.5 ± 0.7	b	9.3 ± 1.0	c	0.24 ± 0.03	cd
		K5	10.4 ± 14.5	NS	9.8 ± 0.4	NS	0.94 ± 0.03	c	11.0 ± 0.3	c	20.4 ± 0,4	b	22.4 ± 0.7	b	9.4 ± 1.0	c	0.26 ± 0.03	d
	Resi	K1	2.7 ± 3.7	NS	9.0 ± 0.4	NS	1.13 ± 0.03	NS	12.8 ± 0.4	a	27.4 ± 0,4	NS	28.0 ± 0.7	a	10.9 ± 1.4	NS	0.18 ± 0.03	a
		K2	2.8 ± 4.1	NS	8.8 ± 0.4	NS	1.12 ± 0.03	NS	10.9 ± 0.4	ab	27.9 ± 0,4	NS	27.4 ± 0.8	ab	11.2 ± 1.4	NS	0.19 ± 0.02	ab
		K3	2.9 ± 3.8	NS	8.1 ± 0.4	NS	1.11 ± 0.03	NS	10.1 ± 0.4	b	27.7 ± 0,4	NS	26.7 ± 0.7	bc	11.0 ± 1.3	NS	0.21 ± 0.02	b
		K4	3.6 ± 5.5	NS	8.1 ± 0.4	NS	1.12 ± 0.03	NS	9.2 ± 0.4	b	27.5 ± 0,4	NS	26.5 ± 0.7	c	10.6 ± 1.2	NS	0.24 ± 0.02	c
		K5	2.7 ± 3.3	NS	7.7 ± 0.4	NS	1.16 ± 0.03	NS	9.4 ± 0.4	b	27.0 ± 0,4	NS	26.4 ± 0.7	c	11.1 ± 1.5	NS	0.26 ± 0.03	c
	Yellow Submarine	K1	4.8 ± 5.4	NS	9.1 ± 0.4	NS	0.95 ± 0.03	a	11.1 ± 0.4	NS	1.2 ± 0,5	a	55.8 ± 0.9	a	11.9 ± 1.3	a	0.20 ± 0.03	ab
		K2	4.4 ± 6.1	NS	8.6 ± 0.4	NS	1.02 ± 0.03	ab	11.5 ± 0.3	NS	-0.0 ± 0,4	b	50.7 ± 0.7	b	13.1 ± 1.7	b	0.19 ± 0.04	a
		K3	5.2 ± 7.1	NS	8.9 ± 0.4	NS	1.13 ± 0.03	b	10.6 ± 0.4	NS	-0.2 ± 0,4	bc	51.1 ± 0.8	b	13.5 ± 1.5	b	0.23 ± 0.02	bc
		K4	6.1 ± 10.5	NS	9.0 ± 0.4	NS	1.14 ± 0.03	b	10.2 ± 0.3	NS	-0.7 ± 0,4	bc	49.8 ± 0.7	b	13.7 ± 1.5	b	0.24 ± 0.02	c
		K5	6.4 ± 10.6	NS	9.0 ± 0.4	NS	1.11 ± 0.03	b	10.3 ± 0.3	NS	-1.1 ± 0,4	c	49.1 ± 0.7	b	13.6 ± 1.3	b	0.27 ± 0.03	c
significance		cult	0.000		0.000		0.000		0.000		0.000		0.000		0.000		0.010	
		K	0.220		0.688		0.000		0.000		0.931		0.000		0.000		0.000	
		cult X K	0.858		0.913		0.000		0.121		0.000		0.000		0.000		0.697	
2015	Primavera	Kd	11.3 ± 1.0	a	8.7 ± 0.3	NS	0.97 ± 0.01	a	13.7 ± 0.6	a	21.7 ± 0,4	b	22.6 ± 0.3	ab	7.5 ± 1.2	NS	0.21 ± 0.02	a
		K1	4.6 ± 1.0	b	9.5 ± 0.3	NS	0.80 ± 0.01	b	15.3 ± 0.6	b	20.1 ±0.4	a	22.3 ± 0.3	a	7.2 ± 0.8	NS	0.13 ± 0.01	b
		K5	9.6 ± 1.0	a	8.2 ± 0.3	NS	1.00 ± 0.01	c	13.1 ± 0.6	a	22.5 ±0.4	b	23.4 ± 0.3	b	7.5 ± 0.9	NS	0.27 ± 0.03	c
	Resi	Kd	2.9 ±1.0	NS	6.2 ± 0.3	b	1.20 ± 0.01	a	10.2 ± 0.7	a	30.2 ± 0.4	NS	27.1 ± 0.3	NS	8.2 ± 1.4	NS	0.20 ± 0.03	a
		K1	2.9 ± 1.0	NS	6.9 ± 0.3	a	1.10 ± 0.01	b	13.5 ± 0.7	b	29.1 ± 0.4	NS	27.3 ± 0.3	NS	9.4 ± 1.1	NS	0.14 ± 0.01	b
		K5	3.1 ± 1.0	NS	4.9 ± 0.3	b	1.30 ± 0.01	a	9.2 ± 0.7	c	29.8 ±0.4	NS	26.9 ± 0.3	NS	8.3 ± 1.7	NS	0.26 ± 0.02	c
significance		cult	0.000		0.000		0.000		0.000		0.000		0.000		0.000		0.391	
		K	0.000		0.001		0.000		0.000		0.000		0.704		0.373		0.000	
		cult X K	0.000		0.562		0.182		0.150		0.160		0.213		0.026		0.085	

Potassium (K) fertilization differentially affects the individual parameters of three cocktail tomato cultivars. Yield and fruit number per plant over the season, as well as fruit weight was determined from four biological replicates. The results of TSS (total soluble solids, g kg^{-1}), TA (titratable acids, g kg^{-1}), and DM (dry matter, g kg^{-1}) comprise six harvests (1, 2, 4, 6, 8 and 10) in 2014 and five harvests (2, 4, 6, 8 and 10) in 2015. The results of color and texture comprise harvest 1 to 12 with four biological replicates for all parameters. K levels increase from K1 to K5 (0.37 g, 0.73 g, 1.47 g, 2.2 g to 3.66 g per week) for each cultivar in 2014. In 2015 K levels were K1 and K5 (0.37 g and 3.66 g per week) and a depletion fertilization treatment (Kd, 3.66 g for 5 weeks). The letters indicate statistically significant differences ($p < 0.05$). NS = no significant difference. P-values are given for a two-way ANOVA between cultivar (cult), K fertilization (K), and the interaction (cult X K) for both years.

Correlation of traits with fruit potassium contents

Correlation of traits with fruit K contents The K concentration in tomatoes increased significantly with a rise in K fertilization. A positive response was also detected for the concentration of several parameters, such as TA or TSS (Table 1). A correlation was performed to test the relationship of the K content in fruit with the individual traits. Only TA was positively correlated with K content in the fruits for all cultivars and years (Table 2). In 2014, DM for Yellow Submarine and firmness for Primavera and Yellow Submarine showed a positive correlation with K content. The color value b* of Primavera and the TSS/TA ratio for Resi were negatively correlated with the K concentration. In 2014, fruit weight was positively correlated with K content in Primavera fruits. Resi and Yellow Submarine showed a positive correlation between the number of fruits and the K content. In 2014, the yield related positively to K content in Primavera and Yellow Submarine. In 2015, the parameters TSS, TA and DM were increased with K content in both cultivars, whereas the TSS/TA ratio was negatively correlated. Firmness decreased in Resi significantly. The color values a* and b* were positively correlated with K concentration in Primavera fruits. However, the yield parameters were not significantly associated with K content in 2015.

Table 2. Pearson correlation of potassium content with quality parameters and tomato fruits yields.

parameter	cultivar	Primavera	Resi	Yellow Submarine	Primavera	Resi
	year	2014	2014	2014	2015	2015
TSS (g kg^{-1})	correlation	0.24	0.13	0.37	0.92**	0.94**
	significance	0.331	0.574	0.108	0.000	0.000
	n	19	20	20	23	22
TA (g kg^{-1})	correlation	0.61**	0.78**	0.53*	0.84**	0.95**
	significance	0.006	0.000	0.017	0.000	0.000
	n	19	20	20	23	21
TSS / TA	correlation	-0.39	-0.78**	-0.38	-0.66**	-0.88**
	significance	0.098	0.000	0.096	0.001	0.000
	n	19	20	20	23	21
DM	correlation	0.47	0.35	0.61*	0.84**	0.87**
(g kg^{-1})	significance	0.120	0.180	0.037	0.000	0.000
	n	12	16	12	23	22
firmness	correlation	0.30*	-0.28	0.31*	0.03	-0.76*
(N)	significance	0.037	0.053	0.041	0.931	0.010
	n	50	49	45	11	10
a*	correlation	-0.08	0.12	0.000	0.44**	0.06
	significance	0.580	0.408	1.000	0.009	0.751
	n	50	49	45	35	32
b*	correlation	-0.46**	-0.13	0.04	0.41*	-0.05
	significance	0.001	0.370	0.821	0.015	0.805
	n	50	49	45	35	32
yield per	correlation	0.54**	0.05	0.39**	0.28	0.19
plant (g)	significance	0.000	0.741	0.007	0.102	0.286
	n	50	49	46	35	32
number of fruits	correlation	0.24	0.45**	0.45**	0.31	0.23
per plant (n)	significance	0.101	0.001	0.002	0.070	0.202
	n	50	49	45	35	32
fruit weight	correlation	0.43**	-0.25	0.09	-0.09	0.05
(g/n)	significance	0.002	0.082	0.557	0.591	0.805
	n	50	49	45	35	32

* The correlation is significant at the level of 0.05 (2-sided) and with two** at the level of 0.01 (2-sided). n = number of observations. The mean fruit weight was calculated for the marketable yield.

Discussion

K treatment successfully led to a fertilizer effect because the fruit K concentration increased with a rise in fertilization (Tables 1 and 2). These results were anticipated because previous studies showed a positive relationship between K fertilization and fruit K concentration (Davies 1964; Chapagain et al. 2003; Serio et al. 2007; Taber et al. 2008). Therefore, significant differences in relation to K application can be attributed to different K treatments.

Effect of K on yield

The effect of K application on the yield of cocktail tomato varieties was strongly cultivar-dependent (Figures 3 and 4). The significant increase of yield for Primavera between the

lowest and the highest K fertilization levels was present in both years, at approximately 45 % in 2014 and 40 % in 2015. A linear relationship with K was also detected in a study by Taber et al. (2008). A non-significant increase of cumulative fruit number (n) was visible for Yellow Submarine. The finding that Yellow Submarine was also positively influenced by higher K application is appropriate because the correlation with K is significant and a significant increase in non-marketable fruits was detected (Table S2). Consistently, over 2 years, Resi showed no yield response to the fertilization regimes. Other studies also showed no relationship between K and yield (Serio et al. 2007; Caretto et al. 2008). An optimum K dose or a saturation of K fertilization was not detected in the present study. Primavera and Resi differed from each other in biomass production, which was analyzed in 2015. The plants of the cultivar Resi produced a higher leaf mass but less fruits compared to those of the cultivar Primavera (Table S3). This was also represented in the leaf-to-fruit ratio, where highly fertilized plants (K5) of Primavera had a ratio of 1.1 and the low fertilized ones (K1) had a ratio of 0.6. Resi had a leaf-to-fruit ratio of 5.8 at K5 and 3.1 at K1. The results indicate that Primavera is a more K-dependent and higher yielding but less biomass-yielding cultivar. Based on the yield and leaf-to-fruit ratio results, we conclude that the effect of K is strongly cultivar-dependent. In salad tomato varieties, several studies have already shown an increase in the yield with a rise in K fertilization (Taber et al. 2008; Liu et al. 2011; Amjad et al. 2014a), whereas a few showed no effect on the yield (Serio et al. 2007; Caretto et al. 2008). Our results suggest that the determination of leaf-to-fruit ratio would help in the interpretation of inconsistent results. Additionally, many of the published studies were performed under widely varying cultivation conditions; for example, in the field (Hartz et al. 2005; Ozores-Hampton et al. 2012), in pots outdoors (Taber et al. 2008) and in hydroponic systems (Fanasca et al. 2006; Serio et al. 2007). However, it is well known that temperature (Gent and Ma 2000) and water availability (Liu et al. 2011) influence yield. These variable conditions make it difficult to compare the performance of different tomato cultivars with each other. In the present study, plants were grown outdoors in pots in two consecutive years under varying weather conditions. According to a two-way ANOVA, mean sunlight was higher in 2015 but the mean temperature was higher in 2014 at the beginning of the summer (Table S4). A colder spring in 2015 reduced yield compared to 2014. Growth and nutritional uptake increase with a higher air and soil temperature (Gent and Ma 2000), whereas sub-optimal temperatures reduce the fruit set (Van Ploeg and

Heuvelink 2005). Consequently, yield differs significantly within these 2 years in accordance with a two-way ANOVA (yield and fruit number, $p<0.001$) (Table S5). However, the reaction of the cultivars to the K treatment was similar in both years.

TSS, TA and DM

We demonstrated that a rise in K application has a positive effect on TSS and TA levels on all three cocktail tomato cultivars (Figure 4). TSS and TA are important quality parameters because they are correlated with the sensory descriptors of sweetness, acidity and good flavor (Jones and Scott 1983). TSS are dissolved solids which, in tomato fruits, consist of approximately 65 % sugar, 13 % acids, and a residue comprising phenols, amino acids, soluble pectin, ascorbic acid and mineral nutrients. TSS is used as a proxy for sugars in fruits, and the values are positively correlated with consumer acceptance (Beckles et al. 2012). The positive influence of rising K fertilization on TSS and TA was shown in many studies on medium- to large-sized tomatoes (Fanasca et al. 2006; Liu et al. 2011; Ozores-Hampton et al. 2012). Also, in the present study, TSS was used as a proxy and increased in all cultivars in both years. The TSS values ranged between 0.6 and 1.1 g kg^{-1}. Cocktail tomatoes, on average, have TSS values in the range 0.9–1.5 g kg^{-1} (Beckles et al. 2012). Taking this range into account, the values of Primavera and Yellow Submarine reached with the fertilizer levels K1 and K2 (0.6–0.8 g kg^{-1}) were too low, whereas those of highly fertilized K5 were more appropriate. The TSS concentrations for Resi were all in an acceptable range (0.9–1.1 g kg^{-1}, Figure 4).

TA concentration was the parameter that positively and significantly correlated with the fruit K concentration in all cultivars over 2 years (Figure 4). Most organic acids are stored in the vacuoles and are responsible for the acidic nature of these fruits (Shiratake and Martinoia 2007). TA decreases in the fruits during the ripening process (Gautier et al. 2008). Malic and citric acid in ripe tomatoes are the main acids that are responsible for TA values (Beckles 2012). Both acids are products of the citric acid cycle (Etienne et al. 2013). According to previous studies, malic and citric acids reduce significantly if a plant suffers K deficiency (Sung et al. 2015); this is consistent with the results of the present study.

Tomatoes consist of more than 90 % water, whereas 5 %–8 % is DM. Dry matter accumulation depends on tomato fruit's sink strength (Heuvelink 1997). Developing fruits are strong sinks for carbohydrates (Wang et al. 1993). The transport of assimilates from the

leaves to other plant organs is favored by K (Haeder and Mengel 1972). Because mainly sucrose molecules are transported via the phloem, positively-charged K^+ ions are the counterion to the negatively-charged sucrose (Hawkesford et al. 2012). In addition, plants with sufficient K tissue concentrations have a higher photosynthetic rate in leaves (Hawkesford et al. 2012). Therefore, we expected an increase in DM with elevated K doses as demonstrated by Caretto et al. (2008). DM increased with a rise in K doses in Primavera in both years and in Yellow Submarine in 2014. Surprisingly, for Resi, a significant difference was found between the lowest and highest K doses only in 2015; this result was also confirmed by a significant correlation between fruit DM and K content in that year (Tables 1 and 2).

Overall, DM accumulation was positively influenced by higher K application. TSS and TA, as important tomato taste parameters, increased with rising K fertilization (Figure 4). With respect to our hypothesis, we can conclude that the accumulation of consumer-related fruit traits TSS, TA and DM are positively influenced by K fertilization in tomato plants.

Color and firmness

For customers, color is an important aspect of the appearance of tomatoes (Francis 1995). Whether the color of a product is acceptable for a consumer depends on a wide range of factors, including the ethnic origin, age and sex of the consumer (Francis 1995). Bright red tomatoes are favored in Europe and the USA (Causse et al. 2010; Piombino et al. 2013; Oltman et al. 2014). The red color values (a*) in our red-fruited cultivars ranged between 20 and 30 (Table 1) and were comparable with the results of Hernández et al. (2007). Significant correlations between the fruit color values and fruit K content were observed only for Primavera for both years (Table 2). A more intense red was detected for highly fertilized fruits. The fruits of Resi generally showed higher a* values independent of K-fertilization regimes. The red color of tomatoes is formed by the pigment lycopene (Arias et al. 2000), although the mechanisms by which K fertilization affects lycopene formation have not yet been clarified (Taber et al. 2008; Liu et al. 2011). In the yellow cultivar (Yellow Submarine) and in the other two red cultivars, the b* values decreased with rising K doses (Table 1). Arias et al. (2000) propose that low b* values reflect the β-carotene concentration, which is partly masked by lycopene in red-fruited cultivars Primavera and Resi. Nonetheless, in another study, a negative correlation between β-carotene

concentration and a rise in K values was detected (Taber et al. 2008). This indicates that higher K levels have a negative effect on the yellow color. The influence on the red color, on the other hand, is rather cultivar-dependent.

Fruit firmness is a complex parameter and several physiological mechanisms, such as turgor, cell wall and membrane chemistry, as well as physical traits (e.g. cell size and shape, cell wall and overall fruit anatomy), play a crucial role in this (Johnston et al. 2002). External and internal factors influence fruit firmness, such as climatic factors (Sams 1999), ethylene and other plant hormones (Johnston et al. 2002), and the genetic background of a cultivar (Sams 1999). Studies investigating tomato fruit softening have demonstrated a ripening-associated cell wall modification (Kramer et al. 1992; Sozzi et al. 1998). Only a few studies have investigated the effect of K fertilization on the firmness of tomatoes. In a multifactorial design, Schwarz et al. (2013) investigated the effects of grafting and K fertilization on two tomato cultivars but could not show any K influence on fruit firmness. This is surprising because tissue K concentration is an important aspect of the firmness and affects the turgor (Hawkesford et al. 2012). The results of the present study demonstrate a significant increase in fruit firmness for Primavera and Yellow Submarine in 2014. This tendency was also found in 2015, although the difference was not significant ($p = 0.51$, Table 1). The fruit firmness of Resi did not respond to K application in both years.

Conclusion

An increasing K concentration in tomato fruits enhanced the levels of TA and TSS in all cultivars. Because these parameters correlated with the sensory descriptor of good flavor, K most likely has a positive effect on tomato taste. All other factors showed a cultivar deepened effect. Especially, Resi hardly showed any K effects, except for the increase of TA and TSS with rising K treatment. On the other hand, DM, color and firmness increased with a rise in K levels in Primavera and Yellow Submarine. Yield and red color intensity improved significantly only in Primavera, a highly productive cultivar. Because several of these responses to an increase in K application are strongly cultivar-dependent, it is concluded that cultivars have different nutrient demands.

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

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Abstract

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; Tavallali 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 (Figure S4). 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 (Table 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). For more details, see chapter two.

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 (Figure S4). 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) (Table S11).

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 K content

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 precolumn (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/nhexane (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 CTO20AC 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 tertmethylbutylether/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 (Table S6, S7, S8, S11, and S12) and t-tests (Table S9, S10, S11 and S13) 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 % (Table S10; 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 (Figure 5). *p*-Coumaric acid was also grouped in the lower part of the PCA. Naringenin 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.

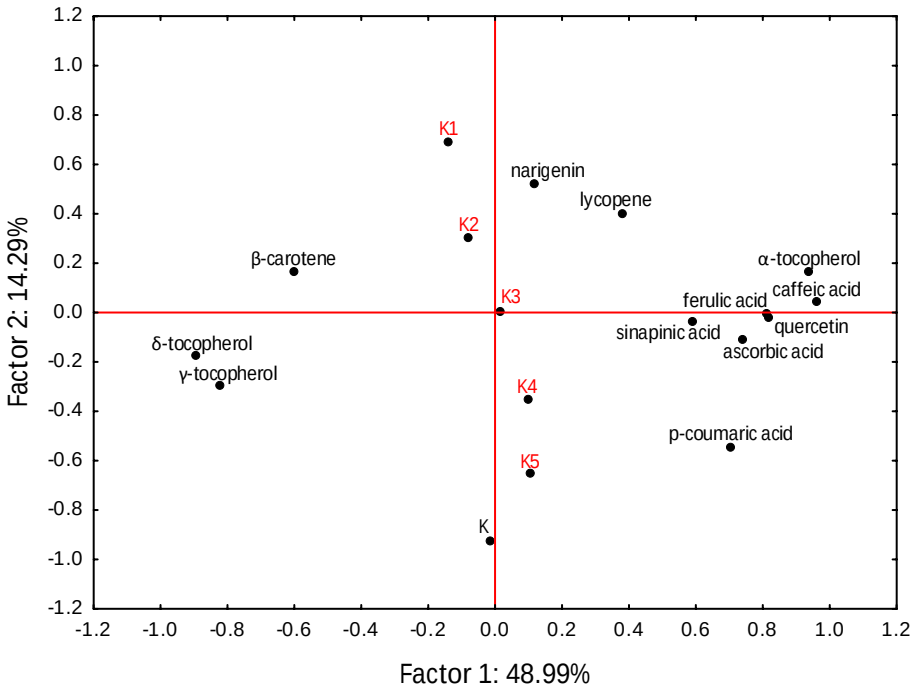

Figure 5. Principal component analysis of the antioxidants in relation to the increasing K levels in 2014. K levels increase from K1 to K5 (0.37 g, 0.73 g, 1.47 g, 2.2 g, and 3.66 g K_2SO_4 per week). K represents the K concentration in the tomato fruit.

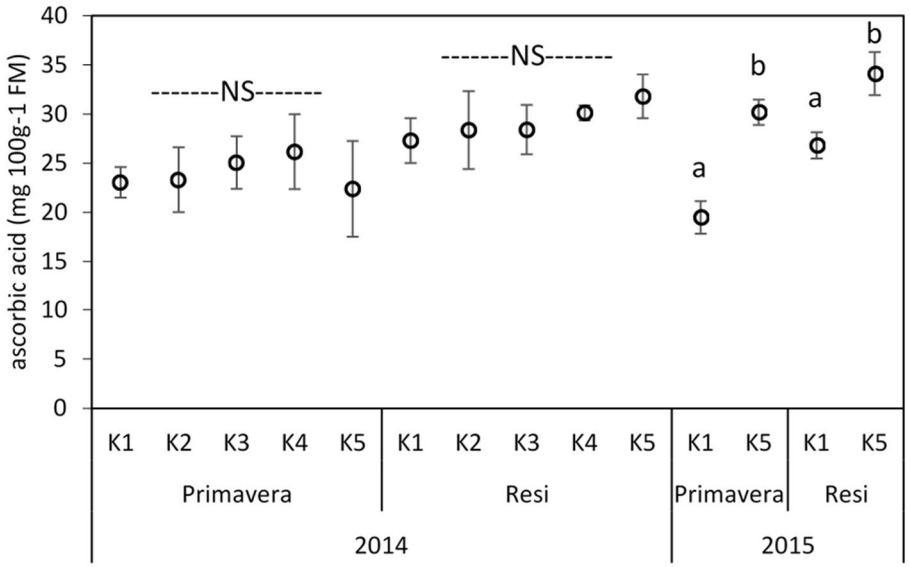

Figure 6. K fertilization differently affects the ascorbic acid concentration of the cocktail tomato cultivars. K levels increase from K1 to K5 (0.37 g, 0.73 g, 1.47 g, 2.2 g, and 3.66 g K_2SO_4 per week) for each cultivar. The mean values and standard deviations were determined from four biological replicates. Letters indicate statistically significant differences and NS indicates no significant difference, according to a Mann-Whitney-U or Tukey-HSD test.

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 (Figure 6). 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 (Table 3). A two-factorial ANOVA revealed a significant interaction between year and K treatment only for Primavera (Table S6). However, the year itself showed no significant influence on the ascorbic acid concentration according to the two-way ANOVA in both analyzed cultivars.

Table 3. 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
naringenin	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.

In 2014, there was no significant change for the phenolic compounds with rising K fertilization (Table 4). Only Primavera showed a significant negative correlation of the fruit K concentration with naringenin in 2014 (Table 3). 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) (Table 3). In 2015, p-coumaric acid as well as caffeic acid

levels increased with rising K treatment in the fruit of Resi and Primavera (Table 4) – in this case, it was also reflected in a significant correlation with fruit K concentration in both cultivars (Table 3). 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 (Table S6). 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.

Table 4. 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	Prima-vera	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.

There were no significant differences for lycopene between the five K fertilization levels in both years (Figure 7). However, as expected, lycopene increased during the ripening of both Resi and Primavera in 2015 (Figure 7). If averaged over both K levels, this effect was significant (Table S8). The β-carotene levels decreased with rising K application only in the fruit of Primavera in 2014 (Figure 7). 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 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 (Table 3). 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 (Table S6). Within the different ripening stages and

averaged over both K levels, β-carotene concentration rose only until the orange ripe stage in both cultivars (Table S8).

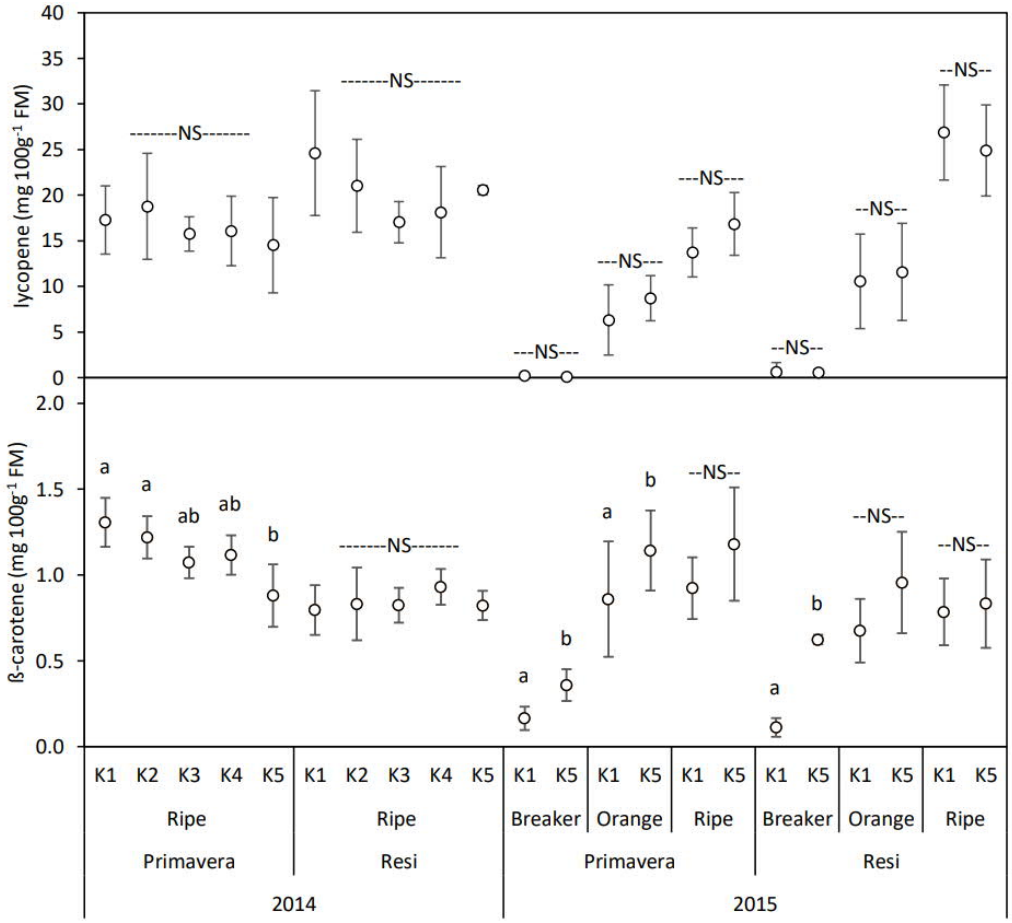

Figure 7. Potassium (K) fertilization differentially affects the carotenoids lycopene and **β**-carotene in the cultivars. Mean values and standard deviations were determined from four biological replicates. K levels increased from K1 to K5 (0.37 g, 0.73 g, 1.47 g, 2.2 g, and 3.66 g K_2SO_4 per week) for each cultivar. Letters indicate statistically significant differences and NS indicates no significant difference, according to a Mann-Whitney-U or Tukey-HSD test.

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 (Table 5). 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 (Table 3). 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 (Table 5). For both cultivars, a significantly negative correlation was detected between K level and γ-tocopherol in 2015 (Table 3). Additionally, the K concentration was negatively correlated with α-tocopherol in Resi and with δ-tocopherol in Primavera. 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 (Table S6). 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 (Table S8).

Table 5. 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	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
		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	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	
		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 al. 2008). Compared with the soilless system used by Fanasca et al. (2006) the fruit K concentrations in our experimen (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 1.5–3.2 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 (Figure S4). 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 (Figure 6 and 7, Table 5). Notably, Primavera did not contain detectable amounts of α- and β-tocopherol, whereas α-tocopherol was the main tocopherol in Resi (Table 5). 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 (Table 3), 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 (Table 3). 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 (Table S9). 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 (Table S6). 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 (Table S6 and S7). 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 (Table 3). 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 (Table S9). In case of caffeic acid, the t-test revealed a significant difference between K1 and K5 for Resi from 2014 (Table S9), 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 (Table S6). 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 (Table S9). 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 (Table S6). In case of βcarotene or γ-tocopherol, even opposite significant correlations with tomato fruit K concentration were determined in 2014 and 2015 (Table 3). 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). 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 (Table S13), although the difference between the months was not significant (Table S12). 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 (Table S6). 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 (Table 3).

Lycopene levels were not influenced by increasing K fertilization in either year (Figure 7). 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 (Figure 7, Table S8), 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 (Table 5). 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.

4. The effect of potassium fertilization on the metabolite profile of tomato fruit (*Solanum lycopersicum* L.)

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Abstract

Tomatoes are an important worldwide vegetable and the macronutrient potassium (K) has vital physiological functions in all plants. For a compressive overview of the induced changes by low, sufficient and high K fertilization we conducted a GCxGC-MS metabolome analysis in tomato fruits of three different cocktail tomato cultivars. A cultivar independent increase was detected for the organic acids and decreased for the amines with rising K fertilization. The sugars, amino acids and several secondary components showed varying tendencies between the cultivars. Many of the secondary components were antioxidants and revealed highest values under K deficiency. The most important and cultivar-independent effect of increased K fertilization was: 1. the rise of TCA cycle metabolites in all cultivar with rising K application and 2. the cultivar-specific effect on several other compounds or compound classes. Indicating that the reaction towards macronutrient stress is quite different between cultivars of one species.

Keywords

Solanum lycopersicum L.; tomato; potassium; minerals; TCA cycle; metabolomics

Introduction

Tomatoes (*Solanum lycopersicum* L.) are among the most important vegetables worldwide. They account for 14.6 % of the vegetable world market with an annual production of 170.8 million tons in 2014 (FAOSTAT 2019). Tomatoes contain several antioxidants and vitamins e.g. ascorbic acid (Capanoglu et al. 2008), tocopherols (Caretto et al. 2008), phenolic acids (Hernandez-Perez and Anderson 1976) and carotenoids (Lu et al. 2008). Therefore, tomatoes and tomato products may promote health (Giovannucci 1999; Rao and Rao 2007; Turati et al. 2015). However, the amount of antioxidants and vitamins in tomato fruits varies considerably due to biotic and abiotic stress factors like nematodes, water shortage, high salinity or irradiation (Gautier et al. 2008; Atkinson et al. 2011; Ehret et al. 2013). In

addition does the nutrition status of plants plays a key role for the chemical composition of tomatoes (Kirkby 2012).

The macronutrient K is essential for several physiological functions in plants, such as translocation of assimilates, activation of enzymes, maintenance of turgescence, and stomata regulation (Hawkesford et al. 2012; Zhao et al. 2018). Many studies found also positive correlation between K fertilization and stress tolerance, such as drought, salinity, cold or pest and pathogen resistance (Cakmak 2005; Amjad et al. 2014; Zörb et al. 2014). In general, crop yield is positively affected by K fertilization (Cakmak 2005; Zörb et al. 2014). A positive effect of high K application on tomato yield has also been described by several authors (Wang et al. 1993; Taber et al. 2008; Amjad et al. 2014). Furthermore, it is known that K positively influences sugars (mostly measured with refractometer and expressed as TSS) and acids (mostly measured by titration and expressed as TA) in tomato fruits (Fanasca et al. 2006; Caretto et al. 2008; Ozores-Hampton et al. 2012). Wright and Harris (1985) demonstrate a positive effect of increasing K fertilizer rate on tomato fruit flavor profile.

Covering a wide range of low-molecular weight compounds, metabolome analyses have the potential to describe the composition of biological systems comprehensively (Hegeman 2010; Jorge et al. 2016). This enables, for example, a better understanding of how the supply with macronutrients like K effects plant metabolism. Comprehensive experiments with *Arabidopsis thaliana* (L.) HEYNH. revealed that K fertilization affects several metabolic pathways. In the shoot of young *A. thaliana* plants K deficiency increases the levels of carbohydrates, including sucrose, reducing sugars, and, to a lesser extent, starch. Additionally, a slight net increase in total protein content and the overall amino acid level was observed (Armengaud et al. 2009). In young tomato leaves K deficiency was found to affect several pathways, mainly sugar metabolism, glycolysis, tricarboxylic acid (TCA) cycle and nitrogen assimilation. An organ-specific decrease of the organic acid in the leaves and an increase in the roots was shown (Sung et al. 2015). Other metabolomics studies on tomato focused on other factors, such as fruit development (Carrari et al. 2006; Tohge et al. 2014) or nitrogen nutrition (Urbanczyk-Wochniak and Fernie 2005). However, the study of Sung and colleagues (2015) is apparently the only one investigating the effect of K on tomato metabolome but here the focus was on young tomato plants. So far, to the best of the author's knowledge, no studies investigating the effect of K fertilization on the metabolome of the agronomic important part, the tomato fruit.

As the macro nutrient K is vital for many physiological functions in plants (Hawkesford et al. 2012) and a limiting factor in many soils (Römheld and Kirkby 2010), there is a need to understand the effect of K on agronomic parameters like crop yield. Further, overall fruit quality - which is closely connected with the fruit metabolite profile - is of increasing interest for consumers. For these reasons, we investigated the impact of K supply on tomato fruit metabolite profile, including possible inter-cultivar differences. In order to cover a wide range of relevant metabolite classes like sugars, sugar alcohols, amino acids, amines, organic acids, sterols as well as unknown compounds, we performed an untargeted metabolome analysis. For this purpose, we used a comprehensive two-dimensional gas chromatography-mass spectrometry platform because of its high separation performance and excellent sensitivity (Wojciechowska et al. 2014; Weinert et al. 2015).

Materials and methods

Growth conditions

For the experiments the three cocktail tomato cultivars Resi´ (R), `Primavera´ (P) and `Yellow Submarine´ (YS) (Dreschflegel GbR, Witzenhausen, Germany) were used. Cultivars were chosen according to their variation in fruit acidity in the following order: Primavera < Yellow Submarine < Resi (unpublished data). Resi and Primavera seeds were provided by Dr. Bernd Horneburg (Georg-August-University Göttingen, Department of Crop Sciences, Division Plant Breeding). Seeds were sown into 94-cells seedling starter trays at the 04.04.2014 (Resi and Primavera) and 05.04.2015. At the 22.04.2014 all plants were transplanted to 11 cm diameter nursery pots (1 L volume). In the starter trays the substrate was comprised of different peats, flesh of coconut and perlite ("Anzuchtsubstrat organisch", Kleeschulte, Rüthen, Germany). The substrate in the nursery pots was peat soil (A 400, Stender, Schermbeck, Germany). Tomato plants were raised under controlled conditions (long day light conditions: 6 am until 22 pm, 22°C during the day and 18°C at night) until their final transplantation. From the 21.05.2014 to 23.05.2014 the transplantation to the final outdoor location at the Georg-August-University Göttingen (coordinates: 51.546456, 9.944742) took place. The tomatoes were planted into `Mitscherlich vessels` (6 L volume) filled with peat ("Gartentorf", Naturana, Vechta, Germany). The plants were arranged in a randomized block design with four replications. Each block had 15 different groups (3 cultivars and 5 K-levels) and each group comprised

of five tomato plants. The five K levels (K1 to K5) had increasing K concentrations (0.4, 0.7, 1.1, 1.4 and 2.2 g K_2SO_4) in the supplied nutrient solution. Potassium was applied weekly together with nitrogen. In week 16 after planting the K-levels K3, K4 and K5 were increased (K3 to 1.5 g K_2SO_4; K4 to 2.2 g K_2SO_4 and K5 to 3.7 g K_2SO_4). To raise the pH, the peat was supplemented with lime ($CaCO_3$) one week before transplanting. Macro- and micro-nutritients were added in liquid form at the final transplantation and at mid-season (week 15 after planting), except for phosphorus, which was fully integrated in solid form to the peat in the Mitscherlich vessels at final transplantation. Tomato plants were watered with deionized water, if needed. The flow through water was collected and poured back. The tomatoes were regularly pruned to one shoot.

Sampling

The fruits were harvested weekly, starting in the middle of July. At the fourth harvest on 7th August 2014, samples were collected for the determination of the mineral content and for the untargeted metabolome analysis. As two independent samples were taken per replicate, the total number of samples per cultivar and K level was eight. In case of Resi, the number of samples per K-level varied between four and eight because the yield of this cultivar was very low (chapter two). For each sample, over 10 -20 fruits were quartered, frozen in liquid nitrogen and freeze-dried (Epsilon 2 – 40, Christ, Osterode, Germany). Lyophilized samples were milled with a ball mill (30 s at 30 Hz; MM 400, Retsch, Haan, Germany) and stored at -80°C. A pooled “quality control” was prepared by combining material from a representative selection of the study samples.

Determination of mineral content

Ground samples were dried at 105°C and 100 mg were weighted into a teflon vessel. The analysis was done as described by Koch et al. (2019).

Determination of amines by HPLC

For the extraction 100 mg of the freeze-dried powder were mixed with 4 mL of extraction solution (0.2 N perchloric acid). The perchloric acid containing 10 µg of diaminoheptan (DAH) as an internal standard. The mixture was placed in the refrigerator for 60 min and shaken every 20 min. Subsequently, 1.5 mL of Polyvinylpolypyrrolidone was added and centrifuged (20 min at 4,000 rpm, Haraeus Megafuge 16R, Thermo Scientific, Waltham, MA

USA). The supernatant was centrifuged again (20 min, 4000 rpm). To 300 µL of the supernatant 200 µl of saturated Na_2CO_3 and 400 µL of the DNS-Cl solution (10 mg of DNA-Cl / mL of acetone) were added for the derivatization. Then the sample were incubated in a shaker without light (60 min, 60°C, 550 rpm, Eppendorf Thermomixer comfort, Hamburg, Germany). Solid phase extraction was carried out with the Baker SPE system at a suction voltage of 5-6 bar. First the C18 separation column was prepared by two column fillings of 2.5 mL of MeOH and two column fillings of water. Then the sample was applied to the column and vacuum-filtered together with two column fillings of water. To elute the amines from the column, 2 mL of MeOH were added. The amine samples were then filtered through a 0.45 µm PTFE filter (VWR, Darmstadt, Germany) into the final vials.

The analysis was carried out with a HPLC system from Jasco (Jasco Labor- und Datentechnik GmbH, Gross-Umstadt, Germany). 15 µL injection volume was drawn by an autosampler (AS-2051 Plus Intelligent Autosampler, Jasco Labor- und Datentechnik GmbH, Gross-Umstadt, Germany), and the analysis time was 59 min. The excitation was carried out at a wavelength of 254 nm, the emission wavelength at 510 nm. A PerfectSil target ODS-3 separation column (MZ Analysentechnik, Mainz, Germany) with a size of 250 × 3 mm was used. Detection was carried out by a fluorescence detector from Jasco (FP-2020 Plus Intelligent Fluorescence Detector, Jasco Labor- und Datentechnik GmbH, Gross-Umstadt, Germany). The eluents used were (A) acetonitrile and (B) 0.1 M Tris buffer (pH 8.5) and water (1: 2).

For the identification and quantification of the polyamines, a calibration curve with reference compounds was created. The reference compounds were histamine, ethylamine, agmatine, tryptamine, isopentylamine, phenylethylamine, diaminopropane, putrescine, cadaverine, serotonin, tyramine, spermidine and spermine. Data were analyzed using the JASCO ChromPass Chromatography Data System software. The limit of quantification (LOQ) was calculated 10 times the noise level and for limit of detection (LOD) 3 times the noise level.

Untargeted GC×GC-MS metabolome analysis

100 mg per sample were weighed in 2 mL Eppendorf tubes (Hamburg, Germany). After the addition of 1,500 µL of methanol to the powder, the samples were spiked with 90 µL of a solution containing seven internal standards (D-pinitol, ribitol, 1-*O*-methyl-2-desoxy-D-

ribose, 5-bromo-2,4-dihydroxybenzoic acid, 5-chlorosalizylic acid, 2-chlorophenylacetic acid and 2-(4-chlorophenyl)ethylamine, each 2.5 or 5 mM in 30 % EtOH) and mixed for 10 min at 35°C and 1,400 rpm. The insoluble matter was sedimented by a short centrifugation and 1,400 µL of the supernatant were transferred to a new tube. The samples were re-extracted a second time, both supernatants were combined and finally centrifuged for 5 min at 16,100×g and 4°C. 10 µL of the supernatant were transferred to screw-threaded GC vials containing 200 µL inserts and evaporated in a vacuum centrifuge for 1 h at 40°C and p < 1 mbar. To remove traces of water, 10 µL of methanol were added and the samples were re-dried again in the speedvac for 20 min. For methoximation, 25 µL of methoxylamine-hydrochloride in pyridine (20 mg/ml) were added and samples incubated for 30 min at 70°C under shaking. Trimethylsilylation was initiated by the addition of 50 µL MSTFA +1 % TMCS and carried out for 1 h at 75°C without shaking. An amount of 20 µL of a linear retention index mixture (saturated fatty acid methyl esters (C7 to C28), each 250 µM in heptane) were added only to daily reagent controls after derivatization. All samples were analyzed within 24–30 h after preparation.

For GC×GC-MS analysis, the system and the method described previously (Hegeman 2010; Wojciechowska et al. 2014) were used with slight modifications: i) The initial temperature of the OPTIC-4 injector was 90°C. ii) Temperature program: 90°C – 2°C/min – 100°C – 4°C/min – 140°C – 3°C/min – 200°C – 5°C/min – 280°C – 40°C/min – 320°C (2.5 min). The total run time was 54.5 min. The analysis was performed in seven day-wise batches which comprised in total 175 runs, including 58 QC runs, 7 blank sample runs and 110 study sample runs. The qMS was tuned before the first and the fifth batch. The septum was replaced after approx. 100 runs.

Data processing and data evaluation

Raw data were processed using the two-step procedure as described by Egert and colleagues (2015). Briefly, the GCMSsolution software (V. 4.11; Shimadzu, Kyoto, Japan) was used for sample-wise peak identification and library matching. The peak data (area and height, retention time, retention index, compound annotation, etc.) and the corresponding mass spectra were compiled as text files. The subsequent processing was done using several R modules and comprised i) import and reformatting of the textual data, ii) a data reduction step aiming to remove non-analyte peaks, iii) the alignment, iv) the merging of

the modulations per analyte per run (demodulation), and v) a correction of drift and batch effects.

After automatic processing, the quality of the GC×GC-qMS data set was evaluated as follows: At first, the integrity of the QC and study sample runs was assessed by calculating for each sample the mean relative deviation of the signal intensities of the internal standards from the mean of all samples in the respective batch. Here, all QC and study samples were confirmed to be within the acceptance limits of 80-120 %. Afterwards, the 409 „raw“ analytes detectable in at least 75 % of the samples of one group (i.e. one K level of one cultivar) were closely inspected in order to exclude known artefacts, not automatically removed sections of noise bands, internal standards as well as coeluting or irreproducible analytes (mean intra-day repeatability RSD ≥ 30 %). 244 analytes were finally considered for statistical testing.

Statistics

All statistical operations were performed with JMP 12.0.1 (SAS Institute GmbH, Böblingen, Germany). First, for principle component analysis (PCA) of the metabolite profiles of all three cultivars, the 233 analytes detected in at least 70 % of all study samples were selected and the remaining missing values (non-detects) replaced by the value 10,000. For cultivar-wise PCA and the ANOVA screening analysis (see below), the selection of analytes and the replacement of missing values were done analogously. Finally, the data matrices contained 224 analytes forPrimavera, 228 for Resi, and 242 for Yellow Submarine.

Although cultivar-dependent differences in global metabolite profile proved to be large, this factor was not considered to be of interest within the scope of this study, For this reason, a one-factorial ANOVA screening analysis was performed for each cultivar separately as described recently (Weinert et al. 2017), with the modification that distribution of the selected metabolites was examined using the Shapiro-Wilk test on residues. This ANOVA screening approach was also used for statistical evaluation of the mineral data (as determined by ICP-OES) and the amine data (as determined by HPLC). The minerals data set contained no non-detects. In case of the amine data, all analytes with more than 30 % missing values were excluded. The few non-detects within the remaining data matrix (mostly less than 10 % per analyte) were ignored.

Results

Changes in mineral content

The mineral content of tomato fruit was determined using ICP-OES. The increasing potassium fertilization resulted primarily in a statistically significant and substantial (between +26 % and +48 %) increase of the potassium content in the tomato fruit (Table 6). This increase was dose-dependent in all cultivars. While the increase was highly linear in the cultivars Primavera (r^2=0.864) and Resi (r^2=0.787), a tendency of saturation was observed with Yellow Submarine (Figure 8). Among the other minerals, magnesium (increased) as well as calcium and phosphorous (both decreased) were also significantly changed in two cultivars each. However, absolute changes were mostly smaller or the effect was not clearly dose-dependent. Additionally, in case of Primavera, sulphur, manganese, zinc, and copper were changed but this effect was neither linear nor dose-dependent. For additional boxplots see supplemental Figure S6.

Table 6. Effect on K fertilization on mineral content of tomato fruit.

Mineral	Primavera			Resi			Yellow Submarine		
	FC	r^2	p	FC	r^2	p	FC	r^2	p
K	1.48	0.86	<0,001	1.39	0.79	<0,001	1.26	0.58	<0,001
Mg	1.04	0.16	0.014	1.26	0.42	<0,001			
Ca	0.78	0.61	<0,001				0.69	0.44	<0,001
P	0.90	0.10	0.059				0.77	0.42	<0,001
S	1.05	0.27	0.001						
Mn	1.01	0.09	0.075						
Zn	0.81	0.12	0.036						
Cu	0.76	0.11	0.042						

Fold changes of more than ± 20 % (>1.2 or <0.8) and correlation coefficients larger than 0.4 are set off by bold face. FC, relative fold change between fertilization levels K1 and K5; r^2, Pearson correlation coefficient; *p*, ANOVA p-value for significance of the correlation.

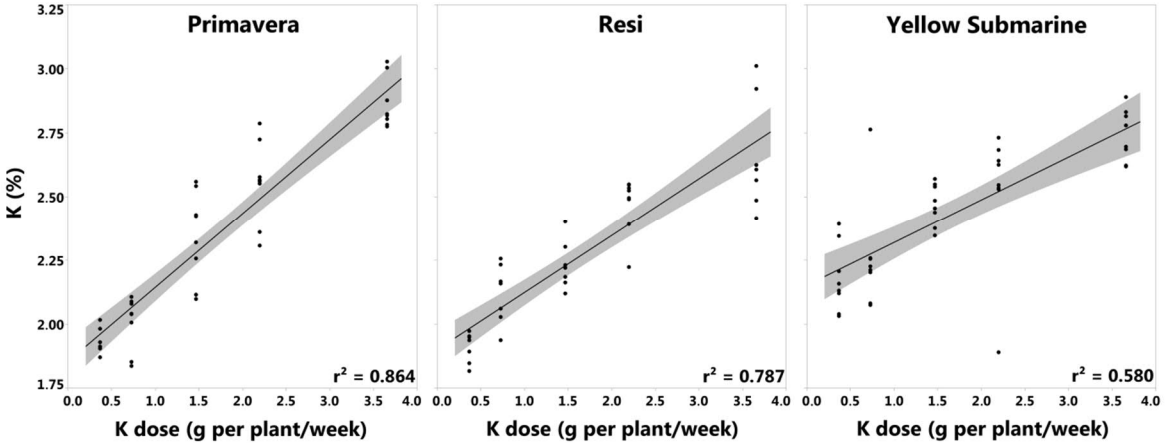

Figure 8. Increase of K content of tomato fruit (percent of dry matter) with increasing K fertilization (weekly K dose in g per plant). – To enable comparability, a linear regression was performed for all cultivars.

Changes in the metabolite profile

An untargeted GC×GC-MS analysis of the tomato fruit metabolite profile was performed. After evaluation of data quality, 244 analytes were considered as genuine metabolites that could be reproducibly semi-quantified.

At first, in order to identify the major sources of biological variation in the data set, a PCA was performed. A clear separation of the samples belonging to the different cultivars was observed (Figure S5 A). In contrast, the metabolite profiles of the samples from the same cultivar but resulting from the different K fertilization levels were more similar. While the most extreme levels K1 and K5 were well-separated in case of the cultivar Primavera (Figure S5 B) and somewhat separated in case of Yellow Submarine (Figure S5 D), no separation was observed for the cultivar Resi (Figure S5 C).

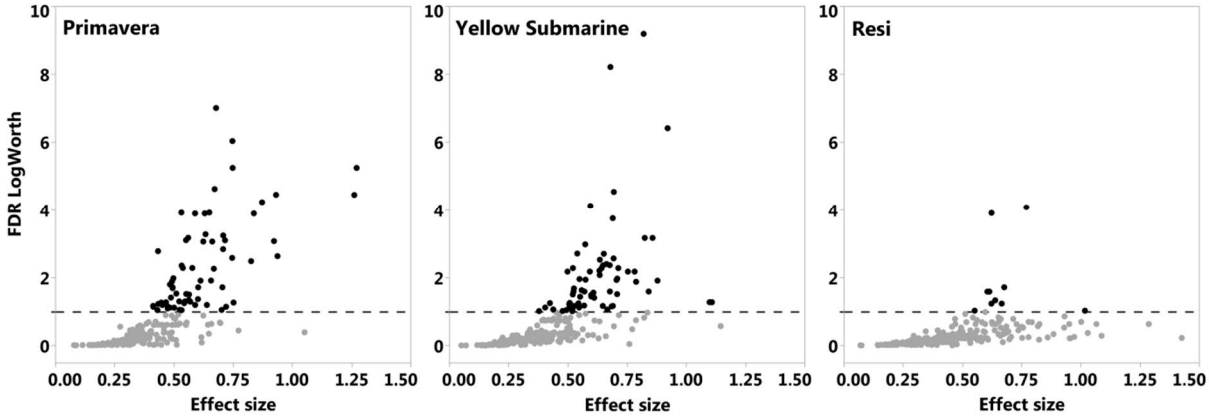

Figure 9. Results of the ANOVA screening highlighting major cultivar-specific differences concerning the impact of K fertilization on the tomato fruit metabolite profile. – The y-axis represents the significance, given as the negatively log-transformed false discovery rate p-values (FDR LogWorth) calculated according to Benjamini and Hochberg.δ The dashed lines denote the significance threshold ($-\log_{10}(0.1) = 1$). Metabolites lying above this line (black dots) were considered as potentially discriminating and further examined using specific downstream testing procedures. The x-axis represents the effect size as a measure of the practical relevance of the observed difference.

A more detailed investigation of the K fertilization effect on tomato fruit metabolite profile was performed using an ANOVA-based approach. Here, the objective was to assess the K response of the single metabolites in the different cultivars separately, thus excluding the dominant inter-cultivar differences (Figure S5 A). Figure 9 highlights the overall outcome of the ANOVA analysis: The K fertilization treatment caused large changes in the fruit metabolite profile of the cultivars Primavera and Yellow Submarine (63 and 57 metabolites significantly changed, respectively) while only 10 metabolites were significantly influenced with Resi. The most important discriminant metabolites are compiled in Table 8. The response patterns of selected metabolites to K fertilization are shown in Figure 10. The

complete results of cultivar-wise ANOVA analyses can be found in supplemental Tables S15-S17.

Among the organic acids, the concentrations of especially citric acid and alpha-ketoglutaric acid increased with increasing K supply. Fruit levels of these two acids were also linearly correlated with K fruit levels, proving a dose-dependent effect (Table 8, Figure 10). Succinic acid and threonic acid were also increased at higher K supply, but this effect was not clearly dose-dependent (Figure 10). Remarkably, only these four compounds were significantly changed in all three cultivars. Beyond that, several other acids were changed in only one or two cultivars. While levels of quinic acid, malic acid, isocitric acid and another citric acid-like compound were increased at higher K supply, the other acids like citramalic acid, dehydroascorbic acid, galacturonic acid and several compounds tentatively identified as acids (Table 8) were decreased at higher K supply.

In case of the amino acids, the effect of K fertilization was even more cultivar-specific (Table 8, Figure 10). Levels of all amino acids in the cultivar Resi were not significantly altered by the K treatment. In contrast, several amino acids decreased significantly at higher K supply in one or both other two cultivars. The largest fold change was observed for asparagine, followed by an S-methylcysteine-like compound and methionine. In case of glutamine, an apparent effect was non-significant due to high variation within the K level groups. Further, oxoproline, cysteine, lysine, leucine and tyrosine decreased significantly in one of the two cultivars. Interestingly, phenylalanine levels increased in the cultivar Primavera and decreased in the cultivar Yellow Submarine. The non-proteinogenic amino acids beta-alanine and gamma-aminobutyric acid showed an increase only in the cultivar Primavera. The levels of other proteinogenic or non-proteinogenic amino acids detected like glycine, alanine, valine, serine, threonine, aspartic acid, glutamic acid, and 2-pyrrolidinone remained unchanged in all cultivars.

Amines were covered by the untargeted GC×GC-MS method as well as a targeted HPLC method. Using GC×GC-MS, a reduction of isopentylamine and *O*-phosphorylethanolamine with increasing K supply was observed with Primavera and Yellow Submarine. Putrescine decreased significantly in Primavera and Resi and non-significantly in the cultivar Yellow Submarine. In contrast, levels of serotonin increased significantly in the cultivar Yellow Submarine and non-significantly in the cultivar Primavera (Table 8, Figure 10). Using the

targeted HPLC method, 6 amines (histamine, ethylamine, diaminopropane, putrescine, tyramine and spermidine) were consistently detected (maximally 12.5 % missing values) and quantified in all cultivars. Additionally, spermine and cadaverine could only be quantified in Resi and Primavera, respectively. As shown in Table 7, a strong and linear decrease in putrescine concentrations with increasing K supply was observed in all cultivars. Spermidine levels decreased significantly only in the cultivars Resi and Yellow Submarine. The concentrations of the other amines remained unchanged.

Table 7. Effect on K fertilization on amines in tomato fruit determined by HPLC.

Amine	Primavera			Resi			Yellow Submarine		
	FC	r^2	p	FC	r^2	p	FC	r^2	p
Putrescine	0.28	0.725	<0.001	0.26	0.794	<0.001	0.53	0.657	<0.001
Spermidine				0.53	0.263	0.027	0.67	0.278	0.030

FC, relative fold change between fertilization levels K1 and K5; r^2, Pearson correlation coefficient; *p*, ANOVA p-value for significance of the correlation.

The major mono- and disaccharides (glucose, fructose and sucrose) were not affected by K fertilization in all cultivars. Nevertheless, a range of other sugar or polyols were changed in the cultivars Primavera and Yellow Submarine (Table 8, Tables S15-S17), especially five minor disaccharides, the trisaccharide, galactinol and several phosphorylated substances. Beyond that, many unknown sugar-like species, mostly larger than monosaccharides and apparently bearing additional groups as indicated by their higher ^{2}D retention, were among these metabolites. The only compound changed specifically in the cultivar Resi was glucose-6-phosphate.

Finally, a range other, in part secondary metabolites was influenced by the K treatment in a cultivar-specific manner, for example chlorogenic acid, naringenin, alpha- and gamma-tocopherol, uridine as well as nicotinic acid (Table 8, Figure 10). Interestingly, the levels of these compounds were mostly higher at lower K supply.

Table 8. Effect of K fertilization is visualized by the results of ANOVA-based statistics for selected metabolites determined by GCxGC MS.

	Metabolite	Primavera			Resi			Yellow Submarine		
		FC	mFC	r^2	FC	mFC	r^2	FC	mFC	r^2
Organic acids	Citric acid TMS_4	1.44	1.44	0.684	1.46	1.46	0.457	1.22	1.22	0.146
	alpha-Ketoglutaric acid MeOX-TMS_2	1.84	1.85	0.570	2.05	2.05	0.510	2.23	2.23	0.517
	Succinic acid TMS_2	1.12	1.45	0.100	1.26	1.42	0.228	1.41	1.45	0.162
	Threonic acid TMS_4	0.99	1.31	0.124	1.46	1.46	0.218	1.50	1.50	0.165
	Quinic acid TMS_5	1.15	1.17	0.347	1.21	1.21	0.352			
	Citric acid-like[a]	2.39	3.16	0.332	1.57	1.57	0.313			
	Ribonic acid or similar	0.77	0.77	0.086				0.72	0.72	0.241
	Citramalic acid TMS_3[a]	0.70	0.60	0.058						
	Malic acid TMS_3	1.22	1.44	0.376						
	Isocitric acid TMS_4	1.48	1.92	0.164						
	Glucuronic acid gamma lactone-like	0.89	0.80	0.244						
	Dehydroascorbic acid isomer	0.84	0.84	0.112						
	Dehydroascorbic acid isomer	0.77	0.77	0.336						
	Dehydroascorbic acid isomer[a]	0.82	0.82	0.125						
	Galacturonic-acid MeOX-TMS_5	0.89	0.80	0.326						
Amino acids	Asparagin TMS_3	0.19	0.19	0.217				0.51	0.49	0.333
	Asparagine TMS_2	0.30	0.30	0.252				0.49	0.49	0.381
	Phenylalanine TMS	1.76	1.82	0.247				0.49	0.49	0.273
	L-Methionine TMS_2	0.61	0.61	0.106				0.63	0.63	0.061
	S-Methylcysteine TMS_2-like	0.23	0.23	0.163				0.48	0.46	0.258
	Isoleucine TMS_2	1.02	1.58	0.014				0.41	0.41	0.182
	beta-Alanine TMS_3	1.48	1.54	0.201						
	5-Oxo-L-proline TMS_2	0.56	0.52	0.090						
	gamma-Aminobutyric acid TMS_3	1.18	1.21	0.383						
	L-Cysteine TMS_3[a]	0.52	0.52	0.131						
	Lysin TMS_4	0.34	0.34	0.171						
	Leucin TMS_2							0.40	0.40	0.175
	Methionine TMS							0.57	0.57	0.157
	Phenylalanin TMS_2							0.50	0.46	0.193
	Tyrosine TMS_3							0.29	0.27	0.268
Amines	Isopentylamine TMS_2[a]	0.37	0.37	0.220				0.46	0.46	0.192
	Putrescine TMS_4	0.14	0.14	0.459	0.10	0.10	0.644			
	O-Phosphorylethanolamine TMS_4	0.35	0.35	0.290				0.32	0.32	0.423
	Serotonin TMS_4							1.72	1.72	0.239
Sugars and polyols	Disaccharide (A1085)	1.23	1.25	0.063				1.44	1.44	0.276
	Galactinol TMS_9	0.30	0.30	0.439				0.56	0.52	0.255
	Disaccharide (A1127)	0.85	0.84	0.340				0.51	0.51	0.433
	Trisaccharide (Maltotriose or similar)	0.71	0.71	0.082				0.69	0.69	0.199
	Unknown sugar-like (A0999)	0.54	0.54	0.515				0.68	0.68	0.170
	Unknown sugar-like (A0908)	0.50	0.50	0.417						
	Disaccharide (A1094)	1.11	1.19	<0.001						
	Disaccharide (A1103)	1.22	1.29	0.008						
	Disaccharide (A1069)							1.14	1.32	0.090
	myo-Inositol phosphate TMS_7[a]	0.80	0.80	0.251						
	Glucose-6-phosphate MeOX-TMS_6				0.75	0.75	0.454			
	Glycerol phosphate TMS_4 or similar[a]							0.75	0.75	0.156
	Glycerol phosphate-like[a]							0.78	0.63	0.059

a trace analyte; rFC: relative fold change (quotient K5/K1); mFC: maximum fold change (quotient of the smallest and the largest group mean); r^2: Pearson correlation coefficient. Green-red scale highlights increasing or decreasing relative fold changes. Green represents rFC above 1 and red represents rFC below 1.

Table 8., continued

Other metabolites	Chlorogenic acid TMS_6	0.43	0.43	0.208				0.57	0.49	0.156
	Uridine TMS_3	0.78	0.72	0.224	0.68	0.68	0.422			
	Naringenin MeOX TMS_3 or similar	0.14	0.14	0.261						
	Nicotinic acid TMS				1.16	0.65	0.004			
	beta/gamma tocopherol TMS							0.68	0.68	0.196
	alpha-tocopherol TMS							0.84	0.78	0.128
	Heptadecanoic acid TMS							0.73	0.70	0.241
	Oleic acid TMS							0.77	0.72	0.226

Discussion

General effect of K fertilization

The aim of this study was to investigate the changes of metabolite profile in tomato fruit induced by increasing K fertilization. First of all, as shown by ICP-OES analysis, K fertilization was successful insofar that an increased K supply lead to a substantially increased K concentration in the tomato fruit (relative increase of up to 48 % or absolute increase of up to 1 % of the dry matter; see Table 6, Figure 8). This effect was anticipated as it was already demonstrated by others (Davies 1964; Chapagain et al. 2003; Serio et al. 2007; Taber et al. 2008). Also the concentrations of some other minerals in tomato fruits changed but to a much lower extend, not consistently in all cultivars (Table 6, Figure S6) and moreover not resulting in specific deficiency symptoms nor in deficient tissue levels (Pujos and Morard 1997). A decrease in fruit calcium content is observed in several studies as K and calcium are antagonists with regard to their uptake (Kabu and Toop 1970; Sainju et al. 2003; Hawkesford et al. 2012). This often results in blossom end rot of fruits (Fanasca et al. 2006; Zhu et al. 2009). However, decreasing calcium concentrations in the fruits of Primavera and Yellow Submarine (Table 6, Figure S6 C) did not cause blossom end rot. In summary, based on the ICP-OES data, we assume that alterations in fruit mineral concentrations were in an acceptable range and that the observed effect of K fertilization on tomato fruit metabolite profile was mainly caused by increasing potassium tissue levels.

As a next step, the metabolomics data set was first evaluated using PCA. A clear separation of the cultivars was obtained (Figure S5 A), indicating a major influence of the genotype. The results of PCA analysis indicated a cultivar-specific response to K fertilization (Figure S5 B-D) which was confirmed by the ANOVA-based statistics (Table 8, Figure 9 and 10).

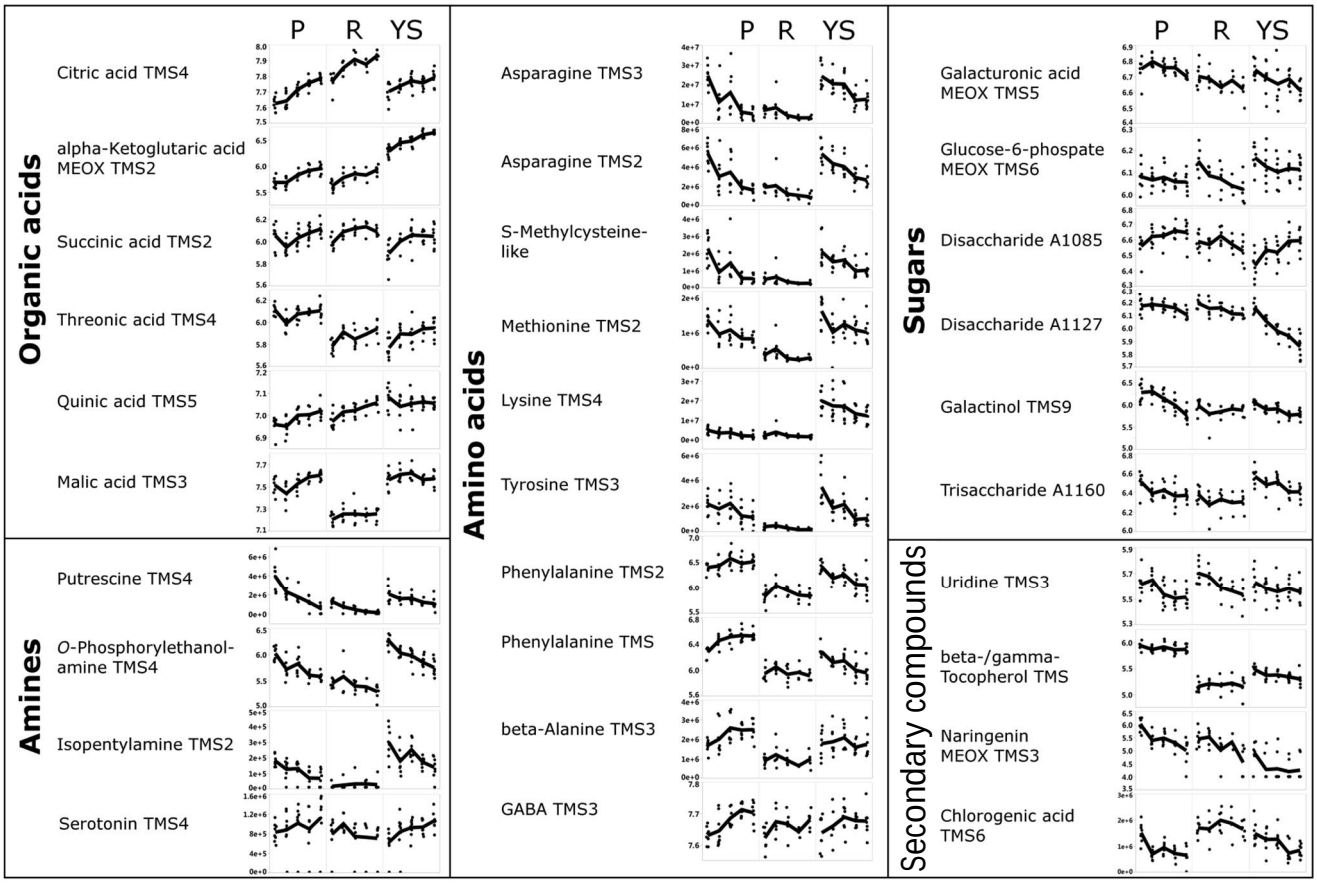

Figure 10. Response of selected metabolites to increasing potassium supply as determined by untargeted GC×GC-MS. – Cultivars: Primavera (P), Resi (R), Yellow Submarine (YS). On the x-axis and within each sub-panel, K fertilization levels are ordered from low (K1, left) to high (K5, right). To enhance comparability, signal intensity (y-axis) was log-transformed if necessary. Confer Table 8.

Likewise, different reactions of tomato cultivars towards altered K fertilization is observed on yield level and for specific fruit quality parameters like secondary plant compounds and organic acids (Caretto et al. 2008; Khan et al. 2014), but so far this has not been investigated at the metabolome level. Based on our results, we suggest that K fertilization i) has a cultivar-independent effect on TCA cycle metabolites and ii) may additionally have a cultivar-specific effect on other compounds or compound classes.

TCA cycle metabolites

One of the striking differences induced by the rising K application was the increased concentration of TCA cycle metabolites. Interestingly, the metabolites citric acid, alpha-ketoglutaric acid and succinic acid were accelerated in fruits of all cultivars while others like malic acid was changed only in the cultivar Primavera. Increasing concentrations of malic and citric acid of tomato fruits with higher K application was already demonstrated (Carañgal et al. 1954; Davies 1964). Malic and citric acid are precursors of several other metabolites like γ-aminobutyrate, flavonoids or via gluconeogenesis glucose (Etienne et al.

2013). Booth acids are also responsible for the acidic nature of fruits and stored in the vacuole in large quantities (Etienne et al. 2013). Nonetheless, it is likely that the K concentration influence the TCA cycle, as the quantity of other metabolites of the TCA cycle were influenced (Table 8, Figure 10). Such an impact on TCA metabolites seems to be a general mechanism, as it was shown in different plant organs of several plant species (Armengaud et al. 2009; Takahashi et al. 2012; Sung et al. 2015). Armengaud and colleagues (2009) assume this effect for two reasons: One is the direct inhibition of pyruvate kinase by low cytoplasmic K and thereby reduced carbon flux towards the TCA cycle. Secondly, the biosynthesis of amino acids is maintained at a net cost of organic acid. Furthermore, another mode of action is possible: The accumulation of negatively charged organic acids in parallel to the increased supply with the K^+ cation could be an unspecific "reaction" of the plant to maintain charge balance at the cellular level (Sung et al. 2015). The reason of this coherence between K concentration and TCA intermediates needs further investigation at the molecular level. However, this study was able to shows that the increase of TCA together with rising K fertilization is also true for tomato fruits.

Amino acids

In contrast to the organic acids, the K fertilization effect on amino acid levels was more specific which suggests that K influences the activity of certain enzymes of the amino acid metabolic pathways directly. In general, the effect of K fertilization on amino acid metabolism is apparently highly cultivar-specific (Table 8, Figure 10). Several amino acids accumulated in the fruits of the cultivars Primavera and Yellow Submarine at low K supply. This phenomenon was especially pronounced in case of asparagine which is very likely due to an inhibition of the K-dependent enzyme asparaginase (Sodek et al. 1980; Sieciechowicz et al. 1988) which hydrolyses asparagine to aspartic acid. In a metabolomics review on plant stress, a general accumulation during periods of biotic and abiotic stress was detected for the branched chain amino acids (BCAAs) leucine, isoleucine and valine. Together with the BCAAs other amino acids, like lysine, threonine and methionine, increase as well during stress. Thease amono acids share the same synthetic pathways, (Obata and Fernie 2012). In this study, several of these amino acids increase under K deficiency in the cultivars Primavera and Yellow Submarine, except for isoleucine in Primavera (Table 8, Figure 10). Indicating that two of the three cultivars show this general stress response.

Another common response to biotic and abiotic stress is the increase of the non-proteogenic amino acid *gamma*-aminobutyric (GABA) (Bouché and Fromm 2004; Saito et al. 2008; Obata and Fernie 2012). Surprisingly GABA, which accumulates during ripening in tomatoes (Takayama and Ezura 2015), is reduced under K deficiency in Primavera and there is no significant tendency in the other two cultivars (Table 8, Figure 10). GABA concentration varies between tomato cultivars and also the increase induced by stress is cultivar dependent (Saito et al. 2008).

Proline is another frequently accelerated amino acid under stress, such as water scarcity, salinity heavy metal enrichment and nutrition deficiency. It has thereby several functions, such as enhancing K^+ uptake for an osmotic balance or forming complexes with heavy metals (Rai 2002). In the tomato fruits of low fertilized Primavera plants, only 5-Oxo-L-proline shows higher values (Table 8, Figure 10). Therefor this general stress response is no universal response in tomato fruits suffering K deficiency.

The amino acids tyrosine and phenylalanine essential in human nutrition (Young and Pellett 1994), were increased in Yellow Submarine but phenylalanine was reduced in Primavera under K deficiency (Table 8, Figure 10). Again, were no significant changes detected by varying K fertilization for Resi. As the reaction to K differs in the cultivars this is another cultivar specific effect. Nonetheless are this amino acids very important for the formation of several secondary components like flavonoids, phenolic acids or alkaloids (Schopfer et al. 2006). This is a surprising result, as some of the amino acids are typically enhanced by biotic and abiotic stress. We assume that this cultivar can adapt better to K deficiency. To obtain similar results even lower K application would be necessary. As the BCAAs were increased, it is possible that the cutlivars cope with abiotic stresses by elevation the BCAAs.

Amines

The aliphatic amines are basic molecules, which are positively charged at physiological pH; they bind strongly to negatively charged nucleic acids, acidic phospholipids and many types of proteins (Bouchereau et al. 1999). Amines decrease in all cultivars with increasing K fertilization (Table 7 and 8). Only putrescine is reduced in all cultivars but not significantly in Yellow Submarine (Table S17). The accumulation of putrescine under K deficiency has been shown for other plant species (Watson and Malmberg 1996; Armengaud et al. 2004; Takahashi et al. 2012). The amines have an adaptive and protective role against stress such

as drought, salt or nutrient deficiency (Galston and Sawhney 1990; Gupta et al. 2013; Minocha et al. 2014). The enhanced production in case of stress functions via the ABA-dependent transcriptional regulation (Obata and Fernie 2012) and is down regulated after resupply of K^+ (Armengaud et al. 2004). Amines are cations and can in case of K deficiency help to maintain the charge balance (Galston and Sawhney 1990). In addition, amines can block outward Na^+ channels and thereby increase the K^+ / Na^+ ratio in the cytoplasm (Minocha et al. 2014). Stress, such as K deficiency, increases the production of ROS which result in stress-induced oxidative stress (Cakmak 2005; Zhao et al. 2018). ROS, as signaling molecules, are important for numerous biological processes but are toxic in high concentration (Baxter et al. 2014). Amines as antioxidants also help to protected the plant cell (Minocha et al. 2014). We suggest that the higher fertilized cultivars have a lower demand to synthesize amines, as they are not experiencing stress related to nutrient deficiency.

Sugars

The different sugar forms show a divers reaction to increasing K fertilization. The main sugars, such as glucose or fructiose were not changed in tomatoes (Figure S10). Whereas some other sugar forms showed a decrease with rising K level in Primavera and Yellow Submarine. Some disaccharides in contrast increase with elevated fertilization in the two cultivars (Table 8, Figure 10). In the fruits of the cultivar Resi only glucose-6-phosphate was reduced with rising K fertilization, none of the others was significantly changed (Table S16). This result was not anticipated, as K is important for long distance transport of sugars (mainly sucrose) in plants (Hawkesford et al. 2012; Beckles et al. 2012) and approximately 70 % of the sugars is produced in the leave. In addition, TSS increased in tomato fruits with rising K fertilization (Fanasca et al. 2006; Liu et al. 2011; Ozores-Hampton et al. 2012). The TSS content is used by several authors as an indicator for sugar concentration, as 65 % of the TSS are soluble sugars (Beckles et al. 2012). Therefor we expected a decrease in the fruits sugar content under K deficiency. In a study by Armengaud and colleagues (2009) the main sugars accumulate in the roots and the shoot of young *Arabidopsis thaliana* plants under K deficiency. However, in this study the sugars did overall not change (Table 8, Figure 10 and S10) indicating that the sugar transport is maintained under K deficiency towards the tomato fruit.

Effect secondary components

Many secondary components are health beneficial for humans. These metabolites are antioxidants or vitamins but decrease with rising K fertilization (Table 8, Figure 10). The vitamins C and E decreased cultivar-specific with increasing K fertilization (vitamin C in Primavera and vitamin E in Yellow Submarine). One exception is nicotinic acids (vitaminB_3), which increases in Resi with rising K doses. As K deficiency increases the production of ROS (Cakmak 2005; Zhao et al. 2018), plants have a higher need of antioxidants. Armengaud and colleagues (2004) showed a down-regulation of genes involved in stress adaptation after resupply of K^+, like the enzymes dehydroascorbate reductases or polyamine synthesis. As these enzymes produce antioxidants, this is a further indication that plants suffering from K deficiency need more antioxidants. In this study, the tomatoes grown with a low supply of K contain more antioxidants (Table 10). Several studies also described a cultivar effect on fruit antioxidants content (Martínez-Valverde et al. 2002; George et al. 2004). In this study the cultivar effect on the antioxidant concentration was confirmed, but more important showed that tomato plants suffering from K deficiency contain more antioxidants in their fruits.

Conclusion

This study indicates that the reaction to high and low K application is not uniform within the three different tomato cultivars. The only effect that was present in all cultivars was the increase of the TCA cycle metabolites with rising K application. This had before been demonstrated by other studies, but mainly in leaves. This study confirm that this is also true for tomato fruits. However, for most other compound classes the cultivar-specific effects were more dominant. Some of the general stress indicators were detected, like a general decrease of the amines and BCAA with rising K level. The accumulation of proline and GABA is also very common during periods of stress, but they were differently or not effected in the three cultivars. In addition, the amino acids, sugars and secondary components were differently affected / not affected by K in three cultivars. Especially the cultivar Resi responded differently, as only ten metabolites were significantly affected. One possible explanation is that this cultivar can tolerate lower K fertilization better. Indicating that minimum K fertilization requirements of the plants is cultivar specific.

5. Discussion

In this chapter, all previously presented results will be discussed in a wider context. Furthermore, the questions of which are the predominant changes and how important different cultivars are for the exploitation of varying results will be answered. Additional results are exhibited in order to support the explanation of the former results.

The acids content increased with rising K application (chapter two and four). This is the most striking change with increasing potassium fertilization in tomato fruits. Several acid concentrations increased, especially citric acid, alpha-ketoglutaric acid and succinic acid, just as TA did with rising K application (Table 8, Figure 4). Several of thoure increased acids are part of the TCA cycle. Rising TCA acids have previously been shown for leaves and roots (Armengaud et al. 2009; Sung et al. 2015). Also, malic and citric acid are stored in large quantities in the vacuoles and are responsible for the acidic taste of fruits (Shiratake and Martinoia 2007; Etienne et al. 2013). Induvidually was the positive influence of K fertilization on the organic acids (Carañgal et al. 1954; Davies 1964) and on TA (Wright and Harris 1985; Fanasca et al. 2006; Amjad et al. 2014) was also confirmed by earlier studies. Here both ist positvily influences, consequently is TA can be used as a good proxy for the organic acids in the tomato fruit. Two main reasons for the connection between the organic acid concentration in tomato fruits and the K treatment were discussed in chapter four. First, pyruvate kinase is directly inhibited by low cytoplasmic K and thereby reduced carbon flux towards the TCA cycle (Armengaud et al. 2009). The second hypothesis proposed that with rising supply of the cation K^+ also the negatively charged organic acids accumulate (Zörb et al. 2014; Sung et al. 2015). Eventually the latter is more reasonable, as large amounts of K^+ cations and organic acids are stored in the vacuoles, leading to ionic charge balance of the cells. In addition, the amines as cations increased in K deficient tomato fruits (Table 8), supporting the maintenance of the charge balance (Galston and Sawhney 1990).

Sugars beside acids are an important aspect of taste. The untargeted metabolomic analysis with GCxGC-MS displayed diverse tendencies for the different sugar forms. Glucose and fructose were not significantly influenced by K based on the results of the metabolomic study (chapter four, data not shown). Similar results were observed when sugars were analyzed with the HPLC (Figure S10). Therefore, these main sugars cannot be responsible

for the increase of TSS in the tomato fruits with rising K application. The majority of sugars and polyols in the tomatoes, assessed in the metabolomic study, were negatively affected by K treatments; only a few disaccharides increased in the fruits (Table 8, Figure 10). This indicates that neither the main nor other sugar forms are responsible for the increase of TSS with rising K fertilization. In any case a relation between K supply and sugar production can be anticipated, as an adequate K supply increases the production via photosynthesis and translocation (Hawkesford et al. 2012). Still the reasons for this discrepancy could not be fully uncovered within the present study. The sugar content in tomato fruits only partly reflects TSS as it accounts for approximately 65 % of TSS while organic acids have a share of 13 %. The organic acids increased as discussed above. It is therefore possible that they are responsible for the increase of TSS with rising K fertilizer rather than the sugars. Components of minor importance for TSS showed varying tendencies with increasing K supply as well, such as a decrease of most amino acids and naringenin (Table 10 and 4), while *p*-coumaric acid, caffeic acid and ascorbic acid showed an increase (Table 4, Figure 5). Nonetheless, other studies showed a good correlation between sugar and TSS in tomato fruits (Jones and Scott 1983; Beckles 2012). This relationship might be reliable, in cases where the acids are not influenced. In this study TSS was not a good proxy for the sugar concentration of tomato fruits and is therefore not recommended as an estimator for sugar determination. However, if TSS is used as a proxy for the sugar concentration in the future also TA should be analyzed to verify their effect on the results.

The sugar and acid concentrations in tomato fruits are important for the tomato taste (Yilmaz 2001; Kader 2008) and high quantities of both are favored by consumers (Causse 2002; Piombino et al. 2013; Oltman et al. 2014). However, an imbalance between sugar and acids reduces the preferred taste. The acid concentration increased with rising K application, as discussed above, whereas the sugars showed diverse tendencies (Table 2, 3 and 10, Figure 1). Nonetheless have cocktail tomatoes already high quantities of sugars (Beckles et al. 2012). Therefore, it is very likely that the taste is positively influenced by the increase o the acids with higher K application; but this needs to be evaluated by trained panelists.

Several studies showed a higher yield of tomato with rising K fertilization (Taber et al. 2008; Liu et al. 2011; Amjad et al. 2014). In a review on plant potassium status and plant stress

response (Wang et al. 2013) the authors concluded that an adequate K nutritional status is vital for plant resistance to biotic and abiotic stress and contributes to optimal growth, yield and quality of a crop. In comparison to the presented observations, these findings fit perfectly to the results of the tomato cultivar Primavera but not to the other investigated two cultivars. Hence, only for the highest yielding cultivar Primavera a yield increase was detected with rising K fertilization (Figure 3). This results of the other two cultivars were surprising, as an increase in yield was expected for all cultivars. A possible explanation can be the difference in biomass production, which was analyzed in 2015 for the cultivars Primavera and Resi. The plants of the cultivar Resi had higher leaf mass but less flowers and fruits compared to the cultivar Primavera (Table S3). This was also represented in the leaf to fruit ratio, where Primavera had a ratio of 2.5 for plants with high fertilizer amounts (K5) compared to Resi with a leaf to fruit ratio of 5.8. Consequently, low K fertilized plants (K1) of Primavera had a lower flower and fruit biomass than the respective high fertilized plants (K5). However, in the cultivar Resi, low K fertilized plants (K1) showed a lower flower biomass but a higher fruit weight compared to the high fertilized plants (K5) (Table S3). This indicates that the plants of Resi receiving low K amounts were very efficient in producing more fruit biomass from a low flower biomass. The cultivar Primavera showed the opposite characteristic; with rising K application, the plants produced more fruit biomass. Nevertheless, under K limitation the enhanced production of Resi was not significant and lower than the yield of low K Primavera plants. The reasons behind this difference in yield and biomass production in relation to K, require further analysis.

Another interesting aspect is that in the high K fertilization level (K5) fruits of the cultivar Primavera had higher K concentrations than fruits of the cultivar Resi, but similar levels in the other two treatments in 2015 (Figure S9). This is noteworthy as the fruit yield in the cultivar Primavera is generally much larger (Table 2 and 3). Thus, Primavera seemed to be more efficient in K uptake, transport, and utilization. For several agronomic important plant species, including tomato, positive correlations between K uptake efficiency and root hair length or density in K-depleted soils have been reported (Zörb et al. 2014). Therefore, it is likely that Primavera can translate a sufficient K supply into the production of increased root hair length, which results in a better K uptake and finally high yield. During times of K starvation, the yield of Primavera were highest compared to the other two cultivars

(Figure 3 and S3). This might be due to enhanced activity of K^+ channels or transporters during K limitation as it was reported that the expression of several K^+ channels or transporters are changed during long-term K starvation (Wang et al. 2013). The cultivar Resi had consistently low yield, which is almost independent of the K fertilizer application.

The cultivar Primavera showed an increase of the red color intensity (a*) with rising K fertilization in both investigated years. Resi on the other hand had more intensive red color values, but the color was not influenced by K fertilizer (Table 2 and 3). As described in the introduction and chapter two and three, the red color of tomato fruits originates mainly from the carotenoid lycopene. However, the lycopene concentration did not increase with rising K application in both red fruited cultivars (Figure 6). This result is surprising, as other studies showed a clear relationship between a* and lycopene (Arias et al. 2000; Hernández et al. 2007; Tavallali et al. 2018). Possibly the anthocyanins could have an influence on the color intensity; they decreased with rising K application in a study by Constan-Aguilar et al. (2015). Several flavonoids, such as rutin or naringenin, also play a role in the color formation of tomato fruits (Ballester et al. 2010). Naringenin for example is yellow, and decreases mostly non-significantly in all cultivars (Table 4) and hence might affect the intensity of the red color value a*. However, the evaluation of which pigment finally is responsible for the more intense red color of Primavera with increasing K application requires further investigation. Resi had more deeply red fruits. The tomatoes of the cultivar Resi are presumably preferred by the consumers, who favor bright red colors in tomatoes (Causse et al. 2010; Piombino et al. 2013; Oltman et al. 2014). The yellow color in the red and yellow fruited tomatoes is primarily produced by ß-carotene (Arias et al. 2000). Only in the cultivar Primavera b* values showed the same tendency as the ß-carotene concentrations (Table 2 and 3). Therefore, it is very likely that the ß-carotene concentration is responsible for the changed color value b* in the cultivar Primavera. However, in all cultivars other pigments like naringenin seemed to influence the red and partly yellow color. Consequently were the concentration of lycopene and ß-carotene not well displayed in the assessed color values.

In the year 2014, there were five increasing K treatments of the plants. Most fruit traits were positively affected until the highest K application K5 (e.g. Figure 1, Table 2 and 3). However, DM only increased until the second-highest level (K4, 2.2 g K_2SO_4 weekly

fertilization) in Primavera and Yellow Submarine (Table 2 and 3). In Yellow Submarine, a few analytes of the metabolomics study also showed the highest concentrations in the treatment K4 rather than in K5 (e.g. an unknown amino sugar, a disaccharide and itaconic acid; Table S17). In the other two cultivars, most of the affected analytes are part of or closely related to the TCA cycle (Primavera: gamma-aminobutyric acid and isocitric acid; Resi: succinic acid; Table S15 and S16). However, in most cases treatment K4 was not differing significantly from treatment K5, indicating that this trend is not very reliable. This observation is of interest as over-fertilization with potassium can cause deficiency of the cations calcium and magnesium as a result of uptake antagonisms (Kabu and Toop 1970; Sainju et al. 2003). In severe cases, calcium deficiency together with environmental stress such as excessive light and temperature can lead to blossom end rot (Ho et al. 1993). In this study blossom end rot was not detected and the concentration of calcium and magnesium were not below critical levels (Figure S6 B), indicating that the applied fertilizer amounts didn't excessively affect the uptake of the other nutrients. Nevertheless, this trend suggests that the applied amount of K fertilizer is sufficient and further application had no enhancing effect on yield and fruit quality traits. Although this is rather speculative, other studies also found an optimum fertilizer amout for yield (Liu et al. 2011, Ozores-Hampton et al. 2012), lycopene (Afzal et al. 2015), tocopherol (Caretto et al. 2008), ascorbic acid, TA, and TSS (Javaria et al., 2012). Hence, further improvement of these parameters is difficult to achieve by elevated K treatment but has to be accomplished by other techniques for instance breeding.

Differences between tomato cultivars were also demonstrated beforehand in other studies (George et al. 2004; Anza et al. 2006; Slimestad and Verheul 2009; García-Valverde et al. 2013). Therefore, in the present study minor differences were expected for both the concentration of different parameters and also for the influences of the increasing K treatment. However, it was demonstrated that the reaction towards different K application varied greatly between the cultivars as the cultivar Primavera was especially sensitive whereas the cultivar Resi showed only few changes. This is depicted in chapter two and four. In chapter three the relationship of K treatment and the antioxidant concentration was studied. Irrespective of the cultivar, only a few influences were detected over both years. Within chapter two the red color intensity and yield increased with rising K

application only in the cultivar Primavera but not in the cultivars Resi and Yellow Submarine (Table 2 and 3). The metabolomic analyses revealed that only ten metabolites in the fruits of the cultivar Resi were affected, while in the other two cultivars over 55 metabolites were altered (Tables S15 –S17). Also, several metabolites were just significantly changed in one cultivar by rising K application (Table 10). This indicates that the factor cultivar is of major importance for the evaluation of the changes induced by increasing K fertilization. As discussed in the introduction, some authors found a relationship between K and certain parameters while others did not. This might thus have to do with the use of more or less sensitive cultivars to K fertilization. For a generalized fertilizer effects more than one cultivar should be tested, considering the sensitivity of different cultivars.

In relation to practical aspects, one might expect that experiments analyzing different parameters such as yield, taste relevant compounds or antioxidants linked to increasing fertilizer treatments will in the end lead to a fertilization guideline. This is however not possible for several reasons. First of all, the response of the tested parameters to increasing K fertilization was very diverse within the three cultivars. The organic acids of the tomato fruits, an important aspect of the taste, increased with rising K application in both years and all cultivars. Hence for taste improvement, a higher K application (K5, 3.66 g K_2SO_4 per week) is advisable. Contrariwise, if an improved yield or fruit color is desired, a clear generalized recommendation is impossible, as only one cultivar showed improvement in these parameters with rising K application. Therefore, cultivars should be tested beforehand for their response to K fertilizer. Additionally, plants of the lowest K treatments (K1) had a reduced vitality towards the end of the seasons. If tomatoes shall be cost-efficient harvested for a longer period of time then a medium application is advisable, such as K3 (1.47 g K_2SO_4 per week) of this study. Consequently, advice can be provided for certain fruit quality traits but cannot be generalized for several traits.

6. Conclusion

The predominat effect of K application on cocktail tomatoes seems to be the cultivar-independent increase of organic acids with K application. Another cultivar-independent trend was the accumulation of most amines in K deficient fruits. Ascorbic acid and the phenolic acids *p*-coumaric acid and caffeic acid concentration rose with K fertilization, but patly insignificant. All other changes were cultivar dependent; especially the cultivars Resi and Primavera showed different reactions towards increasing K application. More sensitive to incrasing K was Primavera, e.g. showing more significant interaction. Only in Primavear was yield positively affected by high K application.

The major pigments lycopene and ß-carotene in red tomato fruits are not well displayed by the color values a* and b*. However, in the investigated tomato cultivars other pigments, possibly the anthocyanins or flavonoids, affect the color. With regard to taste, TA proved to be an appropriate proxy for acids. As major sugars did not respond to K application and minor sugars showed different responses to K treatments, TSS is in this case is not a suitable estimator for sugar concentration. Most antioxidants were probably more affected by abiotic factors, than by the applied K amounts. However, the most consistent K fertilization effects were found for naringenin, *p*-coumaric acid, caffeic acid, and ascorbic acid.

Overall, this study highlighting the impotence to analyze more than one cultivar to obtain a more general picture of the influences of K, as most changes were cultivar dependent and only the organic acids increased in all cultivars with rising K application. Overall, Primavera showed highest yields and several improved quality parameters under elevated K application. Therefore, can Primavera, out of the three cultivers, with sufficient K fertilization be recommended for commercial and home-garden tomato production.

7. Summary

The tomato (*Solanum lycopersicum* L.) is a worldwide important vegetable, with an annual production of 170.8 million tons in 2014. Potassium (K) has several physiological functions in plants, such as translocation of assimilates, activation of enzymes, maintenance of turgescence, and stomata regulation and thereby contributes to fruit yield and quality. The aim of all experiments was to investigate the impact of increasing K application on tomato fruit quality for a better understanding of K's physiological functions. Therefore, different cocktail tomato cultivars (Primavera, Resi, and Yellow Submarine) were studied in two consecutive years in outdoor pot experiments.

Total soluble solids (TSS), titratable acids (TA), dry matter (DM), color, and firmness are important consumer-related quality traits. Especially high concentration of TSS and TA are taste beneficial. In all studied cultivars TSS, TA, and partly DM increased with rising K fertilization. Other parameters, such as color, firmness and yield increased in Primavera in both years, whereas in Resi no further changes were detected. This clear cultivar dependence shows that high K fertilization not necessary enhance these traits.

Tomatoes contain several important water- and fat-soluble antioxidants, like ascorbic acid, phenolics, carotenoids, and tocopherols. The antioxidant concentrations in tomato fruit are affected by K fertilization, but other abiotic factors may alter or even reverse those effects in an outdoor environment. Nevertheless, the tendencies 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 metabolome analysis provides a comprehensive overview of the induced changes by increasing K fertilization on low weight metabolites in tomato fruits. The cultivar-independent increase of TCA cycle metabolites and decrease of amines with rising K fertilization was most prominent. Several other metabolites showed a cultivar-specific effect. Indicating that the reaction towards macronutrient stress is quite different between cultivars of one species.

8. Literature

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9. Supplement

List of supplemental figures

List of supplemental tables

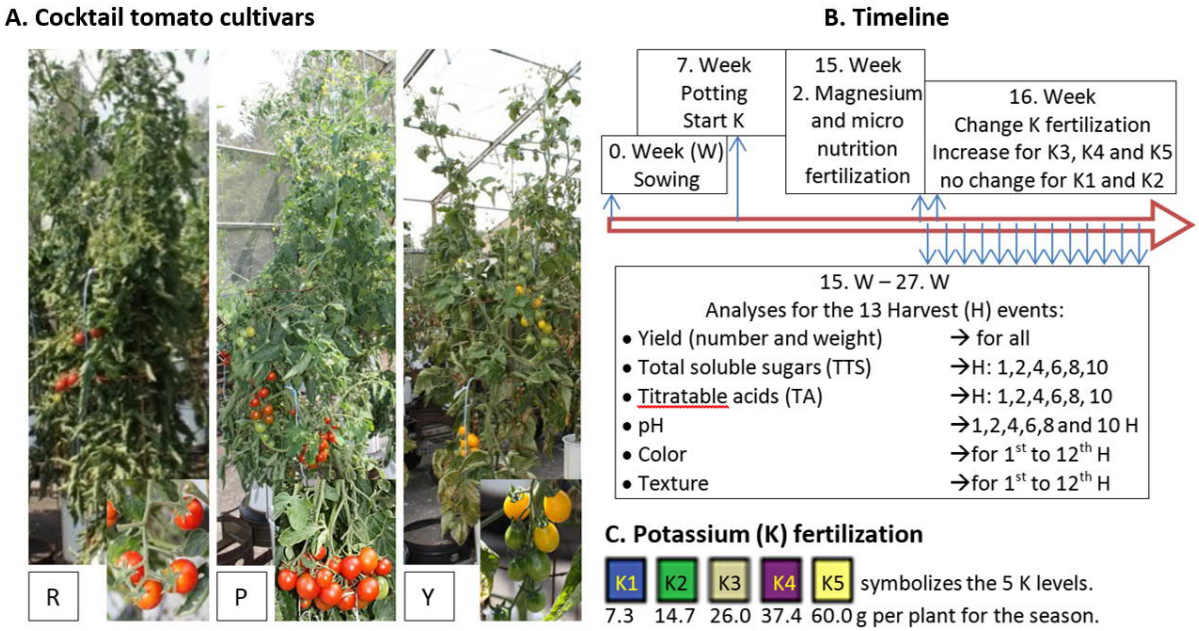


Figure S1. Experimental setup of 2014. A. shows the three cultivars Resi (R), Primavera (P) and Yellow Submarine (Y) and their fruits. B. is the timeline. It marks the important evets during the season, as weeks (W) after sowing. C. displays the five different potassium levels (K1 to K5) and the total and weekly fertilization amounts in g.

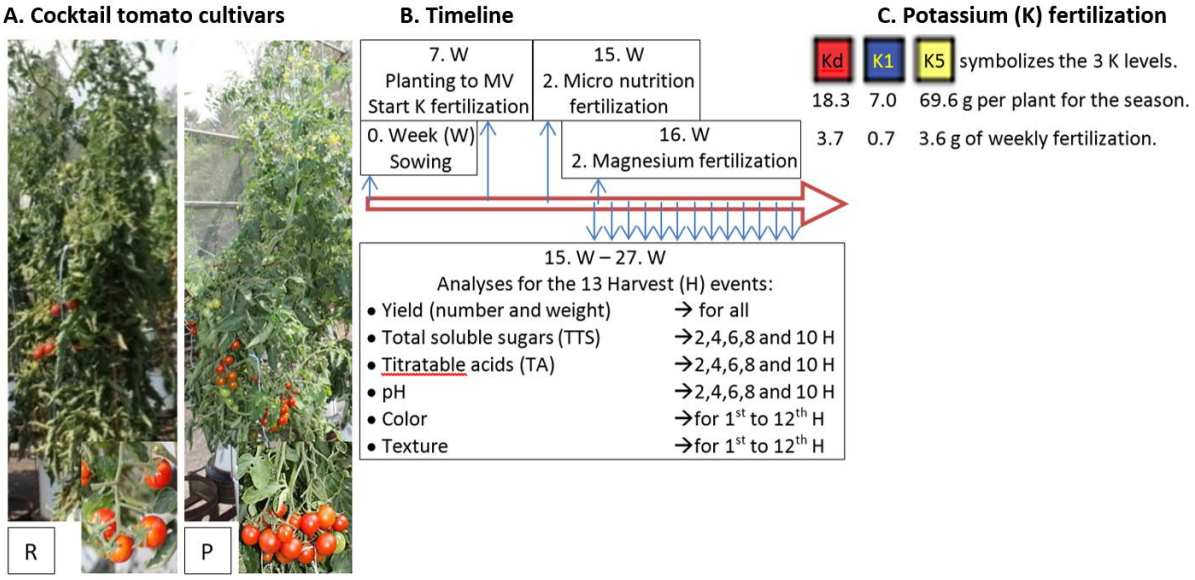


Figure S2. Experimental setup of 2015. A. shows the two cultivars Resi (R) and Primavera (P) with fruits. B: is the timeline. It marks the important evets during the season, as weeks (W) after sowing. C. displays the three different potassium levels (Kd, K1 and K5) and the total and weekly amounts in g. K depletion receives K only for 5 weeks.

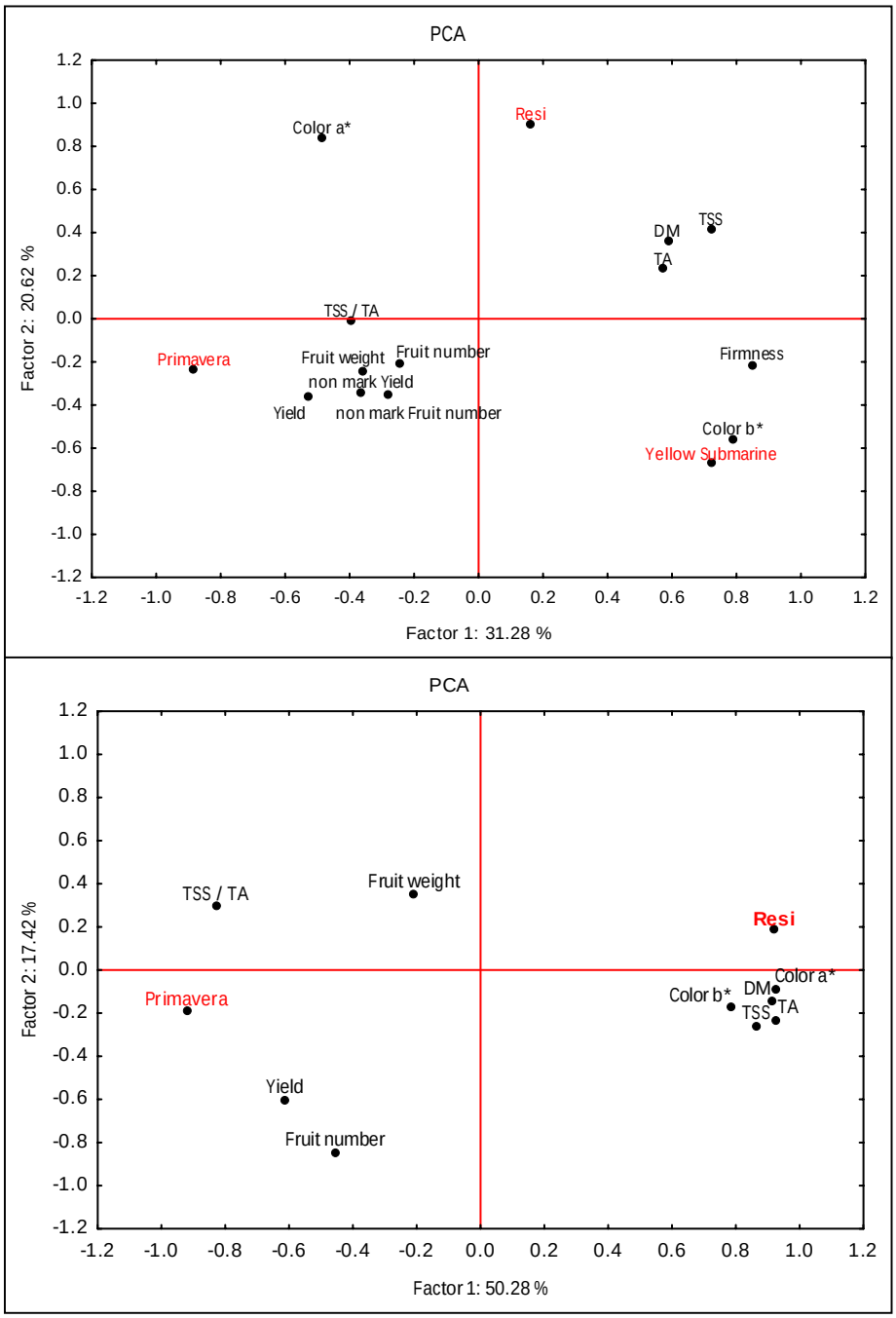


Figure S3. Distribution of the different variables among the cultivars 2014 (A) and 2015 (B). Yield, non-marketable yield were expressed in g per plant, fruit number and number of non-marketable fruits were expressed as n per plant, mean fruit weight (g/n), TSS (total soluble solids, g kg^{-1}), TA (titratable acids, g kg^{-1}), and DM (dry matter, g kg^{-1}), color a* and b* are from the LAB color system, and firmness (N). For each cultivar 50 replicates were present. non mark = non marketable.

Table S1. Macro- and micronutrient fertilization of the cocktail tomato cultivars of 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	$MgSO_4 \cdot 7H_2O$	19.0	twice	week 7 and 15
S	K_2SO_4	sufficient supply by K_2SO_4		
micronutrients				
Cl	$MnCl_2 \cdot 4H_2O$	0.52	twice	week 7 and 15
Fe	Fe-EDTA	1.41	twice	week 7 and 15
Mn	$MnCl_2 \cdot 4H_2O$	sufficient supply by $MnCl_2 \cdot 4H_2O$		
Zn	$ZnSO_4 \cdot 7H_2O$	0.16	twice	week 7 and 15
B	H_3BO_3	0.21	twice	week 7 and 15
Cu	$CuSO_4 \cdot 5H_2O$	0.04	twice	week 7 and 15
Mo	$Na_2MoO_4 \cdot 2H_2O$	$4.72E^{-04}$	twice	week 7 and 15

All tomato plants received the same nutrient concentrations, except for 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 + Kd	$Ca(NO_3)_2 + NH_4NO_3$ (2:1)	61.0 : 10.3	every other week 11 times	starting week 7
N for K1 + Kd	$(NH_4)_2SO_4$	36.8	every other week 8 times	starting week 9
K1	K_2SO_4	7.0	weekly 19 times	starting week 8
Kd	K_2SO_4	18.3	weekly 5 times	week 8 to 13
K5	K_2SO_4	69.6	weekly 19 times	starting week 8
Ca	$Ca(NO_3)_2$	sufficient supply by $Ca(NO_3)_2$		
P	$Ca(H_2PO_4)_2$	13.0	once	week 7
Mg	$MgSO_4 \cdot 7H_2O$	38.0	twice	week 7 and 16
S	K_2SO_4	sufficient supply by K_2SO_4		
micronutrients				
Cl	$MnCl_2 \cdot 4H_2O$	0.52	twice	week 7 and 15
Fe	Fe-EDTA	1.41	twice	week 7 and 15
Mn	$MnCl_2 \cdot 4H_2O$	sufficient supply by $MnCl_2 \cdot 4H_2O$		
Zn	$ZnSO_4 \cdot 7H_2O$	0.11	twice	week 7 and 15
B	H_3BO_3	0.43	twice	week 7 and 15
Cu	$CuSO_4 \cdot 5H_2O$	0.04	twice	week 7 and 15
Mo	$Na_2MoO_4 \cdot 2H_2O$	$9.45E^{-04}$	twice	week 7 and 15

All tomato plants received the same nutrient concentrations, except for potassium (K). K1 and K5 were weekly applied. The third K level (Kd) was a depletion treatment and was five weeks as K5 fertilized. Nitrogen was applied in similar amounts but in different forms: $(NH_4)_2SO_4$ were given to K1 and Kd as sulfur compensation.

Table S2. Non marketable yield and fruit number of 2014 and 2015.

			non-marketable yield per plant (g)		non-marketable fruits per plant (n)	
2014	Primavera	K1	20.6 ± 3.0	NS	1.5 ± 2.0	NS
		K2	32.8 ± 3.1	NS	1.7 ± 1.5	NS
		K3	30.4 ± 2.9	NS	1.6 + 2.3	NS
		K4	49.3 ± 3.2	NS	2.1 ± 2.8	NS
		K5	47.5 ± 3.2	NS	2.2 ± 2.6	NS
	Resi	K1	13.0 ± 3.5	NS	0.8 ± 0.9	NS
		K2	12.9 ± 3.3	NS	0.6 ± 0.6	NS
		K3	13.6 ± 3.6	NS	0.8 ± 0.8	NS
		K4	15.6 ± 3.6	NS	0.7 ± 0.8	NS
		K5	10.4 ± 3.8	NS	0.6 ± 0.5	NS
	Yellow Submarine	K1	2.5 ± 3.4	a	0.7 ± 0.8	NS
		K2	15.3 ± 3.0	b	1.1 ± 1.1	NS
		K3	16.5 ± 3.2	b	1.1 ± 1.1	NS
		K4	19.9 ± 3.0	b	1.1 ± 1.1	NS
		K5	20.0 ± 2.9	b	1.0 ± 1.4	NS
2015	Primavera	Kd	21.4 ± 2.8	NS	3.0 ± 2.6	NS
		K1	18.1± 2.9	NS	2.2 ± 2.1	NS
		K5	28.8± 2.9	NS	3.7 ± 4.0	NS
	Resi	Kd	4.0 ± 3.7	NS	1.3 ± 0.8	NS
		K1	3.6 ± 3.9	NS	1.0 ± 0.8	NS
		K5	3.4 ± 3.7	NS	1.5 ± 0.9	NS

Yield and fruit number per plant over the season 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 to 3.66 g K_2SO_4 per week) for each cultivar. In 2015, a depletion fertilization treatment (Kd) received only in the first 5 weeks K (3.66 g per week). Letters indicate statistically significant differences ($p < 0.05$). NS = no significant difference.

Table S3. Potassium (K) application influences mean leaf, stem, flower, unripe and ripe fruit fresh weight of the tomato plants in 2015.

		Leaf (g)	Stem (g)	Flower (g)	Unripe fruits (g)	Ripe fruits (g)	Fruits (g)	ratio of leaf / fruit
Primavera	Kd	535	213	48	304	115	418	1.3
	K1	210	97	20	222	103	326	0.6
	K5	410	162	40	246	124	370	1.1
Resi	Kd	611	171	30	75	33	108	5.6
	K1	358	107	19	83	33	116	3.1
	K5	485	134	26	58	25	84	5.8

Mean values comprises eight whole plant harvest dates, starting at the day before the final transplanting (19.05.2015) until the 02.09.2015 toward the end of tomato harvest. The K levels are K1 and K5 (0.37 g K_2SO_4 and 3.66 g K_2SO_4 per week), as well as Kd a depletion fertilization treatment received only in the first 5 weeks K (3.66 g K_2SO_4 per week).

Table S4. Sunshine and temperature differences between the 2014 and 2015.

	Day of harvest	Sunshine (h)	Temperature (°C)
	07.08.2014	6.2	21.5
	17.08.2015	7.1	21.0
T		14.8	78.3
Significance (2-sided)		0.043	0.008
Mean difference		6.7	21.3

The mean values comprise the four weeks bevor the harvest. Data was obtained from the German weather services for the weather station Göttingen (latitude and longitude: 51.5003 and 9.9506). Statistical differences were evaluated by a t-Test.

Table S5. Tests of between-subjects effects for the individual fruit traits.

	Dependent variable: weight of marketable fruit per plant (g)			Dependent variable: number of marketable fruit per plant (n)		
	Means of squares	F	Significance	Means of squares	F	Significance
Corrected model	37747.9	18.4	0.000	8888.8	7.0	0.000
Constant Term	2016265.8	982.8	0.000	263991.4	209.3	0.000
F_Code	7792.7	3.8	0.002	1627.8	1.3	0.266
Year	40308.4	19.6	0.000	40688.6	32.3	0.000
Cult	283690.5	138.3	0.000	21415.0	17.0	0.000
F_Code * Year	2186.5	1.1	0.302	1816.2	1.4	0.230
F_Code * Cult	8254.7	4.0	0.000	630.7	0.5	0.875
Year * Cult	4719.8	2.3	0.130	9940.2	7.9	0.005
F_Code * Year * Cult	2702.1	1.3	0.251	1903.8	1.5	0.220
	a. R-square = 0.274 (corrected R-square = 0.259)			a. R-square = 0.126 (corrected R-square = 0.108)		

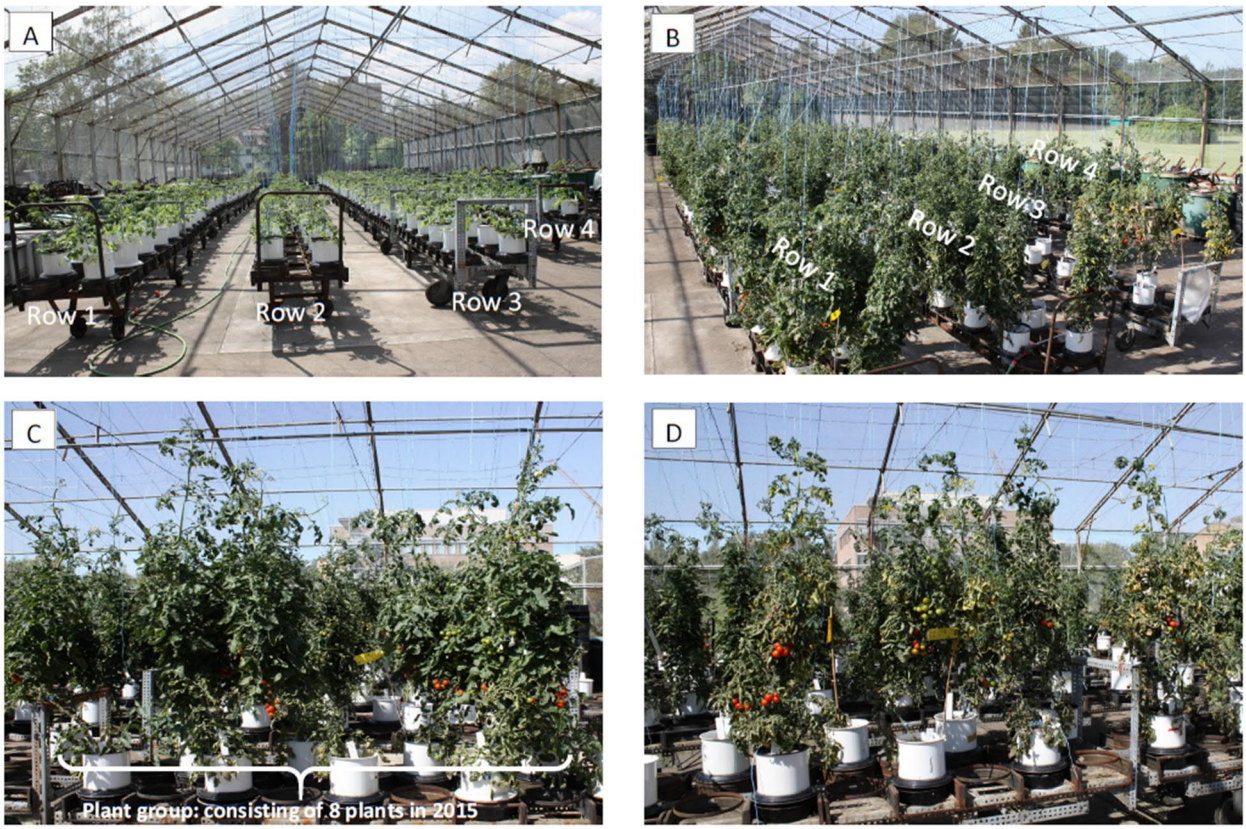


Figure S4. Randomized block design at the outdoor location in Göttingen. Plants are arranged in four blocks [(A) and (B)]. (A) shows the plants at the beginning of the outdoor experiment (May 22 in 2015) and (B), (C), and (D) towards the middle of the growing season (August 5 in 2015). Plants were grown 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 S6. 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 S7. 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 S8. 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 S9. 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				
2014 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				
2015 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 K_2SO_4 and 3.66 g K_2SO_4 per week).

Table S10. 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 $p \leq 0.05$.

Table S11. 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 $p \leq 0.05$.

Table S12. 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 S13. 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≤0.05.

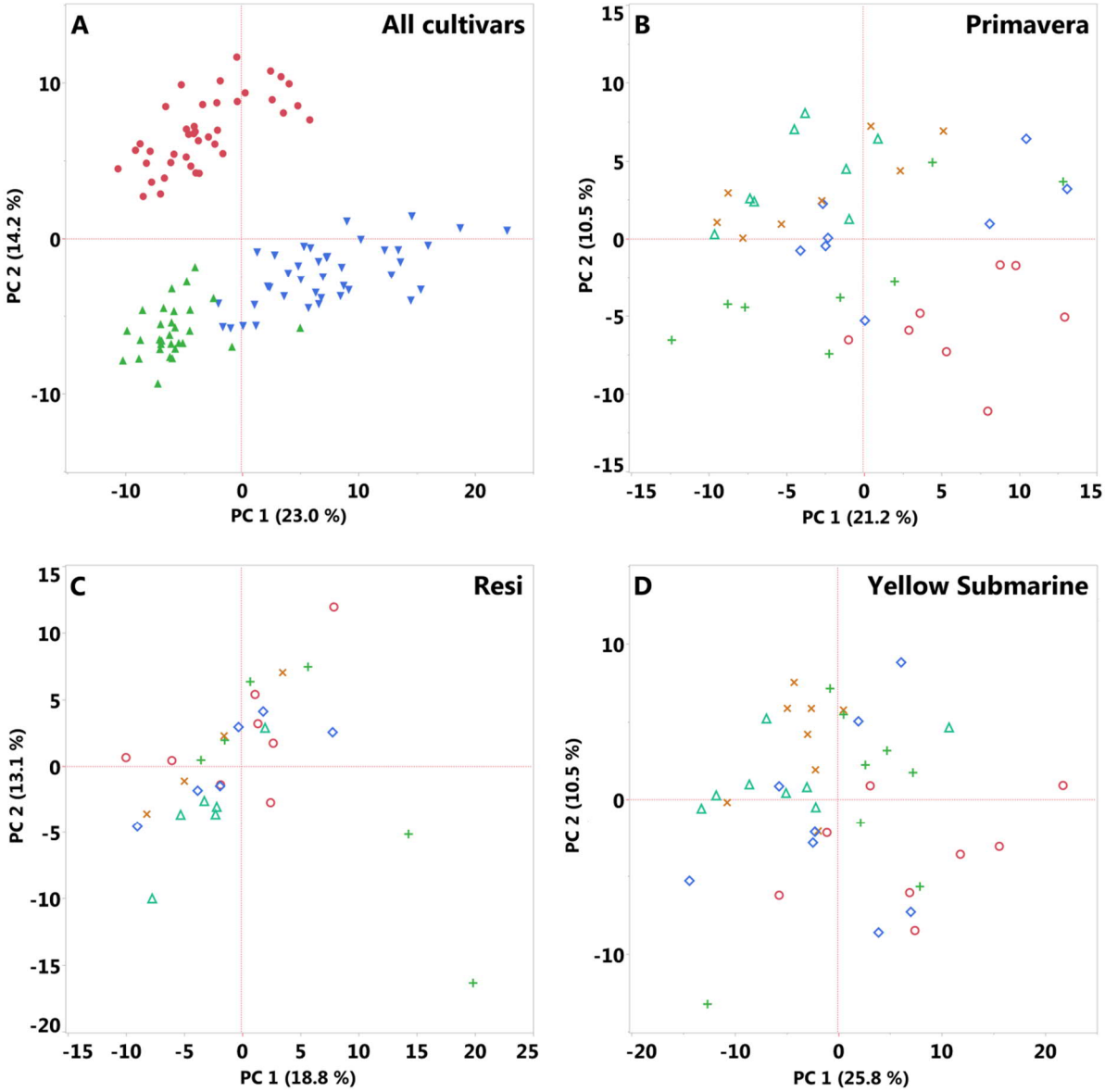


Figure S5. Principal component analysis of the untargeted GC×GC-MS data set. Panel A: Comparison of the metabolite profiles of the three cultivars (● Primavera, ▲ Resi, ▼ Yellow Submarine). Panels B-D: Metabolite profiles of the single cultivars at different potassium fertilization levels (○ K1, + K2, ◇ K3, × K4, Δ K5).

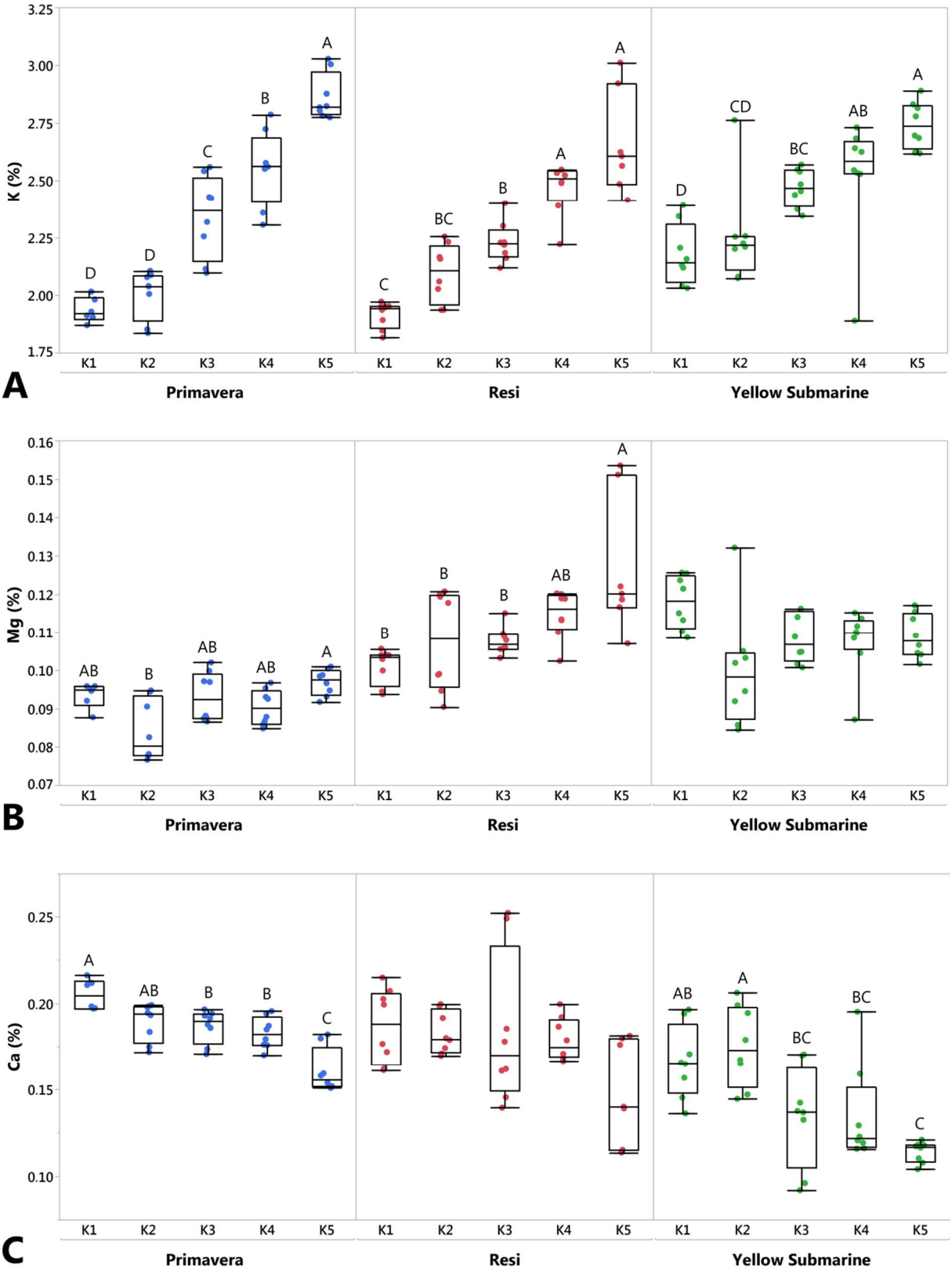


Figure S6. Effect on potassium fertilization on mineral content of tomato fruit. Mineral content is given as percent or ppm of the dry matter. Statistical testing was performed as described in section statistics.

Figure S6., continued

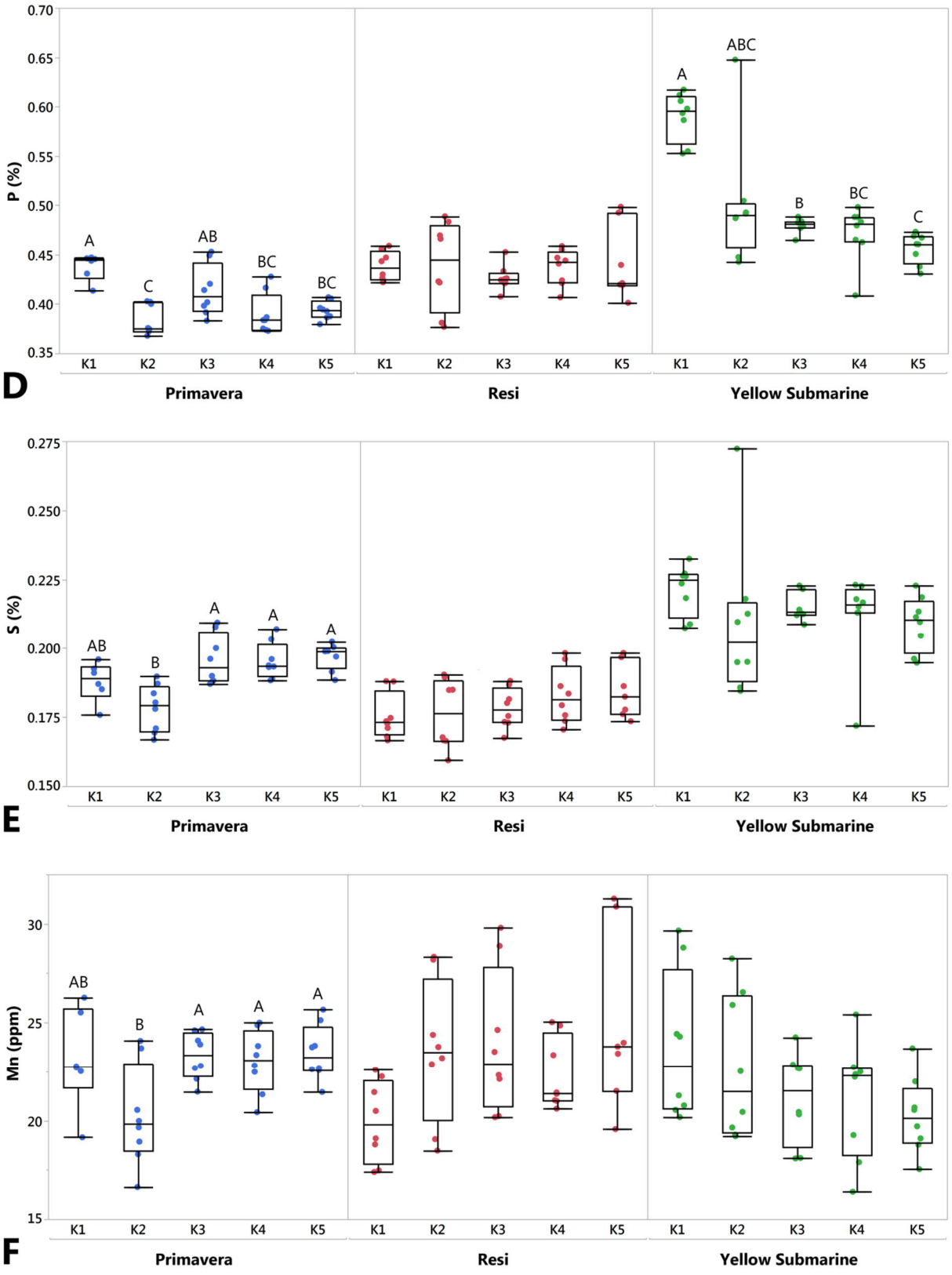
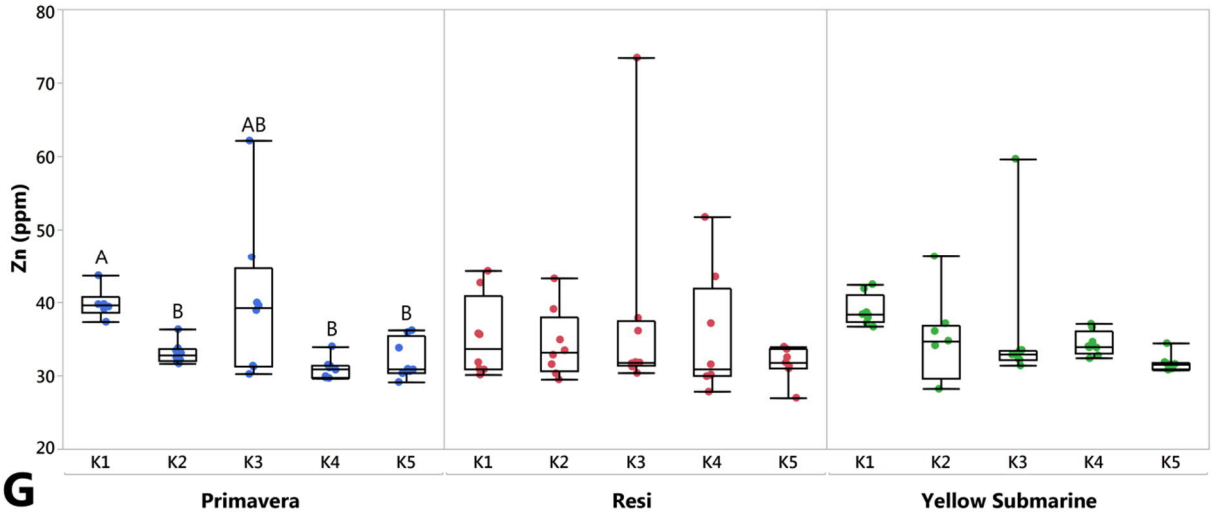


Figure S6., continued



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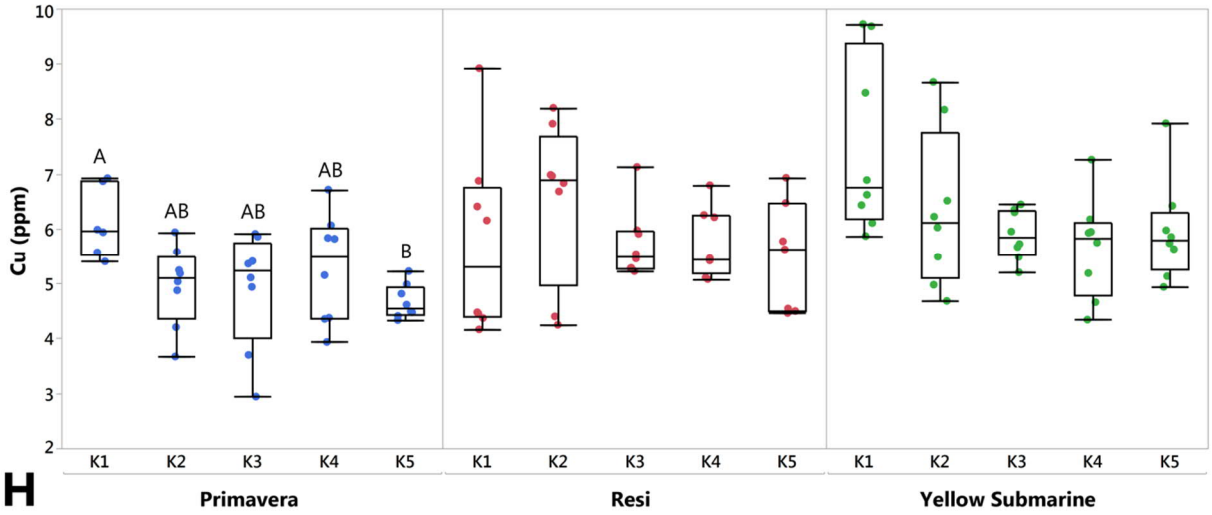

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Table S14. Complete results of ANOVA-based screening statistics for the cultivar Primavera.

ID	Metabolite	1st ANOVA		Testing of assumptions		2nd ANOVA		Post-hoc analysis						Fold changes				Correlations	
		FDR LogWorth	Effect size	Normal disribution[b]	Variance equality[c]	Type	*p*	Type of test	Letter code K1	K2	K3	K4	K5	rFC (K5/K1)	max. rFC	aFC (K5-K1)	max. aFC	r^2	*p*
A0632	Citric acid TMS4	7.00	0.68	0.383	0.802	Standard	<0.001	Tukey	C	C	B	AB	A	1.44	1.44	1.9E+07	1.9E+07	0.684	<0.001
A0329	Malic acid TMS3	3.26	0.63	0.259	0.399	Standard	<0.001	Tukey	BC	C	ABC	AB	A	1.22	1.44	7.2E+06	1.2E+07	0.376	<0.001
A0371	gamma-Aminobutyric acid TMS3	3.16	0.56	0.380	0.499	Standard	<0.001	Tukey	C	BC	AB	A	A	1.18	1.21	7.9E+06	8.9E+06	0.383	<0.001
A0639	Isocitric acid TMS4	1.26	0.75	<0.001	0.296	Kruskal-Wallis	0.004	Steel-Dwass	AB	AB	B	AB	A	1.48	1.92	1.3E+06	2.1E+06	0.164	0.012
A0391	Phenylalanine TMS	3.05	0.66	0.299	0.450	Standard	<0.001	Tukey	B	A	A	A	A	1.76	1.82	1.4E+06	1.6E+06	0.247	0.001
A0664	Quinic acid TMS5	2.25	0.67	0.875	0.976	Standard	0.001	Tukey	BC	C	ABC	AB	A	1.15	1.17	1.3E+06	1.5E+06	0.347	<0.001
A0196	Isoleucine TMS2	1.04	0.70	0.100	0.098	Standard	0.027	Tukey	B	AB	A	AB	B	1.02	1.58	5.5E+04	1.4E+06	0.014	0.475
A1103	Disaccharide	1.29	0.55	0.805	0.831	Standard	0.011	Tukey	B	A	AB	A	AB	1.22	1.29	7.1E+05	9.4E+05	0.008	0.588
A1085	Disaccharide	1.24	0.54	0.051	0.821	Standard	0.014	Tukey	B	AB	AB	A	A	1.23	1.25	8.3E+05	9.1E+05	0.063	0.130
A0297	beta-Alanine TMS3	1.22	0.44	0.186	0.318	Standard	0.015	Tukey	B	AB	A	AB	AB	1.48	1.54	8.1E+05	9.0E+05	0.201	0.005
A0773	Gluconic acid-like	1.51	0.55	0.063	0.560	Standard	0.006	Tukey	A	B	AB	A	AB	0.88	1.30	-3.3E+05	6.2E+05	0.005	0.665
A1094	Disaccharide	1.04	0.43	0.664	0.980	Standard	0.028	Tukey	B	A	A	AB	AB	1.11	1.19	2.5E+05	4.4E+05	0.000	0.978
A0442	alpha-Ketoglutaric acid MeOX-TMS2	4.61	0.67	0.309	0.648	Standard	<0.001	Tukey	C	C	BC	AB	A	1.84	1.85	4.2E+05	4.2E+05	0.570	<0.001
A0219	Succinic acid TMS2	1.40	0.49	0.875	0.724	Standard	0.008	Tukey	AB	B	AB	A	A	1.12	1.45	1.4E+05	4.0E+05	0.100	0.053
A0417	Threonic acid TMS4	1.71	0.60	0.560	0.160	Standard	0.003	Tukey	A	B	AB	A	A	0.99	1.31	-9.2E+03	3.1E+05	0.124	0.030
A0223	Unknown[o]	3.09	0.72	0.425	0.943	Standard	<0.001	Tukey	C	BC	AB	A	A	1.61	1.61	2.6E+05	2.6E+05	0.396	<0.001
A0404	Unknown[o]	2.27	0.58	0.665	0.367	Standard	0.001	Tukey	AB	B	AB	A	A	1.37	2.18	1.1E+05	2.2E+05	0.247	0.001
A0583	Citric acid TMS4-like[a]	2.77	0.43	0.037	0.421	Kruskal-Wallis	0.001	Steel-Dwass	B	B	AB	A	A	2.39	3.16	6.7E+04	8.7E+04	0.332	<0.001
A0924	Unknown[o]	1.26	0.45	0.092	0.318	Standard	0.013	Tukey	A	AB	AB	B	B	0.81	0.81	-1.0E+05	-1.0E+05	0.143	0.019
A0992	Unknown sugar or conjugate[a]	1.90	0.66	0.887	0.433	Standard	0.002	Tukey	A	B	AB	B	B	0.79	0.77	-9.8E+04	-1.0E+05	0.204	0.004
A0111	Isopentylamine TMS2	2.34	0.53	0.040	0.534	Kruskal-Wallis	0.001	Steel-Dwass	A	AB	AB	B	B	0.37	0.37	-1.1E+05	-1.1E+05	0.220	0.003
A0997	Uridin TMS3	1.71	0.50	0.016	0.765	Kruskal-Wallis	0.006	Steel-Dwass	AB	A	AB	B	B	0.78	0.72	-9.2E+04	-1.3E+05	0.224	0.003
A0837	Unknown sugar[a]	1.49	0.56	0.708	0.604	Standard	0.006	Tukey	A	AB	AB	B	B	0.75	0.73	-1.3E+05	-1.3E+05	0.096	0.058
A1098	Unknown sugar-like[a]	1.68	0.50	0.654	0.574	Standard	0.004	Tukey HSD	A	AB	AB	B	B	0.62	0.62	-1.7E+05	-1.7E+05	0.208	0.004
A0322	Citramalic acid TMS3[a]	1.97	0.50	0.384	0.763	Standard	0.001	Tukey HSD	A	B	AB	B	B	0.70	0.60	-1.3E+05	-1.7E+05	0.058	0.146
A0985	myo-Inositol phosphate TMS7[a]	1.13	0.41	0.599	0.720	Standard	0.020	Tukey HSD	A	AB	AB	AB	B	0.80	0.80	-1.8E+05	-1.8E+05	0.251	0.001
A0881	Unknown phosphorylated compound[a]	1.19	0.59	0.452	0.249	Standard	0.017	Tukey HSD	A	B	AB	AB	B	0.66	0.66	-2.0E+05	-2.0E+05	0.065	0.121
A0782	Dopamine-line amine[a]	3.05	0.62	0.434	0.582	Standard	<0.001	Tukey HSD	A	A	AB	B	B	0.28	0.28	-2.0E+05	-2.0E+05	0.327	<0.001
A0749	Dehydroascorbic acid isomer[a]	1.14	0.48	0.507	0.704	Standard	0.019	Tukey HSD	A	AB	AB	B	B	0.82	0.82	-2.1E+05	-2.1E+05	0.125	0.030
A0587	Ribonic acid or similar	2.27	0.54	0.902	0.443	Standard	0.001	Tukey HSD	A	B	AB	B	B	0.77	0.77	-2.4E+05	-2.4E+05	0.086	0.074
A0405	L-Cysteine TMS3[a]	1.19	0.64	0.857	0.871	Standard	0.017	Tukey HSD	A	AB	AB	AB	B	0.52	0.52	-2.4E+05	-2.4E+05	0.131	0.026
A0423	Unknown[o]	2.62	0.94	0.179	0.005	Welch	0.004	Steel-Dwass	A	AB	AB	B	B	0.45	0.43	-2.4E+05	-2.5E+05	0.159	0.013
A1127	Disaccharide	1.33	0.56	0.953	0.110	Standard	0.009	Tukey HSD	A	A	A	AB	B	0.85	0.84	-2.2E+05	-2.5E+05	0.340	<0.001
A0338	Unknown[o]	3.91	0.53	0.203	0.082	Standard	<0.001	Tukey HSD	A	BC	AB	BC	C	0.12	0.12	-2.7E+05	-2.7E+05	0.260	0.001
A0457	Oxoproline-like[a]	2.83	0.71	0.420	0.023	Welch	0.003	Steel-Dwass	A	AB	AB	B	B	0.39	0.39	-2.7E+05	-2.7E+05	0.108	0.044
A0832	Unknown sugar acid-like	1.11	0.48	0.212	0.923	Standard	0.022	Tukey HSD	A	AB	AB	AB	B	0.75	0.75	-3.1E+05	-3.1E+05	0.088	0.070
A0509	Unknown[o]	1.90	0.61	0.506	0.535	Standard	0.002	Tukey HSD	A	B	AB	B	B	0.36	0.36	-3.1E+05	-3.1E+05	0.072	0.103
A0648	Glucuronic acid gamma lactone-like	1.28	0.57	0.286	0.352	Standard	0.011	Tukey HSD	AB	A	AB	AB	B	0.89	0.80	-2.2E+05	-4.5E+05	0.244	0.002
A0352	L-Methionine TMS2	1.79	0.48	0.116	0.368	Standard	0.003	Tukey HSD	A	AB	AB	B	B	0.61	0.61	-5.3E+05	-5.3E+05	0.106	0.046
A0603	*O*-Phosphorylethanolamine TMS4	4.23	0.87	0.012	0.011	Kruskal-Wallis	<0.001	Steel-Dwass	A	B	AB	B	B	0.35	0.35	-7.1E+05	-7.1E+05	0.290	<0.001
A0655	Dehydroascorbic acid isomer	1.04	0.53	0.644	0.798	Standard	0.028	Tukey HSD	A	AB	AB	AB	B	0.84	0.84	-7.3E+05	-7.3E+05	0.112	0.040
A0709	Dehydroascorbic acid isomer	1.16	0.41	0.288	0.564	Standard	0.018	Tukey HSD	A	AB	AB	AB	B	0.77	0.77	-7.6E+05	-7.6E+05	0.336	<0.001
A0908	Unknown sugar	3.91	0.65	0.429	0.281	Standard	<0.001	Tukey HSD	A	AB	B	BC	C	0.50	0.50	-8.0E+05	-8.0E+05	0.417	<0.001
A0999	Unknown	6.03	0.75	0.352	0.695	Standard	<0.001	Tukey HSD	A	AB	B	B	C	0.54	0.54	-8.2E+05	-8.2E+05	0.515	<0.001
A1031	Unknown sugar	2.47	0.83	0.002	0.372	Kruskal-Wallis	0.002	Steel-Dwass	A	AB	AB	B	B	0.70	0.66	-7.4E+05	-8.3E+05	0.121	0.032
A1136	Chlorogenic acid TMS6	4.45	0.93	0.820	0.504	Standard	<0.001	Tukey HSD	A	B	B	B	B	0.43	0.43	-8.7E+05	-8.7E+05	0.208	0.004
A0528	Unknown	2.57	0.75	0.009	0.042	Kruskal-Wallis	0.002	Steel-Dwass	A	AB	AB	B	B	0.27	0.27	-8.8E+05	-8.8E+05	0.155	0.014
A0364	Unknown	3.88	0.84	0.334	0.180	Standard	<0.001	Tukey HSD	A	BC	AB	BC	C	0.15	0.15	-9.0E+05	-9.0E+05	0.219	0.003

Analytes are sorted by the maximum absolute fold change (max. aFC) in decreasing order. Pearson correlation coefficients larger than 0.4 are highlighted by a green background. [a] trace analyte; [b] *p*-value of the Shapiro-Wilk test on residues; [c] *p*-value of Brown-Forsythe test; rFC (K5/K1): relative fold change (quotient of the means of K5 and K1); max. rFC: maximum relative fold change (quotient of the smallest and the largest group mean); aFC (K5-K1): absolute fold change (difference of the means of K5 and K1); max. aFC: maximum absolute fold change (difference of the smallest and the largest group mean).

Table S14, continued

ID	Metabolite	1st ANOVA		Testing of assumptions		2nd ANOVA		Post-hoc analysis						Fold changes				Correlations	
		FDR LogWorth	Effect size	Normal distribution[b]	Variance equality[c]	Type	p	Type of test	Letter code					rFC (K5/K1)	max. rFC	aFC (K5-K1)	max. aFC	r^2	p
									K1	K2	K3	K4	K5						
A1109	Naringenin MeOX TMS3 or similar	4.44	1.26	0.002	0.003	Kruskal-Wallis	<0.001	Steel-Dwass	A	B	B	B	B	0.14	0.14	-9.4E+05	-9.4E+05	0.261	0.001
A1160	Trisaccharide (Maltotriose or similar)	1.52	0.51	0.632	0.329	Standard	0.005	Tukey HSD	A	B	AB	B	B	0.71	0.71	-9.5E+05	-9.5E+05	0.082	0.082
A0740	Unknown dehydroascorbic acid-like	5.24	0.75	0.413	0.417	Standard	<0.001	Tukey HSD	A	BC	B	BC	C	0.52	0.52	-9.5E+05	-9.5E+05	0.302	<0.001
A0748	Galacturonic acid MeOX-TMS5 Isomer 2	1.71	0.71	0.066	0.551	Standard	0.003	Tukey HSD	AB	A	AB	AB	B	0.89	0.80	-6.5E+05	-1.3E+06	0.326	<0.001
A0988	Unknown sugar or conjugate	1.05	0.53	0.256	0.450	Standard	0.026	Tukey HSD	A	B	AB	AB	B	0.72	0.72	-1.3E+06	-1.3E+06	0.040	0.226
A0520	Unknown	3.23	0.71	0.040	0.064	Kruskal-Wallis	<0.001	Steel-Dwass	A	AB	AB	B	B	0.29	0.29	-1.3E+06	-1.3E+06	0.211	0.004
A1124	Galactinol TMS9	3.09	0.55	0.688	0.004	Welch	<0.001	Steel-Dwass	AB	A	AB	BC	C	0.30	0.30	-1.5E+06	-1.5E+06	0.439	<0.001
A0450	S-Methylcysteine-like	3.06	0.92	0.005	0.060	Kruskal-Wallis	0.001	Steel-Dwass	A	AB	AB	B	B	0.23	0.23	-1.7E+06	-1.7E+06	0.163	0.012
A0816	Unknown sugar (isomer 2)	1.29	0.52	0.368	0.877	Standard	0.011	Tukey HSD	B	A	AB	AB	AB	1.03	0.88	4.3E+05	-1.9E+06	0.024	0.351
A0790	Unknown sugar (isomer 1)	1.27	0.47	0.675	0.460	Standard	0.012	Tukey HSD	B	A	AB	AB	AB	1.02	0.94	8.1E+05	-3.0E+06	0.004	0.703
A0717	Lysine TMS4	1.88	0.49	0.758	0.610	Standard	0.002	Tukey HSD	A	AB	AB	B	B	0.34	0.34	-3.0E+06	-3.0E+06	0.171	0.010
A0554	Putrescine TMS4	5.24	1.27	0.009	0.020	Kruskal-Wallis	<0.001	Steel-Dwass	A	AB	B	BC	C	0.14	0.14	-3.4E+06	-3.4E+06	0.459	<0.001
A0462	Asparagine TMS2	3.88	0.63	0.226	0.039	Welch	<0.001	Steel-Dwass	A	B	B	B	B	0.30	0.30	-3.8E+06	-3.8E+06	0.252	0.001
A0368	5-Oxoproline TMS2	1.36	0.60	<0.001	0.123	Kruskal-Wallis	0.006	Steel-Dwass	A	AB	AB	B	B	0.56	0.52	-9.4E+06	-1.0E+07	0.090	0.067
A0506	Asparagine TMS3	3.88	0.59	0.008	0.045	Kruskal-Wallis	<0.001	Steel-Dwass	A	AB	AB	B	B	0.19	0.19	-2.0E+07	-2.0E+07	0.217	0.003
A0925	Unknown	1.29	0.52	0.013	0.519	Kruskal-Wallis	0.013	Steel-Dwass	A	A	A	A	A	1.93	2.08	3.2E+05	3.7E+05	0.213	0.004
A0869	Unknown sugar-like	1.09	0.47	0.028	0.452	Kruskal-Wallis	0.050	Steel-Dwass	A	A	A	A	A	0.79	0.71	-1.4E+05	-2.0E+05	0.148	0.017
A0954	Unknown sugar-like	1.19	0.45	<0.001	0.955	Kruskal-Wallis	0.012	Steel-Dwass	A	A	A	A	A	0.83	0.79	-3.5E+05	-4.2E+05	0.136	0.023
A0828	Unknown sugar-like	1.22	0.46	0.639	0.758	Standard	0.015	Tukey HSD	A	A	A	A	A	0.89	0.84	-5.2E+05	-7.1E+05	0.038	0.242
A0729	Tyrosine TMS3	1.11	0.50	0.133	0.806	Standard	0.022	Tukey HSD	A	A	A	A	A	0.51	0.50	-1.0E+06	-1.1E+06	0.055	0.155
A0846	Unknown sugar-like	1.13	0.72	0.929	0.411	Standard	0.020	Tukey HSD	A	A	A	A	A	0.85	0.85	-5.5E+06	-5.5E+06	0.168	0.011

Analytes with a grey background showed finally a non-significant difference according to post-hoc analysis.

Table S15. Complete results of ANOVA-based screening statistics for the cultivar Resi.

ID	Metabolite	1st ANOVA		Testing of assumptions		2nd ANOVA		Post-hoc analysis						Fold changes				Correlations	
		FDR LogWorth	Effect size	Normal disribution[b]	Variance equality[c]	Type	*p*	Type of test	Letter code K1	K2	K3	K4	K5	rFC (K5/K1)	max. rFC	aFC (K5-K1)	max. aFC	r^2	*p*
A0632	Citric acid TMS4	4.07	0.77	0.580	0.723	Standard	<0.001	Tukey-HSD	C	B	AB	AB	A	1.46	1.46	2.69E+07	2.69E+07	0.457	<0.001
A0664	Quinic acid TMS5	1.58	0.62	0.226	0.995	Standard	0.001	Tukey-HSD	B	AB	AB	A	A	1.21	1.21	2.01E+06	2.01E+06	0.352	0.001
A0442	alpha-Ketoglutaric acid MeOX-TMS2	1.71	0.68	0.629	0.775	Standard	<0.001	Tukey-HSD	B	AB	A	A	A	2.05	2.05	4.42E+05	4.42E+05	0.510	<0.001
A0219	Succinic acid TMS2	1.58	0.61	0.597	0.889	Standard	0.001	Tukey-HSD	B	A	A	A	A	1.26	1.42	2.54E+05	4.03E+05	0.228	0.008
A0417	Threonic acid TMS4	1.02	0.55	0.946	0.767	Standard	0.004	Tukey-HSD	B	A	AB	AB	A	1.46	1.46	2.78E+05	2.78E+05	0.218	0.009
A0203	Nicotinic acid TMS	1.23	0.67	0.377	0.396	Standard	0.002	Tukey-HSD	B	A	AB	B	B	1.16	1.53	4.85E+04	1.60E+05	0.004	0.738
A0583	Citric acid-like[a]	1.32	0.64	0.248	0.462	Standard	0.001	Tukey-HSD	B	AB	A	AB	A	1.57	1.57	8.93E+04	8.93E+04	0.313	0.001
A0997	Uridin TMS3	1.02	1.02	0.450	0.568	Standard	0.004	Tukey-HSD	A	AB	AB	AB	B	0.68	0.68	-1.67E+05	-1.67E+05	0.422	<0.001
A0936	Glucose-6-phosphate MeOX-TMS6	1.23	0.62	0.621	0.863	Standard	0.002	Tukey-HSD	A	AB	AB	B	B	0.75	0.75	-3.59E+05	-3.59E+05	0.454	<0.001
A0554	Putrescine TMS4	3.89	0.62	0.102	0.748	Standard	<0.001	Tukey-HSD	A	B	BC	BC	C	0.10	0.10	-1.13E+06	-1.13E+06	0.644	<0.001

Analytes are sorted by the maximum absolute fold change (max. aFC) in decreasing order. Pearson correlation coefficients larger than 0.4 are highlighted by a green background. [a] trace analyte; [b] *p*-value of the Shapiro-Wilk test on residues; [c] *p*-value of Brown-Forsythe test; rFC (K5/K1): relative fold change (quotient of the means of K5 and K1); max. rFC: maximum relative fold change (quotient of the smallest and the largest group mean); aFC (K5-K1): absolute fold change (difference of the means of K5 and K1); max. aFC: maximum absolute fold change (difference of the smallest and the largest group mean).

Table S16. Complete results of ANOVA-based screening statistics for the cultivar Yellow Submarine.

ID	Metabolite	1st ANOVA		Testing of assumptions		2nd ANOVA		Post-hoc analysis						Fold changes				Correlations	
		FDR LogWorth	Effect size	Normal disribution[b]	Variance equality[c]	Type	p	Type of test	Letter code K1	K2	K3	K4	K5	rFC (K5/K1)	max. rFC	aFC (K5-K1)	max. aFC	r²	p
A0632	Citric acid TMS4	1.40	0.61	0.509	0.415	Standard	0.008	Tukey-HSD	B	AB	AB	AB	A	1.22	1.22	1.1E+07	1.1E+07	0.146	0.015
A0790	Unknown amino sugar isomer 1	1.67	0.53	0.114	0.878	Standard	0.003	Tukey-HSD	B	A	AB	A	AB	1.07	1.11	2.9E+06	4.6E+06	0.138	0.018
A0816	Unknown amino sugar isomer 2	4.11	0.60	0.319	0.243	Standard	<0.001	Tukey-HSD	C	AB	BC	A	AB	1.20	1.31	2.1E+06	3.1E+06	0.230	0.002
A0442	alpha-Ketoglutaric acid MeOX-TMS2	8.21	0.68	0.824	0.740	Standard	<0.001	Tukey-HSD	C	B	B	A	A	2.23	2.23	2.4E+06	2.4E+06	0.517	<0.001
A1085	Disaccharide	2.17	0.59	0.996	0.821	Standard	0.001	Tukey-HSD	B	AB	AB	A	A	1.44	1.44	1.2E+06	1.2E+06	0.276	<0.001
A1069	Disaccharide	1.01	0.48	0.792	0.602	Standard	0.026	Tukey-HSD	B	AB	AB	A	AB	1.14	1.32	3.4E+05	7.6E+05	0.090	0.060
A0998	Serotonin TMS4	1.16	0.65	0.001	0.744	Kruskal-Wallis	0.009	Steel-Dwass	B	AB	AB	AB	A	1.72	1.72	4.4E+05	4.4E+05	0.239	0.001
A0219	Succinic acid TMS2	1.45	0.61	0.930	0.126	Standard	0.006	Tukey-HSD	B	AB	A	A	A	1.41	1.45	3.3E+05	3.6E+05	0.162	0.010
A0417	Threonic acid TMS4	1.58	0.52	0.434	0.569	Standard	0.004	Tukey-HSD	B	AB	AB	A	A	1.50	1.50	3.0E+05	3.0E+05	0.165	0.009
A0223	Unknown[a]	1.24	0.43	0.605	0.693	Standard	0.012	Tukey-HSD	B	AB	AB	AB	A	1.32	1.32	1.5E+05	1.5E+05	0.228	0.002
A0236	Itaconic cid TMS2	1.96	0.71	0.057	0.623	Standard	0.001	Tukey-HSD	B	B	B	A	B	1.09	1.41	1.4E+04	6.2E+04	0.037	0.237
A1125	beta/gamma tocopherol TMS	1.58	0.57	0.039	0.771	Kruskal-Wallis	0.006	Steel-Dwass	A	AB	AB	B	AB	0.68	0.68	-9.9E+04	-9.9E+04	0.196	0.004
A0874	Glycerol phosphate-like[a]	1.24	0.55	0.715	0.468	Standard	0.012	Tukey-HSD	A	A	AB	B	AB	0.78	0.63	-6.1E+04	-1.0E+05	0.059	0.130
A0956	Unknown sugar conjugate	1.11	0.51	0.117	0.809	Standard	0.019	Tukey-HSD	A	AB	AB	AB	B	0.65	0.65	-1.1E+05	-1.1E+05	0.138	0.018
A1098	Unknown sugar-like	1.62	0.56	0.847	0.254	Standard	0.003	Tukey-HSD	A	A	AB	AB	B	0.65	0.65	-1.2E+05	-1.2E+05	0.239	0.001
A0423	Unknown	1.01	0.52	0.084	0.226	Standard	0.027	Tukey-HSD	A	AB	AB	B	AB	0.69	0.67	-1.2E+05	-1.3E+05	0.151	0.013
A0111	Isopentylamine TMS2	2.27	0.71	0.978	0.217	Standard	<0.001	Tukey-HSD	A	BC	AB	BC	C	0.46	0.46	-1.6E+05	-1.6E+05	0.192	0.005
A0852	Heptadecanoic acid TMS	1.04	0.49	0.053	0.354	Standard	0.024	Tukey-HSD	A	AB	AB	B	B	0.73	0.70	-1.5E+05	-1.6E+05	0.241	0.001
A0924	Unknown[a]	2.70	0.54	0.392	0.801	Standard	<0.001	Tukey-HSD	A	AB	B	B	B	0.72	0.72	-1.7E+05	-1.7E+05	0.210	0.003
A0629	Unknown	1.40	0.61	0.566	0.046	Welch-ANOVA	0.009	Steel-Dwass	AB	A	AB	AB	B	0.88	0.68	-5.1E+04	-1.7E+05	0.174	0.007
A0509	Unknown	1.07	0.52	0.257	0.099	Standard	0.022	Tukey-HSD	A	AB	AB	B	B	0.63	0.63	-1.8E+05	-1.8E+05	0.109	0.037
A0285	Methionine TMS	1.44	0.60	0.489	0.435	Standard	0.007	Tukey-HSD	A	AB	AB	B	B	0.57	0.57	-2.0E+05	-2.0E+05	0.157	0.011
A0338	Unknown[a]	1.58	0.68	0.400	0.074	Standard	0.004	Tukey-HSD	A	AB	AB	B	B	0.33	0.33	-2.0E+05	-2.0E+05	0.236	0.001
A1138	alpha-tocopherol TMS	1.25	0.51	0.447	0.060	Standard	0.011	Tukey-HSD	A	A	AB	B	AB	0.84	0.78	-1.5E+05	-2.1E+05	0.128	0.024
A1106	Unknow sugar or conjugate[a]	1.55	0.61	0.600	0.120	Standard	0.004	Tukey-HSD	A	AB	B	AB	B	0.52	0.52	-2.3E+05	-2.3E+05	0.068	0.104
A0881	Unknown phosphorylated compound	2.51	0.64	0.576	0.318	Standard	<0.001	Tukey-HSD	A	AB	ABC	C	BC	0.65	0.63	-2.3E+05	-2.4E+05	0.238	0.001
A0782	Dopamine-line amine	2.17	0.78	0.027	0.305	Kruskal-Wallis	0.002	Steel-Dwass	A	AB	B	B	AB	0.36	0.36	-2.7E+05	-2.7E+05	0.207	0.003
A0589	Glycerol phosphate TMS4 or similar[a]	1.48	0.60	0.304	0.138	Standard	0.006	Tukey-HSD	A	AB	B	AB	B	0.75	0.75	-3.2E+05	-3.2E+05	0.156	0.012
A0587	Ribonic acid or similar	1.87	0.79	0.312	0.471	Standard	0.002	Tukey-HSD	A	AB	AB	B	B	0.72	0.72	-3.3E+05	-3.3E+05	0.241	0.001
A0832	Unknown sugar acid	1.21	0.51	0.615	0.269	Standard	0.013	Tukey-HSD	A	AB	AB	B	B	0.73	0.73	-3.9E+05	-3.9E+05	0.090	0.063
A0785	Unknown	2.35	0.65	0.180	0.903	Standard	<0.001	Tukey-HSD	A	AB	B	B	B	0.77	0.76	-3.8E+05	-4.0E+05	0.253	0.001
A0999	Unknown	1.43	0.55	0.977	0.427	Standard	0.007	Tukey-HSD	A	AB	B	AB	B	0.68	0.68	-4.9E+05	-4.9E+05	0.170	0.008
A0528	Unknown	1.11	0.55	0.160	0.322	Standard	0.019	Tukey-HSD	A	AB	AB	B	AB	0.65	0.56	-4.1E+05	-5.1E+05	0.144	0.016
A0364	Unknown	2.17	0.75	0.181	0.161	Standard	0.001	Tukey-HSD	A	ABC	AB	C	BC	0.48	0.42	-4.7E+05	-5.3E+05	0.186	0.005
A1124	Galactinol TMS9	4.53	0.69	0.349	0.189	Standard	<0.001	Tukey-HSD	A	BC	B	C	BC	0.56	0.52	-4.9E+05	-5.3E+05	0.255	0.001
A0740	Unknown dehydroascorbic acid-like	2.39	0.66	0.505	0.297	Standard	<0.001	Tukey-HSD	A	AB	AB	B	B	0.62	0.62	-5.9E+05	-5.9E+05	0.290	<0.001
A0352	L-Methionine TMS2	1.17	0.57	0.863	0.870	Standard	0.015	Tukey-HSD	A	B	AB	AB	B	0.63	0.63	-6.0E+05	-6.0E+05	0.061	0.126
A0954	Unknown sugar-like	2.27	0.64	0.327	0.766	Standard	<0.001	Tukey-HSD	A	AB	BC	C	C	0.78	0.75	-5.5E+05	-6.1E+05	0.283	<0.001
A0520	Unknown	1.92	0.71	0.020	0.431	Kruskal-Wallis	0.003	Steel-Dwass	A	AB	AB	AB	B	0.53	0.53	-6.9E+05	-6.9E+05	0.225	0.002
A1127	Disaccharide	9.19	0.82	0.692	0.524	Standard	<0.001	Tukey-HSD	A	B	C	CD	D	0.51	0.51	-7.1E+05	-7.1E+05	0.433	<0.001
A0974	Unknown sugar acid-like	1.93	0.58	0.446	0.592	Standard	0.001	Tukey-HSD	A	AB	B	B	B	0.78	0.78	-7.5E+05	-7.5E+05	0.231	0.002
A1136	Chlorogenic acid TMS6	1.95	0.55	0.160	0.937	Standard	0.001	Tukey-HSD	A	AB	AB	B	B	0.57	0.49	-6.4E+05	-7.5E+05	0.156	0.012
A0391	Phenylalanin TMS	2.97	0.57	<0.001	0.640	Kruskal-Wallis	<0.001	Steel-Dwass	A	B	AB	B	B	0.49	0.49	-9.9E+05	-9.9E+05	0.273	0.001
A1083	Monoacylglycerol-like	3.74	0.69	0.788	0.945	Standard	<0.001	Tukey-HSD	A	AB	C	BC	BC	0.56	0.53	-1.0E+06	-1.1E+06	0.373	<0.001
A0450	S-Methylcysteine-like	2.07	0.64	0.004	0.288	Kruskal-Wallis	0.001	Steel-Dwass	A	AB	AB	B	B	0.48	0.46	-1.1E+06	-1.1E+06	0.258	0.001
A0869	Unknown sugar-like	1.58	0.84	<0.001	0.433	Kruskal-Wallis	0.003	Steel-Dwass	A	AB	AB	B	B	0.52	0.51	-1.1E+06	-1.2E+06	0.166	0.009
A1160	Trisaccharide (Maltotriose or similar)	2.19	0.63	0.171	0.421	Standard	0.001	Tukey-HSD	A	AB	AB	B	B	0.69	0.69	-1.2E+06	-1.2E+06	0.199	0.004
A0603	O-Phosphorylethanolamine TMS4	6.41	0.92	0.742	0.478	Standard	<0.001	Tukey-HSD	A	B	BC	BC	C	0.32	0.32	-1.3E+06	-1.3E+06	0.423	<0.001

Analytes are sorted by the maximum absolute fold change (max. aFC) in decreasing order. Pearson correlation coefficients larger than 0.4 are highlighted by a green background. [a] trace analyte; [b] p-value of the Shapiro-Wilk test on residues; [c] p-value of Brown-Forsythe test; rFC (K5/K1): relative fold change (quotient of the means of K5 and K1); max. rFC: maximum relative fold change (quotient of the smallest and the largest group mean); aFC (K5-K1): absolute fold change (difference of the means of K5 and K1); max. aFC: maximum absolute fold change (difference of the smallest and the largest group mean).

Table S17, continued

ID	Metabolite	1st ANOVA		Testing of assumptions		2nd ANOVA		Post-hoc analysis						Fold changes				Correlations	
		FDR LogWorth	Effect size	Normal disribution[b]	Variance equality[c]	Type	p	Type of test	Letter code					rFC (K5/K1)	max. rFC	aFC (K5-K1)	max. aFC	r^2	p
									K1	K2	K3	K4	K5						
A1047	Unknown sugar	1.50	0.52	0.007	0.248	Kruskal-Wallis	0.004	Steel-Dwass	A	A	AB	B	AB	0.77	0.66	-8.7E+05	-1.3E+06	0.204	0.003
A0484	Phenylalanin TMS2	2.36	0.68	0.004	0.545	Kruskal-Wallis	0.001	Steel-Dwass	A	B	AB	B	AB	0.50	0.46	-1.3E+06	-1.4E+06	0.193	0.005
A0187	Leucin TMS2	1.90	0.88	0.253	0.380	Standard	0.002	Tukey-HSD	A	B	AB	B	B	0.40	0.40	-1.5E+06	-1.5E+06	0.175	0.007
A0988	Unknown sugar or conjugate[a]	2.55	0.69	0.047	0.586	Kruskal-Wallis	0.001	Steel-Dwass	A	AB	AB	B	AB	0.64	0.62	-1.6E+06	-1.7E+06	0.287	<0.001
A0196	Isoleucine TMS2	1.27	1.11	0.245	0.362	Standard	0.011	Tukey-HSD	A	AB	AB	AB	B	0.41	0.41	-2.3E+06	-2.3E+06	0.182	0.006
A0729	Tyrosine TMS3	3.16	0.83	0.009	0.161	Kruskal-Wallis	<0.001	Steel-Dwass	A	AB	ABC	C	BC	0.29	0.27	-2.4E+06	-2.5E+06	0.268	0.001
A0462	Asparagine TMS2	3.16	0.86	0.068	0.395	Standard	<0.001	Tukey-HSD	A	AB	ABC	BC	C	0.49	0.49	-2.7E+06	-2.7E+06	0.381	<0.001
A0882	Oleic acid	1.11	0.41	0.310	0.382	Standard	0.019	Tukey-HSD	A	AB	AB	B	AB	0.77	0.72	-6.2E+06	-7.6E+06	0.226	0.002
A0506	Asparagine TMS3	2.69	0.65	0.008	0.681	Kruskal-Wallis	<0.001	Steel-Dwass	A	AB	ABC	C	BC	0.51	0.49	-1.2E+07	-1.2E+07	0.333	<0.001
A1150	Unknown sugar conjugate[a]	1.27	1.10	0.001	0.629	Kruskal-Wallis	0.004	Steel-Dwass	A	A	A	A	A	0.60	0.54	-1.1E+05	-1.3E+05	0.043	0.198
A0992	Unknown sugar or conjugate[a]	1.16	0.53	0.385	0.641	Standard	0.016	Tukey-HSD	A	A	A	A	A	0.81	0.78	-1.1E+05	-1.3E+05	0.111	0.035
A0772	D-Glucaric acid TMS6[a]	1.51	0.71	0.001	0.394	Kruskal-Wallis	0.006	Steel-Dwass	A	A	A	A	A	0.67	0.64	-1.4E+05	-1.6E+05	0.344	<0.001
A0440	Proline-like	1.16	0.69	0.129	0.003	Welch-ANOVA	0.041	Steel-Dwass	A	A	A	A	A	0.58	0.36	-3.2E+05	-4.9E+05	0.041	0.209
A0828	Unknown sugar-like	1.06	0.44	0.880	0.106	Standard	0.023	Tukey-HSD	A	A	A	A	A	0.88	0.85	-7.5E+05	-9.1E+05	0.159	0.011
A0554	Putrescine TMS4	1.15	0.69	0.046	0.035	Kruskal-Wallis	0.014	Steel-Dwass	A	A	A	A	A	0.52	0.52	-9.7E+05	-9.7E+05	0.176	0.007
A0691	Tyrosine TMS2	1.05	0.67	0.002	0.046	Kruskal-Wallis	0.015	Steel-Dwass	A	A	A	A	A	0.17	0.17	-1.2E+06	-1.2E+06	0.211	0.003
A0184	Phosphate TMS3	2.27	0.52	0.009	0.329	Kruskal-Wallis	0.003	Steel-Dwass	A	A	A	A	A	0.87	0.87	-3.4E+06	-3.4E+06	0.202	0.004
A0872	Linoleic acid TMS	1.01	0.38	0.742	0.192	Standard	0.027	Tukey-HSD	A	A	A	A	A	0.79	0.74	-7.5E+06	-9.2E+06	0.265	0.001

Analytes with a grey background showed finally a non-significant difference according to post-hoc analysis.

Table S17. The Plant arrangement of 2014 in randomized bock design.

1. block			2. block			3. block			4. block		
Sample number	Plant number	Cultivar - Treatment	Sample number	Plant number	Cultivar - Treatment	Sample number	Plant number	Cultivar - Treatment	Sample number	Plant number	Cultivar - Treatment
1	1	P K5	16	76	YS K3	31	151	Resi K5	46	226	P K1
1	2	P K5	16	77	YS K3	31	152	Resi K5	46	227	P K1
1	3	P K5	16	78	YS K3	31	153	Resi K5	46	228	P K1
1	4	P K5	16	79	YS K3	31	154	Resi K5	46	229	P K1
1	5	P K5	16	80	YS K3	31	155	Resi K5	46	230	P K1
2	6	Resi K3	17	81	P K2	32	156	YS K4	47	231	Resi K3
2	7	Resi K3	17	82	P K2	32	157	YS K4	47	232	Resi K3
2	8	Resi K3	17	83	P K2	32	158	YS K4	47	233	Resi K3
2	9	Resi K3	17	84	P K2	32	159	YS K4	47	234	Resi K3
2	10	Resi K3	17	85	P K2	32	160	YS K4	47	235	Resi K3
3	11	YS K1	18	86	Resi K1	33	161	P K1	48	236	YS K5
3	12	YS K1	18	87	Resi K1	33	162	P K1	48	237	YS K5
3	13	YS K1	18	88	Resi K1	33	163	P K1	48	238	YS K5
3	14	YS K1	18	89	Resi K1	33	164	P K1	48	239	YS K5
3	15	YS K1	18	90	Resi K1	33	165	P K1	48	240	YS K5
4	16	Resi K5	19	91	P K4	34	166	Resi K2	49	241	P K5
4	17	Resi K5	19	92	P K4	34	167	Resi K2	49	242	P K5
4	18	Resi K5	19	93	P K4	34	168	Resi K2	49	243	P K5
4	19	Resi K5	19	94	P K4	34	169	Resi K2	49	244	P K5
4	20	Resi K5	19	95	P K4	34	170	Resi K2	49	245	P K5
5	21	P K2	20	96	YS K4	35	171	P K3	50	246	YS K2
5	22	P K2	20	97	YS K4	35	172	P K3	50	247	YS K2
5	23	P K2	20	98	YS K4	35	173	P K3	50	248	YS K2
5	24	P K2	20	99	YS K4	35	174	P K3	50	249	YS K2
5	25	P K2	20	100	YS K4	35	175	P K3	50	250	YS K2
6	26	YS K3	21	101	Resi K3	36	176	YS K5	51	251	Resi K5
6	27	YS K3	21	102	Resi K3	36	177	YS K5	51	252	Resi K5
6	28	YS K3	21	103	Resi K3	36	178	YS K5	51	253	Resi K5
6	29	YS K3	21	104	Resi K3	36	179	YS K5	51	254	Resi K5
6	30	YS K3	21	105	Resi K3	36	180	YS K5	51	255	Resi K5
7	31	Resi K2	22	106	YS K1	37	181	Resi K1	52	256	P K2
7	32	Resi K2	22	107	YS K1	37	182	Resi K1	52	257	P K2
7	33	Resi K2	22	108	YS K1	37	183	Resi K1	52	258	P K2
7	34	Resi K2	22	109	YS K1	37	184	Resi K1	52	259	P K2
7	35	Resi K2	22	110	YS K1	37	185	Resi K1	52	260	P K2
8	36	P K1	23	111	P K3	38	186	YS K3	53	261	YS K1
8	37	P K1	23	112	P K3	38	187	YS K3	53	262	YS K1
8	38	P K1	23	113	P K3	38	188	YS K3	53	263	YS K1
8	39	P K1	23	114	P K3	38	189	YS K3	53	264	YS K1
8	40	P K1	23	115	P K3	38	190	YS K3	53	265	YS K1

In each of the four blocks different plant groups (comprising 5 plants) of the three cultivars Primavera (P), Resi, and Yellow Submarine (YS) were fertilized with one of the five K-levels from K1 (grey, 0.37 g K_2SO_4 per week), K2 (green, 0.73 g K_2SO_4 per week), K3 (yellow, 1.47 g K_2SO_4 per week), K4 (blue, 2.2 g K_2SO_4 per week), and K5 (red, 3.66 g K_2SO_4 per week).

Table S18., continued

9	41	Resi K1	24	116	Resi K5	39	191	P K5	54	266	Resi K4
9	42	Resi K1	24	117	Resi K5	39	192	P K5	54	267	Resi K4
9	43	Resi K1	24	118	Resi K5	39	193	P K5	54	268	Resi K4
9	44	Resi K1	24	119	Resi K5	39	194	P K5	54	269	Resi K4
9	45	Resi K1	24	120	Resi K5	39	195	P K5	54	270	Resi K4
10	46	YS K2	25	121	YS K5	40	196	Resi K4	55	271	YS K4
10	47	YS K2	25	122	YS K5	40	197	Resi K4	55	272	YS K4
10	48	YS K2	25	123	YS K5	40	198	Resi K4	55	273	YS K4
10	49	YS K2	25	124	YS K5	40	199	Resi K4	55	274	YS K4
10	50	YS K2	25	125	YS K5	40	200	Resi K4	55	275	YS K4
11	51	P K4	26	126	Resi K2	41	201	P K2	56	276	P K4
11	52	P K4	26	127	Resi K2	41	202	P K2	56	277	P K4
11	53	P K4	26	128	Resi K2	41	203	P K2	56	278	P K4
11	54	P K4	26	129	Resi K2	41	204	P K2	56	279	P K4
11	55	P K4	26	130	Resi K2	41	205	P K2	56	280	P K4
12	56	YS K5	27	131	P K5	42	206	YS K1	57	281	Resi K2
12	57	YS K5	27	132	P K5	42	207	YS K1	57	282	Resi K2
12	58	YS K5	27	133	P K5	42	208	YS K1	57	283	Resi K2
12	59	YS K5	27	134	P K5	42	209	YS K1	57	284	Resi K2
12	60	YS K5	27	135	P K5	42	210	YS K1	57	285	Resi K2
13	61	Resi K4	28	136	YS K2	43	211	Resi K3	58	286	P K3
13	62	Resi K4	28	137	YS K2	43	212	Resi K3	58	287	P K3
13	63	Resi K4	28	138	YS K2	43	213	Resi K3	58	288	P K3
13	64	Resi K4	28	139	YS K2	43	214	Resi K3	58	289	P K3
13	65	Resi K4	28	140	YS K2	43	215	Resi K3	58	290	P K3
14	66	P K3	29	141	Resi K4	44	216	P K4	59	291	YS K3
14	67	P K3	29	142	Resi K4	44	217	P K4	59	292	YS K3
14	68	P K3	29	143	Resi K4	44	218	P K4	59	293	YS K3
14	69	P K3	29	144	Resi K4	44	219	P K4	59	294	YS K3
14	70	P K3	29	145	Resi K4	44	220	P K4	59	295	YS K3
15	71	YS K4	30	146	P K1	45	221	YS K2	60	296	Resi K1
15	72	YS K4	30	147	P K1	45	222	YS K2	60	297	Resi K1
15	73	YS K4	30	148	P K1	45	223	YS K2	60	298	Resi K1
15	74	YS K4	30	149	P K1	45	224	YS K2	60	299	Resi K1
15	75	YS K4	30	150	P K1	45	225	YS K2	60	300	Resi K1

Table S18. Plant arrangement of 2015 in randomized bock design.

1. block			2. block			3. block			4. block		
Sample number	Plant number	Cultivar - Treatment	Sample number	Plant number	Cultivar - Treatment	Sample number	Plant number	Cultivar - Treatment	Sample number	Plant number	Cultivar - Treatment
1	1	PV K1	7	81	Resi Kd	13	161	Resi K1	19	241	Resi K5
1	2	PV K1	7	82	Resi Kd	13	162	Resi K1	19	242	Resi K5
1	3	PV K1	7	83	Resi Kd	13	163	Resi K1	19	243	Resi K5
1	4	PV K1	7	84	Resi Kd	13	164	Resi K1	19	244	Resi K5
1	5	PV K1	7	85	Resi Kd	13	165	Resi K1	19	245	Resi K5
1	6	PV K1	7	86	Resi Kd	13	166	Resi K1	19	246	Resi K5
1	7	PV K1	7	87	Resi Kd	13	167	Resi K1	19	247	Resi K5
1	8	PV K1	7	88	Resi Kd	13	168	Resi K1	19	248	Resi K5
1	9	PV K1	7	89	Resi Kd	13	169	Resi K1	19	249	Resi K5
1	10	PV K1	7	90	Resi Kd	13	170	Resi K1	19	250	Resi K5
1	11	PV K1	7	91	Resi Kd	13	171	Resi K1	19	251	Resi K5
1	12	PV K1	7	92	Resi Kd	13	172	Resi K1	19	252	Resi K5
1	13	PV K1	7	93	Resi Kd	13	173	Resi K1	19	253	Resi K5
2	14	Resi K1	8	94	PV K5	13	174	Resi K1	19	254	Resi K5
2	15	Resi K1	8	95	PV K5	13	175	Resi K1	19	255	Resi K5
2	16	Resi K1	8	96	PV K5	14	176	PV Kd	20	256	Resi Kd
2	17	Resi K1	8	97	PV K5	14	177	PV Kd	20	257	Resi Kd
2	18	Resi K1	8	98	PV K5	14	178	PV Kd	20	258	Resi Kd
2	19	Resi K1	8	99	PV K5	14	179	PV Kd	20	259	Resi Kd
2	20	Resi K1	8	100	PV K5	14	180	PV Kd	20	260	Resi Kd
2	21	Resi K1	8	101	PV K5	14	181	PV Kd	20	261	Resi Kd
2	22	Resi K1	8	102	PV K5	14	182	PV Kd	20	262	Resi Kd
2	23	Resi K1	8	103	PV K5	14	183	PV Kd	20	263	Resi Kd
2	24	Resi K1	8	104	PV K5	14	184	PV Kd	20	264	Resi Kd
2	25	Resi K1	8	105	PV K5	14	185	PV Kd	20	265	Resi Kd
2	26	Resi K1	8	106	PV K5	14	186	PV Kd	20	266	Resi Kd
2	27	Resi K1	9	107	PV Kd	14	187	PV Kd	20	267	Resi Kd
2	28	Resi K1	9	108	PV Kd	15	188	Resi K5	20	268	Resi Kd
3	29	Resi K5	9	109	PV Kd	15	189	Resi K5	21	269	PV K1
3	30	Resi K5	9	110	PV Kd	15	190	Resi K5	21	270	PV K1
3	31	Resi K5	9	111	PV Kd	15	191	Resi K5	21	271	PV K1
3	32	Resi K5	9	112	PV Kd	15	192	Resi K5	21	272	PV K1
3	33	Resi K5	9	113	PV Kd	15	193	Resi K5	21	273	PV K1
3	34	Resi K5	9	114	PV Kd	15	194	Resi K5	21	274	PV K1
3	35	Resi K5	9	115	PV Kd	15	195	Resi K5	21	275	PV K1
3	36	Resi K5	9	116	PV Kd	15	196	Resi K5	21	276	PV K1
3	37	Resi K5	9	117	PV Kd	15	197	Resi K5	21	277	PV K1

In each of the four blocks different plant groups of the two cultivars Resi and Primavera (PV) were fertilized with one of three K-levels from K1 (yellow, 0.37 g K_2SO_4 per week), K5 (red, 3.66 g K_2SO_4 per week), and Kd (orange 3.66 g K_2SO_4 for 5 weeks).

Table S19., continued

3	38	Resi K5
3	39	Resi K5
3	40	Resi K5
3	41	Resi K5
3	42	Resi K5
3	43	Resi K5
4	44	Resi Kd
4	45	Resi Kd
4	46	Resi Kd
4	47	Resi Kd
4	48	Resi Kd
4	49	Resi Kd
4	50	Resi Kd
4	51	Resi Kd
4	52	Resi Kd
4	53	Resi Kd
4	54	Resi Kd
4	55	Resi Kd
4	56	Resi Kd
5	57	PV K5
5	58	PV K5
5	59	PV K5
5	60	PV K5
5	61	PV K5
5	62	PV K5
5	63	PV K5
5	64	PV K5
5	65	PV K5
5	66	PV K5
5	67	PV K5
5	68	PV K5
5	69	PV K5
6	70	PV Kd
6	71	PV Kd
6	72	PV Kd
6	73	PV Kd
6	74	PV Kd
6	75	PV Kd
6	76	PV Kd
6	77	PV Kd
6	78	PV Kd
6	79	PV Kd
6	80	PV Kd
10	118	PV K1
10	119	PV K1
10	120	PV K1
10	121	PV K1
10	122	PV K1
10	123	PV K1
10	124	PV K1
10	125	PV K1
10	126	PV K1
10	127	PV K1
10	128	PV K1
10	129	PV K1
10	130	PV K1
11	131	Resi K5
11	132	Resi K5
11	133	Resi K5
11	134	Resi K5
11	135	Resi K5
11	136	Resi K5
11	137	Resi K5
11	138	Resi K5
11	139	Resi K5
11	140	Resi K5
11	141	Resi K5
11	142	Resi K5
11	143	Resi K5
11	144	Resi K5
11	145	Resi K5
12	146	Resi K1
12	147	Resi K1
12	148	Resi K1
12	149	Resi K1
12	150	Resi K1
12	151	Resi K1
12	152	Resi K1
12	153	Resi K1
12	154	Resi K1
12	155	Resi K1
12	156	Resi K1
12	157	Resi K1
12	158	Resi K1
12	159	Resi K1
12	160	Resi K1
15	198	Resi K5
15	199	Resi K5
15	200	Resi K5
15	201	Resi K5
15	202	Resi K5
16	203	PV K5
16	204	PV K5
16	205	PV K5
16	206	PV K5
16	207	PV K5
16	208	PV K5
16	209	PV K5
16	210	PV K5
16	211	PV K5
16	212	PV K5
16	213	PV K5
16	214	PV K5
16	215	PV K5
17	216	Resi Kd
17	217	Resi Kd
17	218	Resi Kd
17	219	Resi Kd
17	220	Resi Kd
17	221	Resi Kd
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17	227	Resi Kd
17	228	Resi Kd
17	229	Resi Kd
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18	240	PV K1
21	278	PV K1
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22	283	PV Kd
22	284	PV Kd
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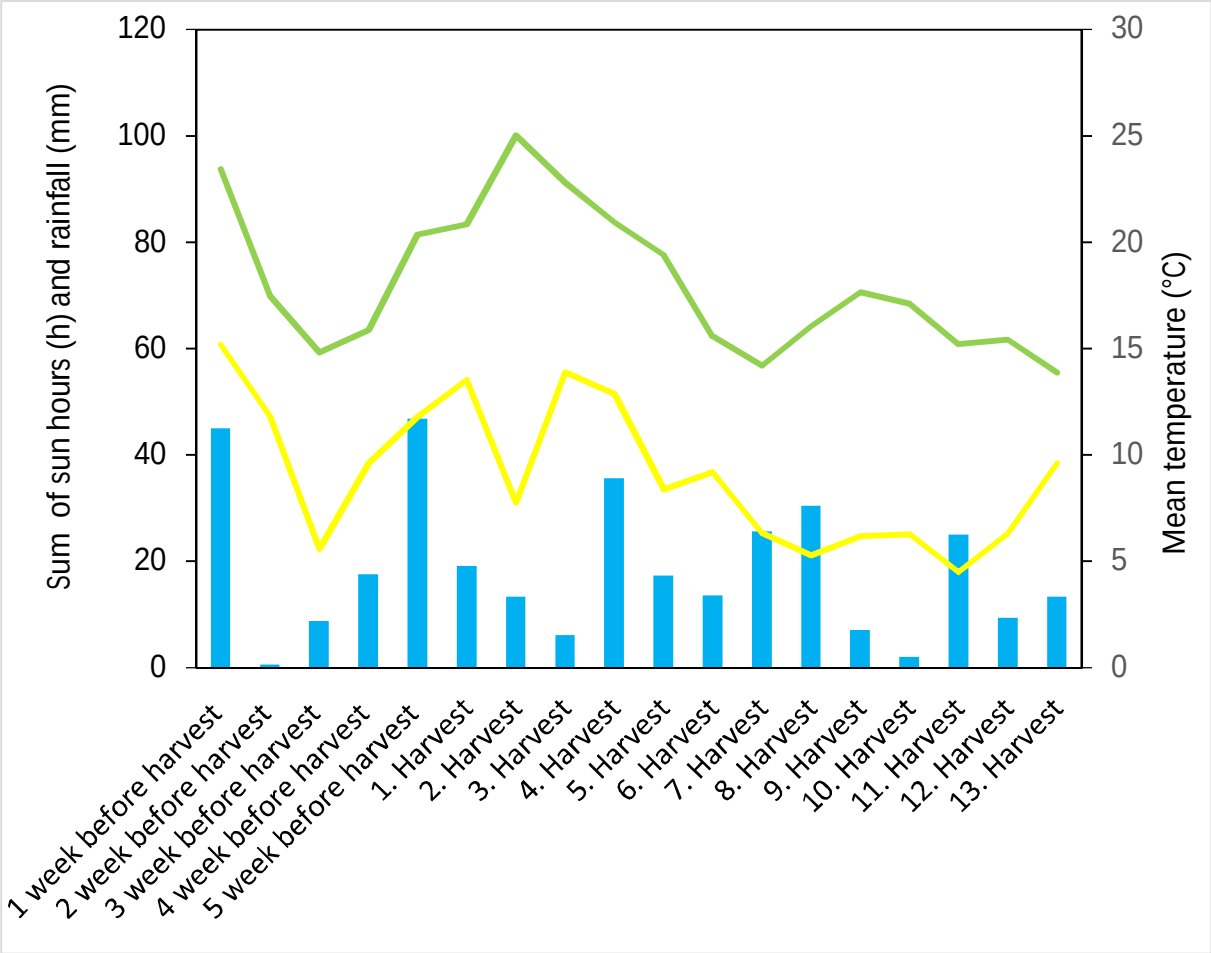

Figure S7. Sum of sun hours (h) sum of rainfall (mm) and mean temperature (°C) of the growing season 2014. Sunshine (yellow line) and rainfall (blue bar) was added up for each weak, while for the temperature (green line) a weekly mean was calculated.

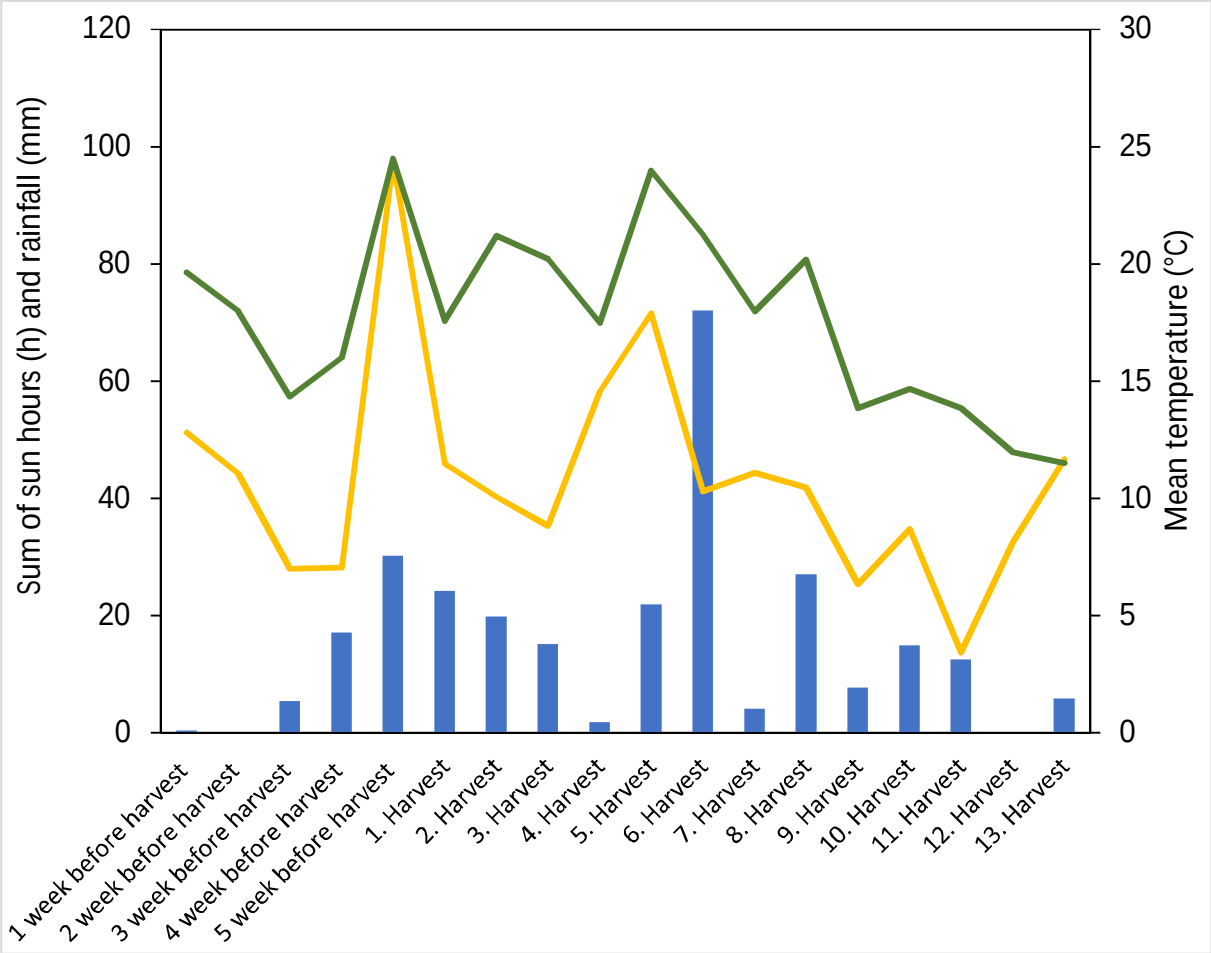

Figure S8. Sum of sun hours (h) sum of rainfall (mm) and mean temperature (°C) of the growing season 2015. Sunshine (yellow line) and rainfall (blue bar) was added up for each weak, while for the temperature (green line) a weekly mean was calculated.

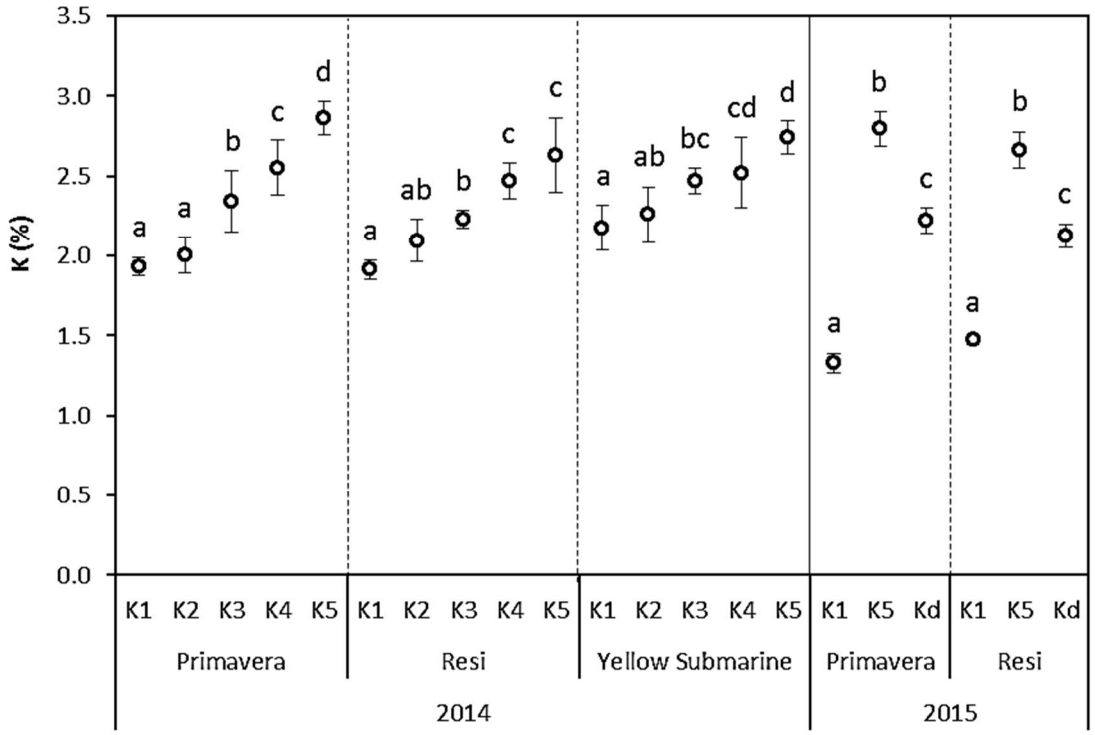

Figure S9. K application differently affects K concentration in the dry matter. K levels increase from K1 to K5 (0.37 g, 0.73 g, 1.47 g, 2.2 g to 3.66 g K_2SO_4 per week) for each cultivar. In 2015, a depletion fertilization treatment (Kd) received only in the first five weeks K (3.66 g K_2SO_4 per week). The mean values were determined from four biological replicates. The standard deviation of means was calculated for all mean values. Letters indicate statistically significant differences.

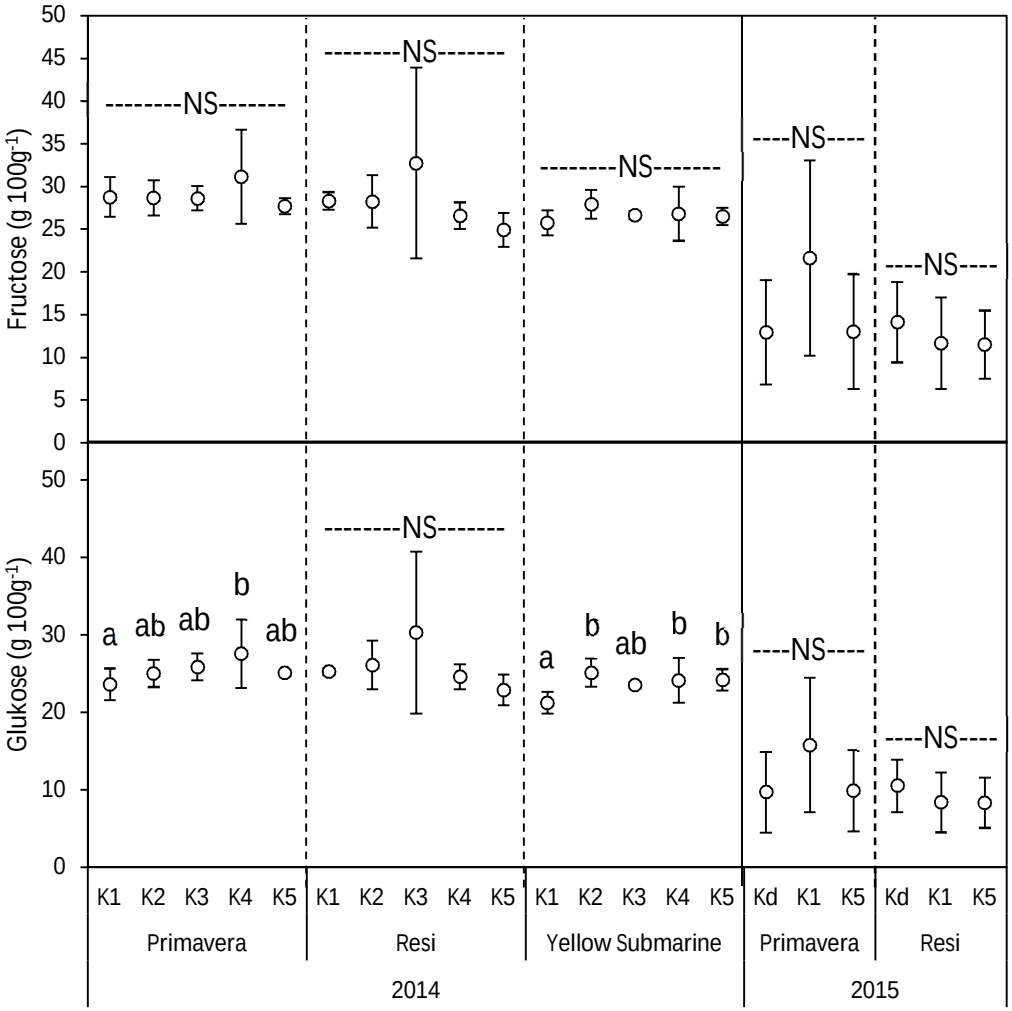

Figure S10. K treatment differently affects fructose and glucoses concentration of three cocktail tomato cultivars. K levels increase from K1 to K5 (0.37 g, 0.73 g, 1.47 g, 2.2 g to 3.66 g K_2SO_4 per week) for each cultivar. In 2015 a depletion fertilization treatment (Kd) received only in the first five weeks K (3.66 g K_2SO_4 per week). The mean values were determined from four biological replicates. The standard deviation of means was calculated for all mean values. Letters indicate statistically significant differences and NS no significant difference.

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Declarations

1. I, hereby, declare that this Ph.D. dissertation has not been presented to any other examining body either in its present or a similar form.

   Furthermore, I also affirm that I have not applied for a Ph.D. at any other higher school of education.

   Göttingen, ...

   .....

   Frederike Sonntag

2. I, hereby, solemnly declare that this dissertation was undertaken independently and without any unauthorised aid.

   Göttingen, ...

   .....

   Frederike Sonntag